Journal of Chemistry and Environment (ISSN: 2959-0132)

Vol.1, No.1

December 2022

Journal of Chemistry and Environment (ISSN: 2959-0132)

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Journal of Chemistry and Environment

Determination of Antioxidant Activity of Acacia Nilotica Linn from different Regions of Baluchistan (Pakistan)

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Abstract

Free radicals are naturally occurring species with unpaired electrons that are formed during normal metabolic processes. Their formation in a high amount may cause oxidation of essential structural molecules of cell, the condition is known as oxidative stress which results in several health issues such as cancer, diabetes, heart diseases, inflammatory diseases, arthritis and a lot more. To overcome these effects, antioxidants, both synthetic and natural ones are consumed by people. Due to recent evidences about the long term harmful effects of synthetic antioxidants the interest towards the natural sources has increased. The aim of the current research is to comparatively analyze the antioxidant activity of acacia Nilotica Linn samples collected from different regions of Makran by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay on which no work has been done so far. The DPPH assay of the samples gave the following results with antioxidant activity values as 18.51851%, 4.47761%, and 8.25958% for the samples of acacia Nilotica Linn from Shahrak, acacia from Turbat, acacia from Pedrak. The results revealed that acacia Nilotica Linn from Shahrak exhibits maximum free radical scavenging activity. More work on chemical composition of the tested samples has to be done.

Keywords: Acacia Nilotica Linn, free radical, antioxidants, DPPH assay

1. INTRODUCTION

Antioxidants poly a significant role for the protection of health. According to scientific confirmation antioxidants decrease the risk for many chronic diseases which include heart and cancer diseases [1]. Naturally occurring major sources of antioxidants are vegetables, fruits, leaves etc [2]. The antioxidant compounds are phenol and polyphenols, flavonoids, carotenoids, steroids and thiol these are natural supplements [3]. The benefits of these antioxidants is that they provide antioxidant defense against oxidative stress and show low chances of causing diseases. The antioxidants of the body are insufficient to protect the body thus supply of external antioxidants is also necessary [4]. Potential approaches for natural antioxidants have been examined in different plant materials. A number of diseases affected by (ROS) which are formed during metabolic processes of cell [5]. In normal conditions for immunity of the biological system since it protects the body from being infected by bacterial and viruses. But their excessive production can harm the tissues [6]. These free radicals cause lipid peroxidation in membrane which is considered to be the starting step towards the cell-injury [7].These free radicals are stabilized by antioxidants by reacting with them before they oxidized any cellular component and in this way they protect the cell from

oxidative injury [8].

Pakistan, which is an agricultural country, rich in aromatic and medicinal plants which are used as traditional medicine for health care [9]. Acacia nilotica is a plant which has known for its useful sources of bioactive properties. Acacia nilotica which gives number of bioactive components that shows anti-hypertensive, anti-platelet, aggregatory, anti-inflammatory, spasmogenic, antispasmodic and vasoconstrictor properties [10]. An inexpensive and a fast simple method for the measurement of antioxidant activity involves the use of (DPPH) which is being used to test the ability of compounds which act as hydrogen donor or free radical scavengers to determine antioxidant activity [11]. The technique of (DPPH) test is based on the (DPPH) reduction which is a stable free radical. The unpaired electrons of free radical (DPPH) shows extreme absorption at 517 nm with (purple color) [12]. As the antioxidant and DPPH react with each other which was the stable radical it becomes paired off because due to the existence of a H-donor and it will became DPPH-H and as concerns the absorptions reduced from the DPPH[13]. The DPPH-H is form, which results in decolonization (yellow color) with respect to total taken electrons [14]. This plant has been testified to own antioxidant properties. So this work has been carry out to assess Acacia nilotica plant for their possible potential to antioxidant action by DPPH Scavenging method [15].

2. EXPERIMENTAL WORK
2.1 Materials and Methods
2.2 Sample collection

Leaves of acacia Nilotica commonly known as desi-kiker were collected from three regions of Makran division of Baluchistan (Pakistan). Each sample were given a number as sample 1, sample 2, sample 3. Sample 1, collected from Shahrak in 25th October 2020, sample 2, collected from Turbat in 30th October 2020, sample 3, collected from Pedrak in 3rd November 2020.

2.3 Chemicals and reagents
All the chemicals and reagents used were of analytical evaluation. Ethanol from sigma Aldrich (www.sigma-aldrich.com), distilled water, and DPPH (2, 2-reagent 95%) from Alfa Aesar, united states.

2.4 Extraction

Air-dried leaves of this plant were ground into powered. 1 gram of each sample was dissolved individually in of 100ml 70% ethanol (absolute). After stirring each sample solution for 5 minutes they were stirred and kept for 5 days at room temperature [11]. After the given period of interval each solution was stirred by magnetic stirrer for 30 minutes and then these were filtered by wattman filter paper to remove the solid residues.

2.5 DPPH radical scavenging assay

Free radical scavenging activities of different leaves extract were measured by (DPPH). In brief, 0.5ml sample solution, 3ml ethanol absolute and 0.3ml of freshly prepared solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution were mixed to get four tests of each sample. Four blanks for each sample were prepare by mixing 3.3 ethanol absolute and 0.5mal sample solution. A control was prepared by adding 0.3mal (DPPH) solution in 3.5ml ethanol and all these were kept in dark for 2hours at room temperature. After that absorbance of control, blacks and tests were measured at 517nm (UV-visible Spectrophotometer (UV 752 (D), China). Ascorbic acid which is a synthetic antioxidant was used as a standard. A 25ppm ethanolic solution of ascorbic acid as standard. The blank and test of ascorbic acid solution were prepared by following the same protocol as was used for the sample and absorbance was measured [12, 13].

2.6 Statistical analysis

Reduction in absorbance is a degree of better potential of substance is scavenging activity. The antioxidant activity is determined and calculated by using the equation 1.

DPPH Scavenging effect (%) or percent inhibition = A°-

2

$A^1 / A^\circ * 100$ (equation. 1)

Where A° was the absorbance of control reaction and A^1 was the absorbance in presence of test or standard sample.

3. RESULTS AND DISCUSSION

The acacia nilotica leaves showed greater antioxidant potential when these are compare with the standard ascorbic acid by DPPH method [14]. The absorbance is measured at 517nm the analysis involves UV-visible spectrophotometer which shows maximum absorbance of sample 1 (18.51851%) and 8.25958%, 4.47761% shown by the sample of Turbat and Pedrak. For standard ascorbic acid value were obtained as 22.28571%.
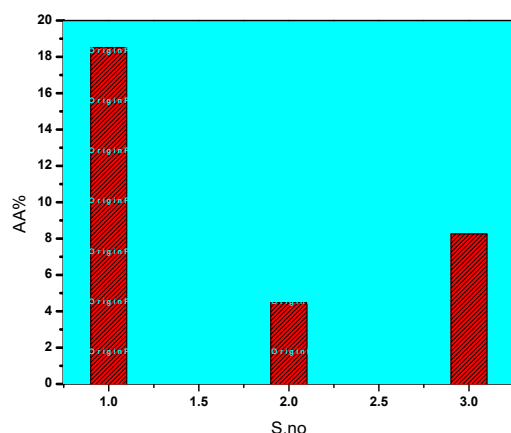

FIGURE 1. Absorbance of different extract of acacia nilotica at 517nm by uv-visible spectrophotometer by DPPH Scavenging assay method.

It means that plant species gives better antioxidant activity by free radical scavenging method as they are compared with standard. So, we can say that acacia nilotica has potential antioxidant activity and it can get a way towards the pharmaceutical uses. The values of control and blank are given in Table 1.Determination of AA% in real samples of acacia Nilotica Linn is given Table 2. AA% of standard (Ascorbic acid) is given Table 3. Absorbance of different extract of acacia nilotica at 517nm by uv-visible spectrophotometer by DPPH Scavenging assay method is given Figure 1.

TABLE 1. Values of control and blanks.

Solution	Absorbance	Mean± SD	Co variance
Blank 1	0.200 0.201 0.202	0.201	0.00082
Blank 2	0.152 0.153 0.154	0.153	0.00082
Blank 3	0.148 0.149 0.151	0.1493	0.00129
Control	0.270 0.271 0.272	0.271	0.00082

TABLE 2. Determination of AA% in real samples of acacia Nilotica Linn.

S. No	Sample type	Absorbance	Mean± SD	Co variance	AA%
1	Acacia from Shahrak	0.420 0.421 0.422	0.421	0.00082	18.51851%
2	Acacia from Turbat	0.344 0.345 0.346	0.345	0.00082	4.47761%
3	Acacia from Pedrak	0.459 0.460 0.461	0.46	0.00082	8.25958%

TABLE 3. AA% of standard (Ascorbic acid).

Absorbance	Meann± SD	Co variance	AA%
0.220 0.221 0.222	0.221	0.00082	22.28571%

4. CONCLUSION

Antioxidants are one of the major requirements of our body in today's world where there are higher risks of disease due to increasing environmental pollution. Since the pollution and several other factors contribute as exogenous source of free radical formation resulting in oxidative stress that results in a number of chronic health issues. For a long period of time the attention of health care institutions and product manufacturers are seeking for the best natural source of antioxidants that are of high value due to their safety assurance. The long term harmful effects of synthetic antioxidants have grabbed the attention of researchers to seek for the safe natural and low cost antioxidant sources. Plants and the derivatives of plants have been proven as the best source of antioxidants. This work

reveals new essential natural sources of antioxidants that are regional acacia samples from three different regions of Makran. The results indicate that all these natural products are significant antioxidants that need to be further analyzed for their chemical composition.

Authors Contribution

M.H supervised research work and has the main idea. ZAA did practical work and wrote the manuscript. FH, AZ, MH, SM, NU and MJ revised the manuscript and provided suggestions. FK is coworker in research. AH coworker in research.

Acknowledgment

Conflict of Interest

The authors declare no conflict of interest. All the authors approved the submission of the manuscript.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

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Journal of Chemistry and Environment

Synthesis, characterization and antibacterial activity of ethylene di-amine and 2-hydroxybenzadehyde Schiff base and its metal complexes

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Abstract

A number of modern techniques have been developed for the synthesis of Schiff bases. We reported the synthesis of ethylene di-amine and 2-hydroxybenzadehyde Schiff base (SB) via the condensation method. To remove phenolic hydrogen to form Schiff base it was reacted with sodium hydroxide and then treated with M(II) chloride (M=Fe, Cu, Zn, Ni and Sn) to fabricate their respective metal complexes. The synthesis of SB metal complexes and detailed functional group characterization were validated via Fourier transform infrared (FT-IR) spectroscopy. In the final SB, FT-IR results revealed a vibrational peak at 1614 cm^{-1}, which was credited to the –C=N part. The absence of a vibration band for –OH vibration on 1613 cm^{-1} and the presence of a novel band in the 659 to 586 cm^{-1} range were due to the metal-oxygen bond, confirming the synthesis of metal complexes. The Schiff base showed high antibacterial activity against *E. coli, Pseudomonas aeruginosa, Salmonella typhi, S.aureus* and *Bacillus* whereas *Streptococcus* was found resistant. Cu, Fe and Sn coordination improved Schiff base activity while Ni coordination did not affect the activity. Similarly, Fe and Sn complex had no effect on *E. coli*. In comparison with standard Ciprofloxacin, the activities of respective metal complexes were low.

Keywords: Ethylene di-amine and 2-hydroxybenzadehyde Schiff base; metal complex of Schiff base; FT-IR; antibacterial activity; standard ciprofloxacin.

1. Introduction

In 1864, a German chemist named Hugo Schiff developed Schiff base (SB) by condensing primary amines and aldehyde [1]. The following compound, R1R2C=NR3, is known as an SB since R1 is an aryl group, R2 is a hydrogen atom, and R3 is an aryl or alkyl group, which is also known as a Schiff base. SBs with aryl substituents are the most stable and easy to synthesize, whereas those with alkyl substituent's are more unstable [2]. SBs and their metal complexes have been used since the mid-nineteenth century. Jorgensen and Warner, the two physicists, addressed the role of SBs and their metal complexes in coordination chemistry [3]. From salicyl aldehyde SBs and their substituted analogues, Pfeiffer and his colleagues built a chain of complexes [4]. The development of SBs complexes, their chelation properties, and stereochemistry are all studied [5]. These are generally bidentate, tridentate, tetradentate or polydentate ligands and form stable complexes with transition metals by forming five or six member ring at the condensation site[6].

1.1 SB metal complexes and their properties

SB based complexes have earned a vital position in coordination chemistry. In this connection, S and N have

been the key elements in the coordination chemistry related biomolecules [7]. SB metal complexes have some intriguing properties of chelation [8, 9] oxygen affinity [10] and hence can be used as a catalyst and in the processing of dyes [11].

1.2 SBs and their metal complexes' biological significance

Malaria is a parasitic disease caused by the Plasmodium genus that kills one million people per year [12]. More than 500 million people are affected according to WHO data, with 90 percent of those affected being children from Sub-Saharan Africa. For controlling malaria, SB based molecules have been effectively applied, especially their Ruthenium complexes from aryl and ferrocyl groups [13]. The development of drug resistance against available antibiotic drugs has been a growing concern for the researcher. This has also led to a significant rise in the mortality rate [14]. SBs made from 2-hydroxy-1-napth-aldehyde and alpha amino-acids possessed exceptional antibacterial action. Apart from these, SBs resulting from salicylaldehyde show strong antibacterial activity against *Mycobacterium tuberculosis* [15].

Some SBs and their complexes like N-salicylidene-2-hydroxyaniline and 3-Fluro salicylaldehydeOxo vanadium (IV) have also been reported with promising antifungal activity. Similarly, SB derived from chitosanareis effective against *Botrytis cinerrea* and *Colletotrichumlagenarrium*[16]. SBs produced from isatin and bisisatin. Abacavir (Ziagen), a pro-drug, has been shown to have potent antiviral and anti-HIV properties [17]. Furthermore, SBs of 2-phenylquinazoline-4(3)H-one have been shown to have potent antiviral properties against corona virus, influenza, and HSV types 1 and 2 [18]. In addition to these activities, SBs have been shown to possess anticancer (Cumarin and pyrazole) [19]. 2, 6-dichloroanilino and 4 amino, 5-di methyl and similarly, some SBs possess activities against insects like bollworm (o-vanillin and its metal complexes). SBs derivative from o-vanillin and their metal complexes

have anti-bollworm properties [20].

Hydrazine carboxoamide and metal complexes of di-oxo Manganese have the anti-fertility ability to alter reproductive physiology [21]. Some of the SBs possess anti-allergic, analgesic, radical scavenging and anti-oxidative activities [22]. Attributed to these reports of SBs against various biotic cultures, herein we report the synthesis of ethylene di-amine and 2-hydroxybenzadehyde Schiff base and its metal complexes and their activities analysis against biological cultures. Fourier transform infrared (FT-IR) spectroscopy was used to classify the prepared SBs, which aided in the interpretation of the experimental findings.

2. Materials and Methods

2.1 Reagents

Sodium Hydroxide (NaOH), 2 hydroxy-Benzaldehyde, Ethylene-diamine, Iron (II) chloride (FeCl2), Copper (II) chloride (CuCl2),Tin (II) chloride (SnCl2), Zinc Chloride (ZnCl2), Nickel (II) chloride (NiCl2), Ethanol, Methanol, and Chloroform, Except for metal chloride salt, which was purchased from Sigma Aldrich, these chemicals were extremely pure and purchased from Fluka.

2.2 Methods

The condensation method was used to synthesize SB and its metal complexes while the antibacterial properties of these compounds were investigated using the agar well diffusion process.

2.3 Instruments

By using an electrochemical melting point apparatus, the difference in melting points of precursors and final products was calculated. FT-IR was used to analyze the chemistry and nature of bands and functional groups noted in a range of 4000 to 400cm^{-1} by using KBr-disc methods.

2.3 Synthesis of SB and respective metal complexes

The synthesis of SB and its metal complexes are schematically shown in Figure 1.

2.4 Synthesis of 2,2' (1E, 1E')-(ethane-1,2-diylbis (azan-1-yl-1-yidene) bis(methan-1-yl-1ylidene)-diphenol

First, ethylene diamine was reacted in 1:2 ratios with 2

hydroxy Benzaldehyde in a two-necked flask linked to a reflex condenser and Deane-Stark apparatus.

The extra solvent was evaporated while the methanol was refluxed for 3-4 hours, and the yields were washed away by ethanol and re-crystallized with chloroform. Figure 2 depicts the chemical reaction for SB synthesis.
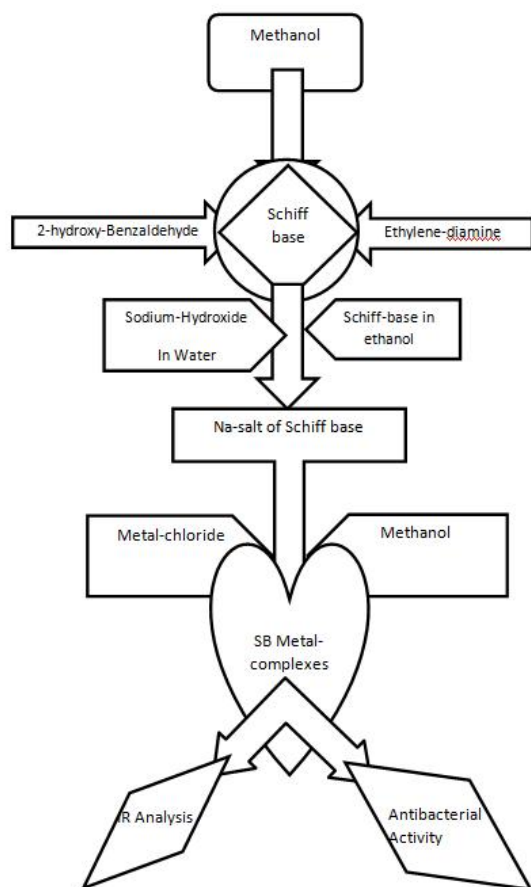

Figure 1: Overall pattern adopted throughout this study

2.5 Synthesis of the Sodium salt of SB

The solutions were combined with intense stirring and continuous heating for 1 hour after sodium hydroxide and SB were dissolved in water and ethanol in 1:2 ratios. The solvents were evaporated at 120°C, leaving a white substance that was cleaned, collected then preserved for coming experimentations. Figure 3 illustrates the chemical reaction of SB sodium salt.

2.6 SB synthesis of copper complex

Copper (II) chloride was added in a 1:2 ratio to a hot methanolic solution of SB-Na salt, then the mixture was

refluxed for 6-7 hours and then chilled overnight while the additional solvent was vaporized under reduced pressure. With the aid of chloroform, the finished product was re-crystallized.

2.7 Synthesis of the iron complex with SB

For the Creation of the SB-iron complex, a similar procedure was followed as that for the copper complex, and the reaction scheme is summarized in figure 5.

2.8 Nickel complex synthesis with SB

The nickel complex of SB was prepared by applying an equimolar solution of Nickel (II) chloride to a hot metha-nolic solution of SB-Na salt and refluxing it for 6-7 hours. With the aid of chloroform, the finished product was re-crystallized. Figure 6 shows the chemical reaction.

2.9 Synthesis of Tin and Zn complexes with SB

Similar procedures for the preparation of Sn and Zn complexes with SB were followed while their respective reaction schemes are shown in Figures 7 and 8.

2.10 Anti-bacterial activities evaluation

The anti-bacterial activity of SB and respective complexes were used against Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus, Streptococcus*, and Gram-negative bacteria such as *Bacillus subtilis*, *Escherichia c*oli, *Pseudomonas aeruginosa* and *Salmonella typh)*. Bacterial cultures were inoculated on media, and SB and their metal complexes solutions were poured into these cultures under aerobic condition and incubated for 24 hours at 37°C. The plates were analyzed for inhibition zones after 24 hours (IZ).

3. Results and discussion

The SB was synthesized by condensing ethylene-di-amine with 2-hydroxy-benzaldehyde (salicyl-aldehyde) in 1:2 ratios, and then react it with various metal salts to make its metal complexes. The empirical and structural formula of SB and its metal are summarized in Table 1.

3.1 SB and its metal complexes physical parameters

The Schiff base has a melting point of 113 to 115°C, while its metal complexes have a melting point of 262-293°C.Table no 2 lists the physical characteristics of SBs
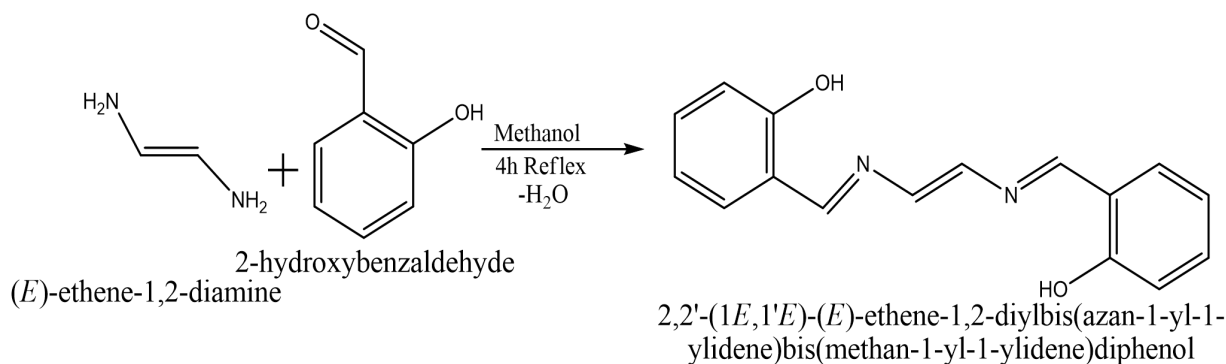
Figure 2: Chemical reaction for the synthesis of 2,2'(1E, 1E')-(ethane-1,2-diylbis (azan-1-yl-1-yidene)bis(methan-1-yl-1ylidene)-diphenolpreparation
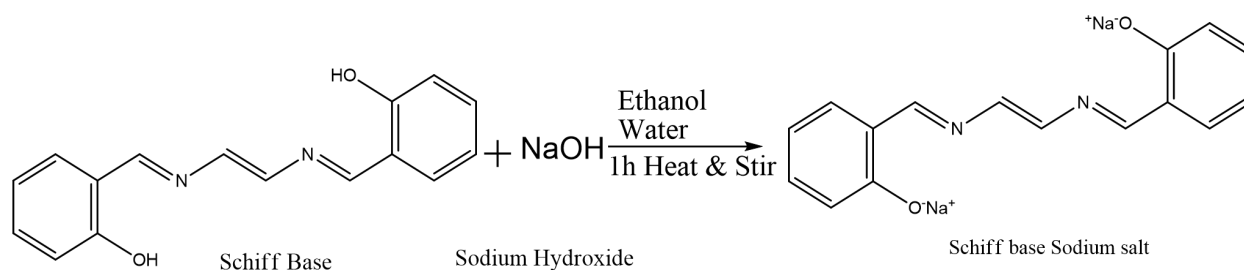

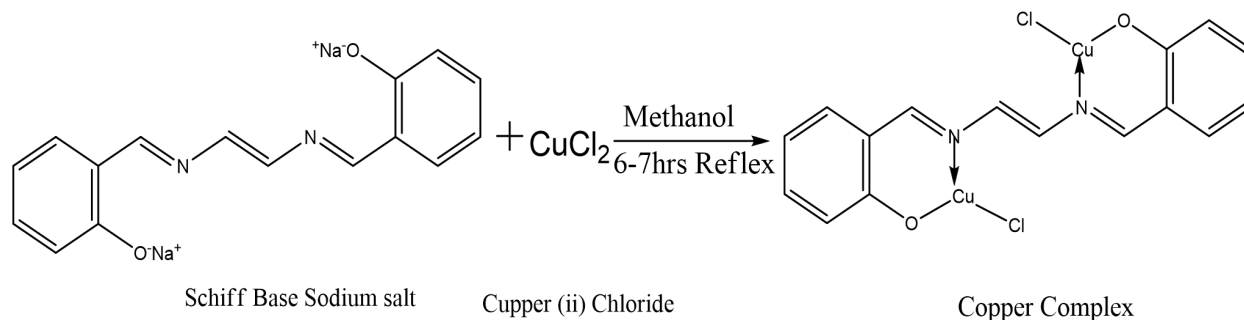**Figure 3:** Schiff base reaction with sodium salt

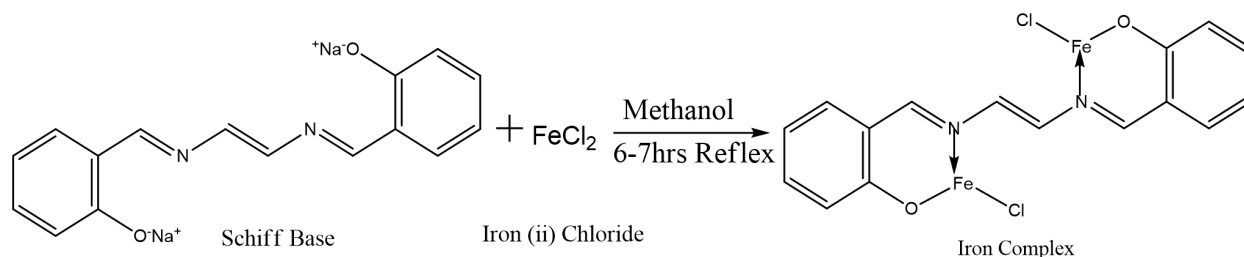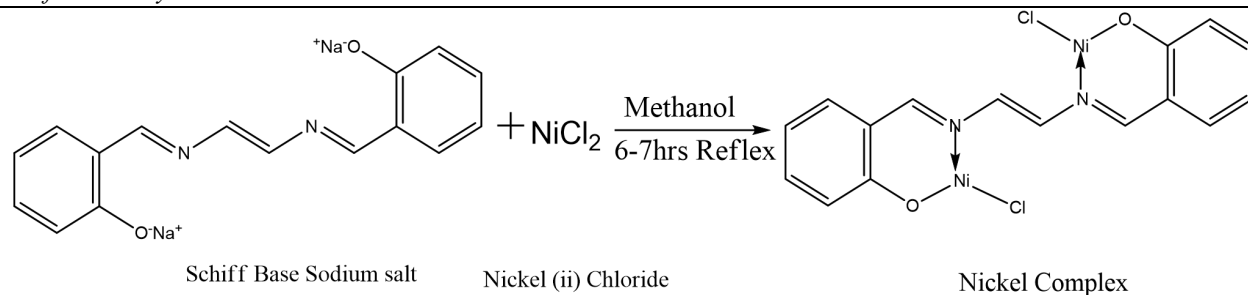**Figure 4:** Schiff base Na-salt with copper chloride reaction

Figure 5: Schiff base with Iron chloride reaction

Figure 6: Schiff base Na-salt with Nickel chloridereaction

Figure 7: Schiff base Na-salt with Tin chloride reaction

Figure 8: Schiff base Na-salt with Zn chloride reaction

and their complexes, such as color, melting point, molecular weight, and physical state. FT-IR in the range of 4000-400 cm^{-1} was used to perform spectroscopic analysis of SB and metal complexes, yielding valuable information about the chemistry and functional groups of different SBs and their corresponding complexes. Table 3 shows some of the most significant FT-IR bands of the synthesized SBs and metal complexes.

3.2 FT-IR characterization of SBs and their respective metal complexes

FT-IR research revealed a new vibration peak at 1614 cm^{-1} assigned to –C=N-, created by the reaction of ethylene diamine's amine (-NH2) and carbonyl-group (-C=O) of 2-hydroxyl-benzaldehyde. The hydroxyl group was attributed to a strong band in the FT-IR spectra of SB in the range of 3578-

3450 cm^{-1} (-OH). The absorption bands at 3086, 1588 and 1436 cm-1 were attributed to the carbon-hydrogen and aromatic carbon-carbon (C=C) double bonds correspondingly, while the absorption band at 1249 cm^{-1} was attributed to a carbonyl group. The existence, absence, and shifting of certain bands in FT-IR analysis determined the active preparation of SBs and their metal complexes. The absence of a vibration band for –OH vibration at 1614 cm-1 and the occurrence of a new band in the series of 659-586 cm^{-1} were due to the formation of metal complexes, confirming their synthesis [23]. Further groups at 536-516 cm^{-1} and 439-406 cm^{-1} are attributable to metal-chloride band, and imin- nitrogen similar by metal ion (N →M) as well confirmed the successful synthesis of metal complexes [24]. The shifting of the band for the imine group (-C=N-) to a lower wave number due to the shifting of electron density from

9

Nitrogen to metals ions in the FT-IR spectra of metal complexes is a clear confirmation of metal nonmetal coherence and production of metal complexes.

3.3 Anti-bacterial action

The synthesized compounds were dissolved in DMSO with a 1:10 ratio to test the biological activity of SB and its metal complexes. After 24 hours of incubation at 37°C, the diameter of IZ is visually determined. The PC was Ciprofloxacin, and the NC was DMSO.

3.4 Antibacterial activity of synthesized compounds against *Escherichia coli, streptococcus and Bacillus cereus*

By using the good diffusion process, the SB and its metal complexes were added to the cultures above. The synthesized complexes were effective against *E. coli* and *Bacillus cereus*, but had no effect on *Streptococcus* growth, which was unaffected by any metal complexes except SB, which has negligible activity. The IZ measures are tabulated in table 4.

Table 1: Titles, empirical and structural formulation of SBs and respective metal complexes

Serial	Titles	Empirical formulation	Structural formulation
1	2, 2'- (1E,1E)- (ethane- 1,2-diylbis(azan-1yl-1-ylidene)bis-(methan-1yl-1ylidene)-diphenol	$C_{16}H_{14}N_2O_2$	
2	(2- (E-(2- (E- 2-hydroxy benzalieneamine)ethylimino)methyl) phenoxy)di-cupper (II) di-chloride	$C_{16}H_{12}C_{12}Cu_2N_2O_2$	
3	(2-(E-(2-(E-2-hydroxybenzalieneamine)-ethylimino)methyl)phenoxy)di-iron (II) di-chloride	$C_{16}H_{12}C_{12}Fe_2N_2O_2$	
4	(2-(E-(2-(E-2-hydroxybenzalieneamine)-ethylimino)methyl) phenoxy)di-nickel (II) dichloride	$C_{16}H_{12}C_{12}Ni_2N_2O_2$	
5	(2-(E-(2- (E-2-hydroxybenzalieneamine)-ethylimino)methyl)phenoxy)di-tin (II) dichloride	$C_{16}H_{12}C_{12}Sn_2N_2O_2$	
6	(2-(E- (2-(E-2-hydroxybenzalieneamine)-ethylimino)methyl)phenox) di-zinc (II) dichloride	$C_{16}H_{12}C_{12}Zn_2N_2O_2$	

Table 2: SBs and their metal complexes physical parameters

Serial	Molecular-Formulas	Physical-state	Color	Molecular weight(g/mol)	Melting point(°C)	Yield%
1	$C_{16}H_{14}N_2O_2$	Solid	Yellowish	227.28	114-116	88
2	$C_{16}H_{12}C_{12}Cu_2N_2O_2$	Solid	Light blue	462.26	234-236	77
3	$C_{16}H_{12}C_{12}Fe_2N_2O_2$	Solid	Brownish	446.83	292-294	72
4	$C_{16}H_{12}C_{12}Ni_2N_2O_2$	Solid	Green	452.58	268-270	76
5	$C_{16}H_{12}C_{12}Sn_2N_2O_2$	Solid	White	573.56	207-209	68
6	$C_{16}H_{12}C_{12}Zn_2N_2O_2$	Solid	White	466.02	228-230	77

Table 3: FT-IR statistics of SBs and their metal complexes

S.No	Complexes	-O- H	=C –H Aro	-C= N	-C =C Aro	=C –O	M –O	M- N	N→ M
1	SB	3579-3451	3086	1614	1589 1437	1249			
2	Cu(ll)	-	3095	1598	1539 1406	1227	586	516	406
3	Fe(ll)	-	3109	1586	1545 1391	1231	616	551	439
4	Ni(ll)	-	3131	1571	1569 1376	1211	659	511	426
5	Sn(ll)	-	3111	1585	1581 1372	1222	650	536	411
6	Zn(ll)		3127	1579	1576 1381	1216	631	523	416

Table 4: IZ of synthesized complexes against *E. coli, streptococcus and bacillus cereus*

Serial	Complexes	Dilution 1:10	Ciprofloxacin(Positive control)	DMSO(Negative control)
Esherichia Coli				
1	SB	6mm	19.6mm	0 mm
2	Cu- complex	4.4mm		
3	Fe-Complex	Resistant		
4	Ni-complex	3.7mm		
5	Sn-complex	Resistant		
6	Zn-complex	4.2mm		
Streptococcus				
1	SB	0.5mm	14mm	0mm
2	Cu-complex	Resistant		
3	Fe-complex	Resistant		
4	Ni-complex	Resistant		
5	Sn-complex	Resistant		
6	Zn-complex	Resistant		
Bacillus Cereus				
1	SB	5.1mm	15.4mm	0mm
2	Cu-complex	2.7mm		
3	Fe-complex	6.7mm		
4	Ni-complex	4.1mm		
5	Sn-complex	3.7mm		
6	Zn-complex	5.2mm		

Table 5: IZ of SBs and its metal complexes against *Staphylococcus Aureus, pseudomonas aeuroginosa and salmonella typhi*

Serial	Complexes	Dilution 1:10	Ciprofloxacin (Positive control)	DMSO (Negative control)
		Staphylo-coccus aureus		
1	SB	3.1mm	15.6mm	0mm
2	Cu-complex	6.3mm		
3	Fe-complex	3mm		
4	Ni-complex	2.3mm		
5	Sn-complex	2.6mm		
6	Zn-complex	4.3mm		
		Pseudomonas aeuroginosa		
1	SB	3.2mm	16.3mm	0mm
2	Cu-complex	3.1mm		
3	Fe-complex	7.3mm		
4	Ni-complex	2.3mm		
5	Sn-complex	4mm		
6	Zn-complex	3mm		
		Salmonella typhi		
1	SB	3.7mm	12.4mm	0mm
2	Cu-complex	4.5mm		
3	Fe-complex	7.1mm		
4	Ni-complex	3.2mm		
5	Sn-complex	3.4mm		
6	Zn-complex	2.7mm		

The copper complex has high activity against *staphylococcus aureus*, followed by zinc complex, and Ni complex has low activity, while the iron complex has high activity against *Pseudomonas aeuroginosa* and *salmonella typhi*, as shown in Table 5.

The Agar well diffusion method was used to design an in-vitro antibacterial study of SBs and their metal complexes for some specific bacteria [25].

The impact of the synthesized SBs and their metal complexes against the growth of *streptococcus* was negligible; however marginal activity shown by some SBs was greatly dependent on the central metal atoms upon coordination with SB against *Bacillus Anthraces.* After reacting iron with SB, the activity of the iron complex increased, while the zinc complex has the same activity as pristine SB. The cell wall surrounded by a lipid membrane favored the route of lipid-soluble constituents which is a vital aspect of controlling anti-microbial action. The IZ showed that the copper complex has the highest activity against *Staphylococcus aureus*, followed by the Zinc complex, and Ni complex has the lowest activity among the synthesized compounds. The iron complex possesses high activity as related to other synthesized compounds upon exposure to *Pseudomonas aeruginosa*. The activity of SB was enhanced by coordinating Fe and Sn metals while it remained unaffected by the addition of other metal ions.

SBs and their metal complexes inhibited the growth of *Salmonella typhi* as iron complex has high activity with IZ of 7mm followed by copper complex with IZ 4.3 mm while zinc complex with 2.6 mm IZ has the least activity. The coordination of SB with transition metal complexes is the main reason for the increased activity [26]. The different variations in the activity of complexes may be due to the impermeability of microbe's cells or can be the difference in ribosomes in microbial cells [27].

4. CONCLUSION

This study focused on the synthesis of SBs and their metal complexes which were in turn applied for the treatment of various pathogenic bacteria as tested by standard biotic cultures and their results were compared with standard antibiotics (Ciprofloxacin). With the exception of *streptococcus*, which showed resistance, the SBs and their metal complexes exhibited strong anti-bacterial activity against selected bacteria. Metal complexes synthesized with SBs may be a feasible alternative to currently available antibiotics.

Authors Contribution

M.J and M.H supervised the research work and has the main idea and wrote the manuscript. ZY and ZQ revised the manuscript and provided suggestions.

Conflicts of Interest

The authors declare no conflict of interest. All the authors approved the submission of the manuscript.

Acknowledgment

Data Availability statement

The data presented in this study are available on request from the corresponding author.

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Comprehensive Review on Synthesis of Abox Material and its Catalytic Applications

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Abstract
Perovskites are materials with crystal structures comparable to perovskite (mineral). The backbone of perovskite is calcium titanium oxide ($CaTiO_3$). Perovskite oxides with the general formula ABO_x are highly important. In the general formula of perovskite ABX_3, A & B are cations, where X is an anion that binds with two cations. Perovskites have proven their versatility in catalysis, photovoltaics, solar cells, electrode conducting material, etc. Due to their unique structural properties and applications, they are compatible with elements having metallic approximately 90% of the periodic table. This review discusses the synthesis and catalytic application of perovskite oxides. There are five sections to this review: (a) a brief description of perovskite oxides, (b) the synthesis of perovskite oxides with various properties, (c) general characterization, (d) catalytic applications, and (e) conclusions and future perspectives.

Keywords: perovskite oxides, synthesis, catalysis.

Graphical Abstract

1. Introduction

The term perovskite is used to explain oxides produced from Ca-Ti-O_3 with the structural formula ABO_3 or A_2BO_4. Oxides of A_2BO_4, are often known as perovskite-like oxides. They are synthesized by alternating ABO_3 and AO layers. The cations A and B were replaced by a new cation with a different oxidation state and radius, and the material noted for its structural stability. The oxidation state of the B-site cation and the number of oxygen vacancies may be regulated when a desired external cation is used, providing a practical and workable technique for connecting physicochemical characteristics with the catalytic ability of the material [1].

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According to several experts, perovskite oxides have been used to generate a range of oxygenated molecules [2].Due to their significant thermal stability, the exceptional activity of oxidation and low cost, perovskites has examined for a range of automobile exhaust purification, fuel cells, N_2O decompositions and Water Gas Shift WGS reactions. Perovskite oxide having lanthanide at the A site and transitional metals at the B site is more frequently utilized within heterogeneous catalysis, possibly to use transition metals' catalytic capabilities. Noble metals are recommended as a catalyst owing to their excellent stability in these circumstances. Whereas transition metals have good catalytic activity because of their electronic structure, transitional metals could oxidize in higher temperatures within oxygen presence [3]. Larger ionic radius cations have oxygen atoms and there is 12-fold coordination accommodating sites A and a cation having lower ionic radius with 6-folded coordination accommodating sites B. Perovskite oxides have a structure of the cubical crystal. B is located in the cubic closet packing's octahedral gaps formed by A and O [3].

As stated in "Witness to grace" Professor John began his research to explore perovskite materials 60 years ago and explored perovskite materials for their applications for storage and energy conversion [4]. Most catalysts used are made of mixed metal oxides. Out of mixed metal oxides, a perovskite-type oxide was highlighted. Despite ionic radius requirements, another requirement is electro-neutrality, which means the sum of charges on cations is equal to the charge on anion, which can be achieved only by proper distribution of charge on A, B, and O, respectively. According to Pena and Flerro et al., [5] perovskite oxide materials, such as ABO_3, have exceptional properties of physical like $(BaTiO_3)$ Ferro electricity, $(SrRuO_3)$ ferromagnetism, $(LaFeO_3)$ weak ferromagnetism, $(YBa_2Cu_3O_7)$ superconductivity and thermally conductive. They also examined the adsorption features of many perovskite materials by studying their shape to find their active sites, which they then investigated for catalytic activity. Perovskite oxides with different morphologies are shown in Fig (1).

Catalysis is a reaction in which the outcome is influenced by a chemical known as the catalyst, which is not consumed during the reaction. Catalysis can be either homogeneous or heterogeneous. The mixture reaction and the catalyst are in the uni-phase in homogeneous catalysis, whereas the mixture reaction and the catalyst are in separate phases in heterogeneous catalysis. Both types of catalysis have high activity, but heterogeneous catalysis has low selectivity than homogeneous catalysis.

Heterogeneous catalysis requires high reaction temperature than homogeneous catalysis. However, our main aim in this study is to focus on the preparation method of perovskite oxide and explore its catalytic application, which provides the basis for all.

1.1 Perovskite Materials' (Properties of Electronic, Magnetic, and Optical)

The study of oxides of perovskite magnetic properties has attracted a lot of curiosity, among other things. There have already been findings of magneto-optical materials, sensors, catalysts, fuel cells, and other applications. Transition metal perovskite oxides have recently gotten attention because of their significant magnetic properties. Because of the great interaction of electron-electron to the manifold 3D, and their physical strength, these perovskite oxides have remarkable magnetic properties.

Perovskites are used within solar cells, and light detectors and light-emitting diodes are examples of this, for example, optoelectronic applications (LEDs). Because of their outstanding optical features, which are required for both light absorbers and light-generating materials, they are prominent. Higher coefficients of absorption and configurable bandgaps of direct, for example. As a result, in the dye of solid-state comparison sensitized solar cells, the thickness of TiO_2 within perovskite solar cells is relatively lower. It could enhance to some extent, that perovskite solar cells are commercialized and developed.

2. Perovskite Oxides Synthesis with different morphologies

2.1 Perovskite Oxides synthesis in Bulk

Mixed oxides of perovskites are generated by simply annealing a metal oxide mixture at a high temperature, which is a straightforward process. This synthesis is environmentally beneficial because it occurs without the release of any hazardous gases as a result of the reaction. Although it is necessary to homogenize the metal oxide precursors for them to react completely and generate pure perovskite oxides, this is not required. In this case, the mixture could be processed in ethanol with a ball mill or stabilized ZrO_2 balls. When good mechanical attributes are required, this approach generates a sample with a lesser surface area and a greater particle size, which is often used in ceramics [1].

Because catalysis is a surface reaction, it necessitates strong contact between the catalyst and the substrate. And, to put catalysis into practice, perovskite oxides must have a larger surface area and a smaller particle size. As a result, high-surface-area perovskite oxides must be synthesized. Because the precursor and synthesis processes have complex organic that is employed to correlate, burn, and finally, metal oxide particles disperse, replacing metal oxides with soluble metal nitrates is one of the most successful approaches. Citric acid combustion, for example, uses metal nitrates as a precursor and citric- acid as a complex organic to metal coordinate ions. Because of its simple operating technique and high catalytic performance, this technique is frequently used to make oxides of perovskite catalysts within catalysis [1].

Undesirable gases like NO_2 and CO_2 are emitted in the process due to nitrates and organic compounds. As a result, synthesis should be done in a fume cupboard or somewhere with good ventilation. It should also be highlighted that because the organic complex contains a carbon source, the creation of carbonates is unavoidable. Carbonates may influence the material's catalytic effectiveness in some reactions; hence their amount should be kept as low as feasible [1].

2.2 Nano-Sized Perovskite Oxides

Following is the procedure for making perovskite oxides from polyvinyl pyrrolidone (PVP):

A solution of water containing metal stoichiometric nitrates and Poly-Vinyl-Pyrrolidone was heated to 100 degrees Celsius. Perovskite-type oxide precursor produced after 1 hour of drying at 150°C. The polymerized precursors were burned in the presence of air for 6 hours at 300–600 degrees Celsius, yielding perovskite-type oxides [6].

Also in the PVP technique, a reaction of solid-state and a procedure of citrate was used to make $La_{0.6}Sr_{0.4}Mn_{0.6}Fe_{0.4}O_3$. La_2O_3, MnO_2, $SrCO_3$, and Fe_2O_3 were used as starting particles for the solid-state reaction. The powders were mixed well in an agate mortar, and the mixture was sintered in the air for 10 hours at 400–1300°C. Nitrates of metal and citric-acid-monohydrate ($C_6 H_8 O_7.H_2O$; 2 times total cation moles; double the times of total moles of cations) dissolve in water and agitate at about 100°C till gel formation. A precursor was created after gel drying for 1 hour at about 150°C. The precursor was calcined within the atmosphere for 10 hours about 4–8 hundred degrees Celsius. Temperature raised with a continuous rate of about 20^oC min^{-1} for calcination [6].

According to SEM analysis, the oxide manufactured with PVP was 20nm to 30nm in size, whereas those made with the process of citrate and the reaction of solid state were 50 nm and 10 nm in size. $La_{0.6}Sr_{0.4}Mn_{0.6}Fe_{0.4}O_3$ obtained from PVP had the lowest sized particle and the biggest surface-area specific of the several synthetic techniques, probably due to calcination could be done for low temperatures of about 600 ^0C [6]. The typical preparation technique for perovskite-type oxides using polyol mediates synthesis is presented in scheme 1 [7].

2.3 Porous Perovskite Oxides

2.3.1. Synthesis in solid state

To manufacture perovskite oxides, a reaction of solid state is typically utilized, characterized by a simpler process, higher calcination temperature, mass-production capabilities, and lesser manufacturing cost, among other things [8]. In a typical synthesis, the solid raw components are thoroughly mixed before being calcined at a higher temperature for a long enough period to generate a suitable phase of perovskite oxide.

A calcination temperature of over 900 °C is required to produce phase-pure perovskite oxides due to the significance of

overcoming the diffusion barrier for perovskite phase formation during the synthesis [8-10]. Particularly for perovskite oxides, including several components and alkaline earth elements, higher calcination temperatures and a lengthy calcination duration of several hours are necessary to produce a high-purity perovskite phase [11-13]. On the other hand, such high synthesis temperatures usually lead to poor sintering and nearly pore-free products. A lower phase formation temperature is required efficiently synthesize porous oxides of perovskite utilizing the reaction of solid-state methodology, and achieving good perovskite phase purity at a lower annealing temperature is a severe difficulty [14].

Solid-state synthesis with the help of high-energy ball milling (HEBM) [13,15-20] and Solid-state reaction injected into the molten salt [21-24] are two recently founded solid-state reaction processes that have been updated. In the solid-state synthesis of HEBM-assisted, known as mechano-chemical manufacture, mechanical energy is frequently used to aid the reaction of solid-state. In process of preparation, mechanical force is used to break down the reactants and products into small particles and appropriately mix them. As a result, the phase formation diffusion barrier can be reduced effectively, the preparation temperature may be properly reduced, and the product surface specific area can successfully be increased. Various perovskite oxides have been successfully generated using this process to utilize as electrodes in metal atmosphere batteries and Solid Oxide Fuel Cell SOFCs [25-28]. The ball-milling period, ball mass ratio to powder solid and other operational factors influence the product surface specific area, pores structure, and size of crystalline [26-29]. High Energy Ball Milling HEBM-assisted solid-state synthesis still has poor porosity which is a severe disadvantage [14]. When a sample is placed under stress calcination, the pores structure is prone to collapse. Kaliaguine's team changed the HEBM-assisted solid-state synthesis technique by adding various alkali additives to the 4–105 m^2 g^{-1} ball-milling process to make porous perovskites, which maximized porosity [30,31]. As a result, they all have outstanding catalytic characteristics in a variety of procedures. To our knowledge, no study on

applying this fascinating technique to the fabrication of perovskite electro-catalysts in ORR or OER processes has been published, emphasizing that much more research is needed in this domain. If this novel method was used to create porous perovskites as ORR/OER electro-catalysts, the impurity from the alkali additive could potentially be introduced into the perovskite phase and the potential change in activity could be attributed to such impurity doping because most elements can be doped into the perovskite lattice [14]. Inadequate porosity is produced by the reformative HEBM-assisted synthesis method described above because insufficient amounts of alkali are supplied. The molten salt synthesis method, which uses a large amount of alkali metal salts to generate crystallized, chemically purified single-phase powder has gotten more attention in recent history due to its simple, versatile, cost-effective approach [21-24, 32-34]. The molten salt additions serve as a soft template as well as a heat source [33-35]. In synthesis low melting point salts are combined to reactants in non-solvent freshly ground form crystals and the combined precursors are then heated to temperature over the salts melting point. Because reactants have a defined solubility in liquid salts, the molten salt methodology is used [18.19,31]. As catalysts for the formation of higher alcohols, porous $LaCo_{1-x}Cu_xO_{3-\delta}$ (LCCu) perovskite oxides were produced in 2007. In this synthesis process, Elements of Group I was utilized as addictive alkalis, successfully controlling the growth grain of phases of perovskite while acting as a soft template. The LCCu perovskite displayed a large specific surface area and many holes when the alkali (0.08wt% of alkali ions) was washed away in water, indicating increased catalysis activity for high alcohol preparation by syngas. Furthermore, the surface-specific areas of the resultant compound changed with a radius of cationic metals of alkali utilized and the increase is in order of Li < Na ($\approx$ Cs) < K< Rb respectively**.** During the ball milling procedure porous La $Fe_{0.8}$ $Cu_{0.2}O_3$ and $LaCo_{1-x}Fe_xO_3$ were also used as perovskite oxides and was well synthesized in the presence of numerous additives and displayed a higher surface specific area for synthesis which uses convection, diffusion processes, permits for speedy transfer of mass transit in the

phase of liquid and allows reactant to mixed on an atom scale [21,36-37]. The salt of a resulting sample could be eliminated by washing it with water that is de-ionized after pyrolysis resulting in porous products.
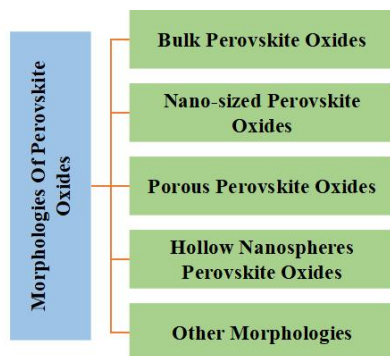

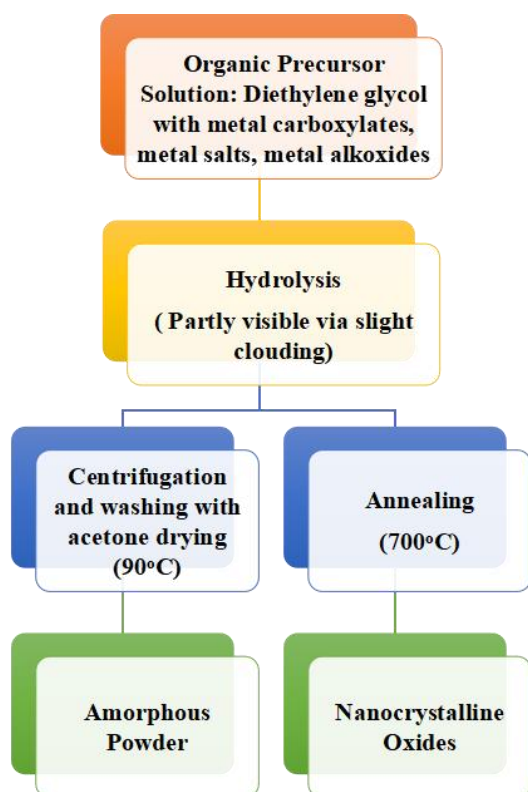**Figure. 1** Perovskite Oxide different structures

The ratios weight of metal salts of alkali (e.g., $ZnCl_2$, NaCl, KOH, and $NaNO_3$) to solid precursors have ranged from 1:1-15:1 in prior investigations [32-40]. According to some experts, a high ratio can aid in mesopores formation [35-40]. As a result, particle size, morphology, porosity, and other parameters may be effectively modified with molten salt. For decades, molten salt synthesis has been effectively utilized to generate perovskite oxides, particularly ferroelectric oxides such as $BaTiO_3$ and $SrTiO_3$ [32]. Furthermore, molten salt synthesis was used to create single-crystalline $BaZrO_3$ particles, allowing for rational control of the structure of perovskite oxides [41]. Aside from these, other perovskite oxides can be made using this process [21, 32-34, 37-39].

Li and his colleagues reported a molten salt solid-state precursor methodology for the fabrication of $LaCoO_3$ perovskite. To conduct the precursor reaction, they mixed solid-state $Co(NO_3)_3.6H_2O$ and $La(NO_3)_3.nH_2O$ with a preset amount of KOH after several hours of calcination nanoparticles in porous form having diameters of 15nm to 40 nm were formed [39]. By significantly altering the calcination period various porous nanostructured $LaMnO_3$ including spheres and cubes were well created employing a co-assisted molten salt method involving $NaNO_3$ and KNO_3 [24]. $LaMnO_3$ took on a porous spherical shape after calcination at 550°C for 4 hours with the pore's mean size of 34.7 nm. SEM and TEM images are shown in Fig 2-(b,c,d,e). After increasing the time of reaction up to 6 hours, reveal single-crystal porous cubic $LaMnO_3$ particles. The production mechanisms of several $LaMnO_3$ nanostructures are prepared as shown in Fig (2-f). XRD pattern of $LaMnO_3$ is shown in Fig (2-g). It was discovered that shape significantly impacted activity in the catalytic elimination of toluene.

Song et al. recently synthesized $La_{0.6}Sr_{0.4}Co_{0.2}Fe_{0.8}O_{2.9}$ porous using a standard molten salt process in which the mixed oxide precursors were combined with a 2:1 weight ratio eutectic salt mixture of NaCl and KCl [21]. Fig (2-h) shows scanning electron microscopy (SEM) pictures of porous nanoparticles interconnected with a macroporous cores diameter of 200 nm. The porous arrangement made a huge interfacial surface, exposing more active sites and even perhaps strengthening OER catalytic activity. When compared to the benchmark IrO_2 precious metal-based catalyst, the $La_{0.6}Sr_{0.4}Co_{0.2}Fe_{0.8}O_{2.9}$ porous catalyst had a lower over-potential of 345mV and a density of current of 10mA cm^{-2} (360mV). Molten salt techniques are also used to make Sr, Mg doping in perovskite form $LaAlO_3$ porous for electrolyte SOFC and $La_{0.8}Sr_{0.2}MnO_3$ perovskite powders for the SOFC cathode [22].

However, according to what has been observed, excellent molten salt synthesis perovskite oxides still make up just a tiny proportion of the whole perovskite oxide group. This could be owing to some variables, including the relatively high temperatures at which phases occur (usually > 1000°C) or the likelihood of molten salt reacting and reactants. Even though most modified solid-state approaches do not involve pore size management, they have been shown to improve the surface area and reduce particle size. Several modified solid-state synthesis methodologies have been utilized to target pure phase formation, controllable particle sizes, and associated specific areas. However, due to the unforeseen and random pore-forming processes, controlling the porosity and/or pore size is difficult. More research is needed to better understand phase formation and pore growth in molten salt synthesis, which would greatly improve the design of porous perovskites for applications such as electro-catalysis [14].

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Scheme 1. Synthetic technique for perovskite-type oxides using polyol mediates the synthesis.

2.3.2. Wet Chemical Process

Wet chemical techniques for developing porous metal oxides have been widely used and have made significant progress in recent decades [42-55], while multi-metal oxides still need to be studied further. Porous materials have been developed using surfactants and templates. After the template, the surfactant was removed under mild conditions, and with additional calcination procedures, porous solid material can be well generated [14].

Wet chemical synthetic methods such as the process of sol-gel, methodology of complexation, combustion solution technique, route of hydrothermal, synthesis of electro-spinning, and a few others have been developed. Controlled morphology and porous structures, as well as perovskite oxide nanoparticles, are of particular interest [14,21,54-60].

2.4. Hollow Nano-spheres Perovskite Oxides

Nanospheres with hollow interiors are a newer type of nanosphere. Perovskite has a bigger surface area and energy than mixed perovskite because of its hollow structure, allowing for the doubled face (inner and exterior) interaction with the reactant. Good perovskite oxide performance of catalysis may be achievable as a result. Hollow perovskite is made using a variety of techniques, including One approach for simulating the material using a spherical template, like spheres of carbon [61] or directly synthesizing hollow materials using organic guiding agents and a hydrothermal technique [62,63]. Scheme 2 depicts the synthesis of LCMO nanospheres using template-assisted growth.

The carbon spheres might be made by either exfoliating organic precursors (such as glucose) or replicating hollow perovskite oxides utilizing a silica template, which would be utilized as a template (secondary) to duplicate the hollow oxides of perovskite in the first stage. This image shows the usage of a carbon spheres template with silica as a template secondary for the synthesis of hollow oxides of perovskite [64]. Firstly, sucrose was added to the template of silica, which was subsequently carbonized, to create a carbon-silica composite. The composite of carbon silica etched in the 48% of aqueous HF solution, revealing carbon copy, removal of template of the silica. The pores on the carbon copy were then filled with a pre-prepared solution with the required metal cations. Finally, the mixture was dried and calcined at particular temperatures to achieve the desired result. Zhang et al. [63] suggest a quick procedure. The hydrothermal approach is described in the next paragraphs. The nitrates of metals, citric acid, P123, and urea were initially mixed in ethylene glycol, water, and ethanol. The solution was placed in an autoclave and boiled at about 100°C for 48 hours after which it was completely dissolved. The product solid was centrifuged and dried before being calcined for 8 hours at 600°C in the flow of air to get perovskite oxides.

On the other hand, the carbon spheres template technique necessitates manufacturing carbon spheres before the creation of hollow perovskite oxide, making the process more complicated overall. Because of the homogeneous particle size of the silica template, spheres of carbon, eventually, oxides of perovskite having particle that is uniform in size could be

Fig. 2-a) Relation among radius of cationic metal of alkali with a surface area of oxides of perovskite generated. Having the consent of ref. [18] this image has been reproduced. Copyright 2007 Journal of Catalysis b) SEM c) Images showing transmission electron microscopy (TEM) of a porous spherical $LaMnO_3$ sample. d) Scanning Electron Microscopy Analysis and e) Having TEM, porous cubic images of $LaMnO_3$ materials were captured. f) suggested the production process of different $LaMnO_3$ Nanostructures in molten salt media, where the structure of LaMnO3 was modified from porous-cubic nanoparticles. This image has been reproduced with the permission of ref. [24]. Copyright 2014 ACS applied materials and interfaces g) XRD patterns h) SEM images of the synthesized porous nanostructured perovskite $LaO.6SrO.4CoO.2FeO.8O_2.9$. Inset (h) the equivalent enlarged image. Reproduced from ref. [21]. Copyright 2018 Nano Res.

Scheme 2 The creation of LCMO nanospheres using a template-assisted approach is depicted in a series of schematic illustrations [64].

duplicated. Moreover, the particle of perovskite oxides could change from silica to having the required particle size [1].

2.5 Other Morphologies

With the evolution of materials research and the growing need for innovative applications, oxides of perovskite have different techniques, such as nanoporous perovskite oxide, a nanoplate,

nanofibers, nanotubes, flower-like, and cubic, have been created. These various morphologies would allow perovskite oxides to have a wider range of surface characteristics and uses. We went over nanoporous perovskite oxides in great depth.

2.5.1. Nanoporous Perovskite oxides

Hard template, soft template, hydrothermal, colloidal crystal template, and electrospinning procedures are some of the most common ways to make nanoporous perovskite oxides. We only go into detail on the synthesis of soft and hard templates and colloidal crystal templates.

(a) Soft-Template Method

Soft templates have been highlighted [65] in the creation of materials of nano-porous like silica of mesoporous like MCM-41 [66], and SBA-15 [67]. One of the most efficient ways to make mesoporous oxides is to use evaporation-induced self-assembly (EISA). Direct co-condensation methods are typically combined with soft templates. The EISA approach has four steps: (a) Synthesis of a homogenous initial sol including the soft templates and inorganic precursors in the proper stoichiometry; (b) During the dip-coating methodology, solvent evaporation caused inorganic precursors to self-assemble into micelles with a poorly compacted network and a steadily increasing strength over Critical Micelle Concentration CMC. (c) Additional inorganic condensation and film equilibration with its surroundings are responsible for the final mesostructured adjustment. (d)Temperature treatment is produced before consolidation, template removal, and chain crystallization. Even though "EISA" technology was utilized to generate mesoporous oxides of single-metal several times, it was only utilized to make mesoporous perovskite oxides a few times. The cations from the first gel mixed homogeneously on a molecular scale throughout the entire procedure to generate mesoporous oxides of perovskite having pure phases. In as-prepared samples, however, variations within the solubility of components that are non-volatile in the evaporation of solvent procedure frequently result in the separation of phase or secondary phases. Furthermore, because the EISA method

uses a lower temperature for the breakdown of organic surfactants than for the crystallization of perovskite oxides, mesostructures destroy due to a lack of support at calcination of high temperatures, allowing amorphous phases or impurities to emerge. Despite these difficulties, a few papers have been published on the EISA technique for the preparation of mesoporous oxides of perovskite [68]. In 2004 Grosso and company utilized a semi-commercial template of organic for making mesoporous oxide of perovskite films [68] Brezesinski and company [69] effectively generated many Nano porous oxides of perovskite films with honeycomb 3D structures using the "EISA" technique of associated dip coating onto a polar substrate with several amphiphilic block copolymers with high thermal stability. Although the methodology of EISA, when paired with dip coating can make nanoporous perovskite oxides, it is often challenging and limited to small-scale production. A modified EISA method for manufacturing nanoporous perovskite oxides without the need for precipitants or chelating agents was recently devised by certain researchers which eliminates the necessity for dip-coating. These chelating chemicals would result from the further solution that is homogenous and greater dispersion of cation in the process of evaporation, yet they would have an impact on surfactant self-assembly and interactions with ions of metal. As a result, hierarchically nanoporous perovskite oxides such as $BaTiO_3$, and $SrTiO_3$ may be generated using these improved EISA techniques [70,71].

Because of the great ability of hydrolysis of titanium precursors, there is still much research utilizing the self-assembly of soft template technique for generating higher structured mesoporous oxides of perovskite. $BaTiO_3$ oxide of perovskite having mesostructure inside crystallites was produced directly from solution using the cationic surfactant cetyltrimethylammonium chloride using a simple sol precipitation technique (C16TMAC) [72]. Yan and company developed a higher ordered mesoporous $ZnTiO_3$ with a greater pore volume, a large surface area, and narrow pore dispersion size utilizing the method of sol-gel in combination with EISA with ethanol and F127 Pluronic being structure guiding reagents [73].

(b) Method of Using a Hard Template

Nanocasting, also known as repeated templating, is a process for manufacturing nanostructured materials with unique features. A method employed in this procedure is to fill metal precursors into porous templates made of mesoporous carbon or mesoporous silica, which are subsequently calcined and etched away using acid or alkaline etching [74].

The template of the hard approach utilized to make nano-porous oxides of perovskite for the past decade includes mesoporous $LaNiO_3$ utilizing template SBA-15 and $LaFe_xCo_{1-x}O_3$ utilizing KIT-6. There are several distinctions between hard-template procedures for perovskite and single metal oxides due to the usage of multiple precursors metal for oxides of perovskite. Mesoporous silicas (such as SBA-15, MCM-48, and KIT-6) and mesoporous carbons (such as CMK-3, CMK-1) are frequently used as "hard templates," as seen in [75]. After calcination, agents of Chelating were added to the metal salt precursor solution to obtain a stoichiometrically correct homogenous metal salt precursor solution. When silica is removed with NaOH or HF aqueous solution, mesoporous oxides of perovskite with an organized structure of mesoporous and a high specific surface area are formed. An image of TEM of mesoporous oxide of perovskite is shown in

Because of the complicated interactions between silica and ion of metal precursors filtrated, it can be difficult to fill the mesoporous silica at once, necessitating the observation of a comprehensive impregnation of metal precursors over long periods. Larger oxides of perovskite particles are frequently found outside the pores of mesoporous silica. As a result, various innovative approaches have been devised to increase metal precursor impregnation while reducing external pore loading, such as functionalizing mesoporous silica templates to a group of organic compounds [76]. Similarly, the mesoporous perovskite oxides reported using mesoporous silica as a hard template are confined to little varieties, namely those having composition $LaB_{1-x} B'_xO_3$ (B, B′= Mn, Co, Fe, Ni). Chelating chemicals such as citric acid must be added to metal nitrate precursor solutions to form pure-phased

perovskite oxides in lesser calcination temperatures. Another drawback of the hard template method is that correctly draining the silica utilizing a solution of NaOH/HF is difficult, silica residue impacts the hard-characteristic template progress [77]. These issues can be solved by utilizing mesoporous carbon as a template-hard since high-temperature calcination can completely obliterate the carbon template. Based on a silica Aerosil, the process of nano casting in a micro-mesoporous carbon resulted in $LaFe_{1-x}Co_xO_3$ perovskite oxides with a high specific surface area Fig 4(A) [78]. The inorganic precursors are converted into perovskite oxide nanoparticles in the process of calcination at 800°C in air, while the carbon is removed by oxidation. However, there are several drawbacks to employing mesoporous carbon as a template, including inadequate aqueous precursor solution wetting of the pore walls and a low decomposition temperature. Normal impregnation fails to properly fill the pores resulting in perovskite particles developing outside the pores, which is the underlying problem with the hard-template approach. As a result, it will be necessary to build more user-friendly solutions. Due to high interfacial tension in mesoporous structures, the double-solvent method which combines a significant amount of hydrophobic solvent with an aqueous metal precursor solution with a pore volume, may provide an effective method for enhanced metal precursor penetration [79].

The mesoporous structure of $LaFeO_3$ material is shown in Fig (4B). Silica modification of the surface with different groups that are functional on the interior or exterior surfaces will promote metal precursors to be impregnated due to interactions between metal precursors and functional groups [73,80].

(c) Method for Creating Colloidal Crystal Templates

Another typical production method for the crystalline technique uses nano-porous materials of perovskite having 3D organized macro-pores. Inorganic porous materials with sizes of pores ranging from nanometers-micrometers have been successfully produced using organic polymer spheres. Depending on the synthesis approach, three methods for manufacturing periodic structures of macro-porous using a colloidal crystal template are presented in Fig (5) [81]. The vacant gaps between

monodisperse spheres packed close together e.g., Polystyrene (PS) or Polymethyl methacrylate (PMMA) and in-situ precursor solidification are filled or covered with liquid metal precursors in these colloidal-crystal-template techniques. The three-dimensionally organized macroporous (3DOM) structures may be made by removing the templates and calcinating them at a high temperature. Inverse opals have a three-dimensional interconnected structure that allows big molecules to move swiftly and gas to diffuse quickly.

Two benefits of employing the template of colloidal crystal technique for generating nanoporous oxides of perovskite are the capacity to manufacture ordered nanoporous perovskite oxides and employing calcination at a high temperature. The 3DOM $La_{0.7}Ca_{0.3}MnO_3$, $La_{0.7}Ca_{0.32-x}Sr_xMnO_3$ was created by Hur et al. [82] by dissolving stoichiometric metal acetates and 2-methoxy ethanol in HNO_3 then gently dumping the thick solution until the millimeter PMMA template was completely immersed. The SEM and TEM images of $La_{0.7}Ca_{0.3}MnO_3$ are shown in Fig (3A, B). Sintering at 800°C in an oxygen environment was used to remove PMMA colloids. On the other hand, making a metal alkoxide solution is labor-intensive and expensive. Using a colloidal-crystal-template technique, researchers created 3DOM oxides of perovskite precursors including solutions of ethylene glycol methanol of different metal nitrates.

Zhao and co-created 3DOM $LaCo_xFe_{1-x}O_3$ perovskite oxide using this method [83]. Various Surfactants of Organic, chelating compounds are utilized to equally distribute nitrates of metal throughout the crystal colloidal template process in the formation of oxides of 3DOM perovskite. Dai and co, for example, employed a surfactant-assisted PMMA templating procedure to make 3DOM $La_{0.6}Sr_{0.4}FeO_3$ with a mesoporous or nano void-like framework, and the findings demonstrated that the nature of the solvent and surfactant affects the surface area and pore structure of the end product [84].

According to researchers processing the $La_{0.6}Sr_{0.4}FeO_3$ a precursor in the development of $La_{0.6}Sr_{0.4}FeO_3$ 3DOM would be favored at 500°C by N_2 for amorphous carbon and so in the air about 7500°C. Dai's group synthesized many types of 3DOM perovskite oxides using citric acid, PEG (Polyethylene Glycol), and triblock copolymer (pluronic P123) including $EuFeO_3$, $Eu_{0.6}Sr_{0.4}FeO_3$, $LaMnO_3$, $La_{0.6}Sr_{0.4}MnO_3$ and La_2CuO_4 [85].

The noble metal nanoparticle-assisted crystal colloidal template approach was used to create 3DOM oxides of perovskite in a single step. 3DOM, for example, supports silver nanoparticles. PMMA was employed as a template in a dimethoxytetraethylene glycol (DMOTEG) solution to generate $La_{0.6}Sr_{0.4}MnO_3$ with larger areas of surface (38.2-42.7 m^2/g), and the DMOTEG-mediated procedure resulted in size-controlled silver nanoparticles that were also stabilized against agglomeration without the need of extra styrene [86].

In a methanol solution by combining stoichiometric quantities of $La(NO_3)_3$, $Pd(NO_3)_2$, and $Mn(NO_3)_2$ with PEG and lysine in an aqueous HNO_3 solution, Wang and co-created a 3DOM Pd-$LaMnO_3$ composite [87]. The 3D porous structure may still collapse or be lost during or after the template removal due to the delicate material nature, which has considerably lower wall thickness than the pore size. As a result, the stability of the pore structure of 3DOM perovskite oxide should be checked frequently throughout manufacture and usage.

Another disadvantage of the crystal colloidal template strategy is the cost and time required to create templates of polymer, which confine the practical applicability of metal 3DOM oxide of perovskite. To generate pure phase 3DOM perovskite oxides, organic matter or detergent must be added to the possibility for molecules that are organically interacting with CCT must be addressed when using a precursor solution of metal ion to evenly divide the ions of metal [73]. Different Synthetic protocols with their corresponding positive and negative aspects are shown in Scheme 3.

3. Methods for Making 1D Perovskite-Type Oxide Nanostructures

In the last ten years, many uni-dimensional oxide nano-structures of perovskite have been produced. The most commonly used techniques are "top-down", and "bottom-up". There are two types of preparation procedures for 1D perovskite nanostructures.

Figure. 3 (A) La0.7 CaO.3 MnO3 spherical array duplicated from the carbon template, as seen in SEM. A TEM picture of a La0.7 Ca0.3 MnO3 sphere with a hollow sphere feature is shown in the inset. **(B)** TEM picture of hydrothermally produced LaCaMnO3. Reproduced with permission from ref. [1]. Copyright 2014 ACS catalysis

Figure. 4(A) The mesoporous LaFe$_x$Co$_{1-x}$O$_3$ perovskite oxides were made using a co-nano casting process using a rigid template of mesoporous KIT-6, **(B)** LaFeO$_3$ that has been developed as a mesoporous material. Reproduced with permission from ref. [73]. Copyright 2018 Chemical Science

The first method does not use a template, but the second does. The following section examines recent advances in manufacturing unidimensional oxide nano-structures of perovskite.

3.1 Template Free Synthesis

Hydrothermal or solvothermal preparation, the salt of molten approach, and the process of electro-spinning having used to build uni-dimensional oxide nano-structures of perovskite without the need for a template.

For example, Joshi and co [88] created perovskite of single crystalline BT ($BaTiO_3$), ST nano-wires using a solution-based approach of free templates. Piezoelectric (PZT) single crystalline nanowires were also made using the template-free hydrothermal method [89]. The templates need not be removed after the perovskite oxide nanowires are generated because this approach does not require organic templates. BT, ST, and PT single crystalline nano-wires are made using the methodology of molten salt. Precursors' surface and interface energies, as well as the molten salts used, are crucial in the synthesis of molten salt nanowires. To make monocrystalline BT nanowires, the metal of alkali titanates is employed as precursors of synthetic in a modified hydrothermal method. Tetragonal Pt uni crystalline nano-wires with a necklace-like shape have also been made via electrospinning. Their lengths range from tens to various tens of micrometers, while their diameter range from 100-200nm. Sol-gel electrophoresis was also used to make tetragonal PZT nanofibers for mechanical energy harvesting nanogenerators [90-92]. Despite its time-consuming and low-throughput nature, the advantage of this technology is that the nanostructures generated can be morphologically controlled. Perovskite nano-tubes' oxides are also made using free template methods like hydro-thermal manufacturing (PONTs). For example, the hydrothermal method w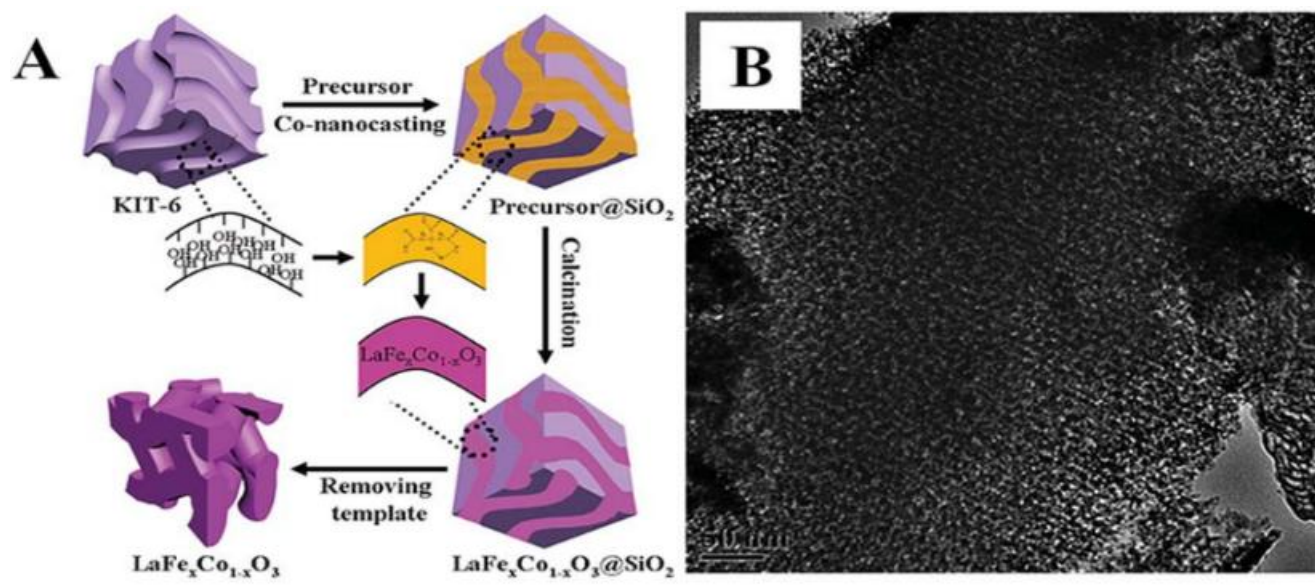as utilized to make BT and BST nanotube arrays on titanium substrates. Constant BT crystalline and ST PONTs were also made employing a low-temperature hydrothermal method using TiO_2 nanotubes as a bonafide precursor material [93-95].

3.2 Synthesis using Templates

The template-assisted approach is an excellent way to mass-produce regular nanostructured arrays in large numbers. The most frequent templates so far have been colloidal monolayers, anodic aluminum oxide, block-copolymers, and nanoimprint molds. Unidimensional oxide of perovskite nano-structures have been created using template-assisted procedures, which have the following benefits: (1) regular nanostructured arrays with a high density; (2) a high surface-to-volume ratio; and (3) finished product dimensions with excellent control over the template channels. With success in the procedure, the oxide of perovskite $La_{0.825}$ $Sr_{0.175}$ MnO_3 nanowires having a polycrystalline perovskite structure was also generated [96,97]. Perovskite oxide nanotubes are created utilizing a sol-gel template-based approach in addition to perovskite oxide nanowires. Hernandez et al. [98] published ground-breaking research using (Anode Aluminium Oxide) AAO templates and the so–gel process to synthesize perovskite $PbTiO_3$ (PT) and $BaTiO_3$ (BT) nanotubes. PZT and multiferroic $BiFeO_3$ BFO nanotubes were made using the same approach. Even though the nano-tubes sizes and shapes can be feasibly controlled through templates, template-based approaches produce polycrystalline nanotubes in general, attributed to nucleation of heterogeneous on walls pore; this process produces tiny uni-crystalline oxide of perovskite nano-tubes. Anti-ferroelectric PZ PONTs were formed utilizing pulsed laser deposition in templates of AAO made up of nanoparticles with diameters ranging from 3 to 7nm and a wall thickness of roughly 10nm. Sol-gel electrodeposition would be utilized to produce PZT nanotube arrays, in which the channels of the templates of AAO are filled with PZT-prepared sol driven from electrophoretic DC voltages. As a result, the filling effect was substantially improved. Manganite nanotube arrays of perovskite were categorized by utilizing microwave irradiation and AAO template-assisted synthesis [99]. This approach allows for the production of arrays of nano-tube at lesser temperatures.

Perovskite $La_{0.59}$ $Ca_{0.41}$ CoO_3 nanotubes are also made using sol-gel templates. A template-inorganic precursor and low-

temperature calcination were also used to make perovskite $LaNiO_3$ nanotubes [100]. $LaNiO_3$ nanotubes have a polycrystalline structure with very tiny crystals ranging in size from 3–5 nm [95].

4. 2 D Perovskite-Type Oxide Nanostructure Preparation Methods

Thin-film, arrays of nano-dot, lamellae patterns, nanosheets, nanoplates, and nanowalls are only a few examples of perovskite oxide 2D nanostructures essential in today's microelectronics. As a result, numerous methods for generating perovskite oxide 2D nanostructures have been discovered in the last few years. This section discusses the two-dimensional perovskite ferroelectric nano-structures oxide based upon planar-structures and oxide of perovskite nanosheets, as well as oxide of perovskite thin films and multilayers.

4.1 Multilayer Perovskite Oxide Thin Films

The process that transforms the gaseous state of atoms, molecules, or ions into substrate films or multilayers is known as the oxide of perovskite thin film or multilayer growth. For growing oxide of perovskite thin-films/multilayers, PVD physical vapor deposition methods like PLD, magnetron-sputtering RF, and chemically methodologies like CSD, CVD, and MOCVD, as well as MBE, are all commonly used methods (MBE). This section briefly discusses PLD, CSD, CVD, MOCVD, as well as MBE.

4.2 Laser Deposition using Pulses (PLD)

Smith and co initially used the PLD approach to create dielectric thin films in 1965 [99] and it has since become a prominent thin film growth method. The capacity of the PLD technique to yield film compositions that are almost equal to those of the target, despite the target's complicated stoichiometry, is its most crucial property. Several thin films of oxide of perovskite or multi-layers are generated by altering the PLD process parameters. The literature has an excellent review of the epitaxial development of thin films oxide of perovskite and super lattices.

4.2.1. Chemical Solution Deposition

Because of its low cost, ease of setup, and ability to coat large areas, Chemical Solution Deposition (CSD) is a promising approach for generating thin films. It was first developed for the oxide of thin films of perovskite in the mid-1980s. To date, the CSD method has been used to make a large number of oxide of perovskite thin films. Four processes are involved in the production of oxide of perovskite thin films: Preparation of the precursor solution, spin coating/dip-coating of solution on the substrate, deposited solution of pyrolysis in lesser temperatures, and high-temperature crystallization of the films [101-103].

4.2.2. Chemical Vapour Deposition (CVD) & Metal Organic Vapour Deposition (MOCVD)

CVD is a widely used method for producing high-quality and performance thin films of perovskite oxides across a vast area (CVD).The synthesized materials must have a high vapor pressure when used as a precursor in the CSD process. The substrate compulsorily increases temperature to a specific temperature to improve the reaction's deposition and adatom mobility. To successfully install complicated multi-component thin films oxide of perovskite with homogenous compositions across a vast region, the utilized precursors must have matching thermal properties and acceptable vapor pressures. Improved film quality control has been accomplished using modified CVD methods based on liquid or aerosol generation injection. Liquid injection CVD was used to deposit PZT perovskite, lanthanum-barium-manganite (La_{1-x} Ba_x MnO_3) thin films, whilst aerosol and plasma-assisted CVD was used to create La_{1-x} Sr_x MnO_3 perovskite thin films [104].

Perovskite oxide thin films and super lattices are made using metal-organic chemical vapor deposition (MOCVD). Improved film stoichiometry control, greater crystallization quality, and the ability to cover complicated structures and wide areas are just a few of the benefits of this procedure over standard physical deposition methods. Some of the MOCVD variants that have been developed to satisfy a range of applications include MOCVD at low pressures, MOCVD at atmospheric pressures, MOCVD with direct liquid injection, and MOCVD with plasma enhancement. In the MOCVD injection process, the creation of micro-droplets of solution of the precursor is

controlled with a high-speed electro valve pumped in the system of the evaporator. The injection frequency and timing are used to change the optimal growth rates of different deposited materials. Injection MOCVD is now being utilized to produce ferroelectric oxide of perovskite thin films including Barium strontium Titinate (BST), PT, PZT, and BFO, as well as oxide of perovskite super lattices like $(BT/ST)_n$ & $(LSMO/STO)_n$ [105].

4.2.3. Molecular-Beam Epitaxy (MBE)

In the same way that atomic spray painting employs alternatively shuttered elemental sources to maintain cation stoichiometry carefully, the MBE process for producing perovskite oxide thin films uses alternatively shuttered elemental sources to produce high-quality perovskite oxide thin films. Major problems in MBE multicomponent oxide synthesis in only a few applications include regulating oxide substrates terminated at well-defined ionic planes and monitoring the deposition of individual molecular/atomic layers. Reflection high energy electron diffraction) RHEED is frequently utilized for in situ monitoring of the developing surface in MBE. MBE has previously been utilized to fabricate high-quality thin-films oxide of perovskite and epitaxial heterostructures [95].

5. Planar Perovskite Oxide Nanostructures in 2 Dimensions

5.1 Top-Down Methods

A diversity of "top-down" 2D perovskite oxide nanostructured materials based on planar topologies have been constructed thus far using methodologies such as transmission electron microscopy, electron beam lithography (EBL), and nanoimprint lithography (NIL). Alexe and his colleagues were the first to construct a slightly elevated ferroelectric memory based on multiple perovskite ferromagnetism oxide nanostructure formations. Regular arrays of $SrBi_2 Ta_2O_9$ & PZT nanoisland capacitors with lateral dimensions less than 100 nm were safely manufactured using the EBL procedure.

After the BT nanodots were cut from the BT single crystal using Focused Ion Beam FIB technology, the zone structures inside the BT dots were evaluated [95,106].

5.2 Bottom-up Methods

Bottom-up techniques, including syntax synthesis, have been employed to create two-dimensional oxide of perovskite nanostructures based on lateral arrays of nanodots in addition to top-down solutions. Using template-assisted "bottom-up" synthetic technologies, nanosphere lithography was applied to produce BT, PZT, and $SrBi_2Ta_2O_9$ ferroelectric oxide nanodots with narrow size and distribution. Lee et al. [107] utilized ultrathin AAO membranes as a stencil mask and PLD method to build PZT arrays on Pt/ MgO substrates. Ultrahigh density ferroelectric memory might be allowed by Pt/PZT/Pt nano-capacitors with a density of 176 Gb/$inch^2$ [95].

6. Methods for making 3D Oxide Nano-structures of Perovskite-Type

Both "bottom-up", and "top-down" methodologies can be employed to create 3D nanostructures. "Top-down" nanostructure production options include using FIB technology to cut away bulk ferroelectric material and construct logically and continuously ordered nanosized structures. Exact placement and effective control over the shapes and sizes of the nanostructures formed are two major advantages of using "top-down" methodologies for FIB milling to make 3D perovskite oxide nanostructures. On the other hand, the FIB milling machine has a range of negative effects, including slower milling and patterning frequencies, thus causing difficulties for volume patterning nanostructures, specifically bigger ones [108].

Furthermore, difficulties have occurred at the nanoscale as a result of impact ions causing damage to the sample surfaces. Because the features of 3D oxide of perovskite nanostructures are highly influenced by their morphologies and ordered alignments, large-scale nanostructure arrays with the required shape and structure are important. A glass substrate, electron

Figure. 5 Three colloidal-crystal-template methods for producing periodic macroporous structures. Top: Precursor material is infiltrated into a preformed colloidal crystal, which is then treated to generate the 3DOM structure once the template is removed. Middle: To make a 3DOM structure homogenous, once the template is removed the nanoparticles (NPs) and templating spheres are co-deposited. Bottom: Hollow shells are produced by regularly arranging core-shell components. This diagram has been reproduced with the permission of ref. [73]. Copyright 2018 Chemical Science

Scheme 3 Synthetic approaches for nanoporous perovskite metal oxides are summarized from ref. [73].

Figure. 6 Patterns of XRD of SrPdO3 created from a combustion process at varied pH levels (A) for various fuels (B) SrPdO3, SrPd3O4, and SrCl2. Miller indices (h, l, k) are given in black for SrPdO3, red for SrPd3O4, and the sign (*) for SrCl2... Reproduced with permission Ref. [110]. Copyright 2014 Electrochimica Acta

Table 1. Lattice parameters of SrPdO$_3$ synthesized by different methods

	Lattice Structure	Particle Size (nm)	Lattice Parameters (A)	Lattice Volume (A^3)	Theoretical Density (g/cm^3)
Standard SrPdO$_3$ (ICCD card, 00-025-0908)	Orthorhombic		a=3.977 b=3.350 c=12.82	179.98	4.47
Citrate-nitrate method	Orthorhombic	34.0	a=3983 b=3.541 c=12.80	180.59	4.45
Urea-nitrate method	Orthorhombic	45.4	a=3.954 b=3.563 c=12.82	180.63	4.45
Glycine-nitrate method	Orthorhombic	25.7	a=3.972 b=3.527 c=12.83	179.69	4.47

Fig. 7 Images showing SEM analysis of **(A)** 'LaNiO₃', **(B)** 'LaCoO₃', **(C)** 'LaFeO₃' and **(D)** LaMnO₃ was produced using a microwave-assisted citrate technique at 720W for 30 minutes, magnified 35,000 times. From ref. [112]. Copyright 2014 Electrochimica Acta

Figure 8. Images showing HRTEM analysis of **(A)** 'LaNiO₃', **(B)** 'LaCoO₃', **(C)** 'LaFeO₃', and **(D)** For 30 minutes, microwave-assisted citrate was employed to generate LaMnO₃ @ 720W. according to the source ref. [111]. Copyright 2008 Journal of magnetism and magnetic materials

Figure. 9 FTIR spectra of LaFeO₃ produced with permission From ref. [113]. Copyright 2008 Bulletin of materials sciences

Figure 10: La (3d), Fe(2p), and O(1s) XPS spectra in $La_{0.9}FeO_3$ and $LaFeO_3$ samples. From ref. [114]. Copyright 2009 Bulletin of the Korean chemical society

beam lithography, and scanning probe lithography are used to achieve results [95].

7. Perovskite Characterization

The distinct phases of the perovskites generated may be distinguished by X-ray powder diffraction (XRD). Another approach for determining the structure of perovskites is single-crystal XRD. Thermal analytic techniques such as TGA, DTA, and DSC can be used to study the thermal stability of the produced perovskites. SEM & TEM, on the other hand, may disclose a variety of structural and surface properties of the perovskites created [109]. We chose a variety of perovskites from earlier research as examples for characterization investigations in this study.

7.1. SrPdO₃ perovskite

a. XRD

Galal et al. [109] Fig. (6A) show the XRD patterns of $SrPdO_3$ synthesized at pH values of 2, 7, and 10 for 3 hours at a calcination temperature of 750 °C. The Intensified Charge Couple Device (ICCD) results for $SrPdO_3$ were compared to those of the XRD. The real and theoretical pH 2 values are very close. This helps form the primary orthorhombic phase of perovskite of $SrPdO_3$ (major diffraction peak 110) and the secondary phase of $SrPd_3O_4$ emerged (210). In prepared samples of pH 7 & 10, only the $SrPd_3O_4$ phase was visible, while the (110) peak vanished. XRD patterns of $SrPdO_3$, $SrPd_3O_4$, and $SrCl_2$ are shown in Fig (6B).

Consequently, the optimal pH for generating $SrPdO_3$ is pH 2.

XRD may also be used to optimize the kind of (citric-acid, urea, and glycine) $SrPdO_3$ made with this material. In all cases, $SrPdO_3$ was the dominant phase, albeit with differing percentages of $SrPdO_3$ (110) compared to $SrPd_3O_4$ (210). The proportion was greater in the case of urea, but low in the case of citric acid. Table 1 lists the structural parameters that were calculated as well as well-matched theoretical data [109].

b. SEM and TEM

Perovskite nanoparticles' morphology and physical features may be explored using SEM and TEM. The SEM of the formed perovskites was strongly influenced by the preparation environment, synthesis methodology, types of A- & B-site ions of metal, and doped A- and/or B-sites. Galal and co. [109] employed 720W as the working power for 30 minutes of microwave irradiation to synthesize $LaNiO_3$, $LaCoO_3$, and $LaFeO_3$ are all oxides of nickel, cobalt, iron., and $LaMnO_3$ using the microwave-assisted citrate technique.

The SEM images of several perovskites i-e$LaNiO_3$, $LaCoO_3$, $LaFeO_3$, $LaMnO_3$ are displayed in Fig (7). $LaNiO_3$ had a surface as compact with a greater degree of order, whereas $LaCoO_3$ and $LaMnO_3$ had spherical grain agglomerations with smaller grain sizes in $LaMnO_3$ and greater grain sizes in $LaCoO_3$ and $LaMnO_3$, respectively. $LaFeO_3$ has a unique shape, with a porous surface filled with bonelike particles. $LaFeO_3$ has greater electro-catalytic activity than other perovskites in the hydrogen evolution process [109].

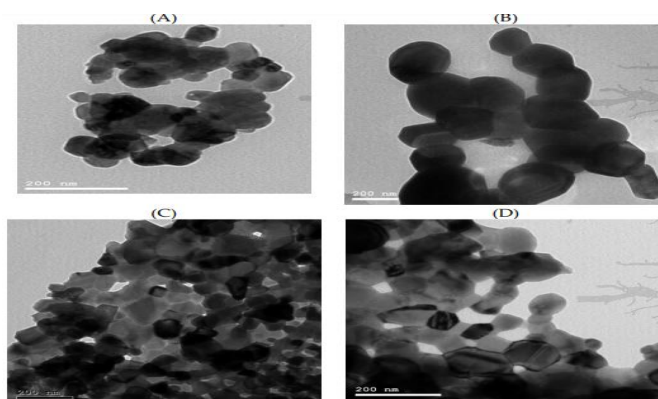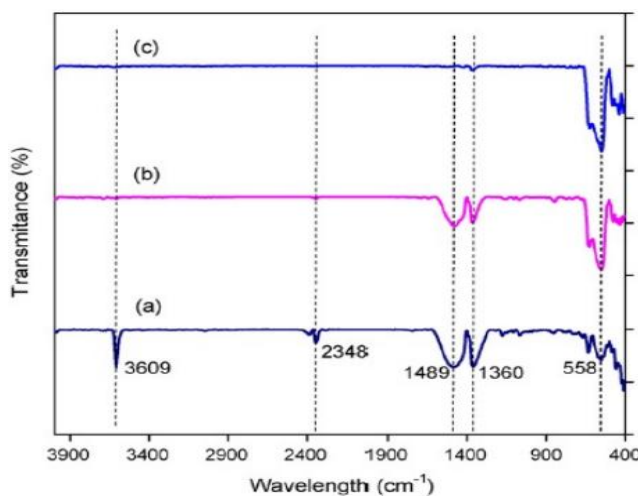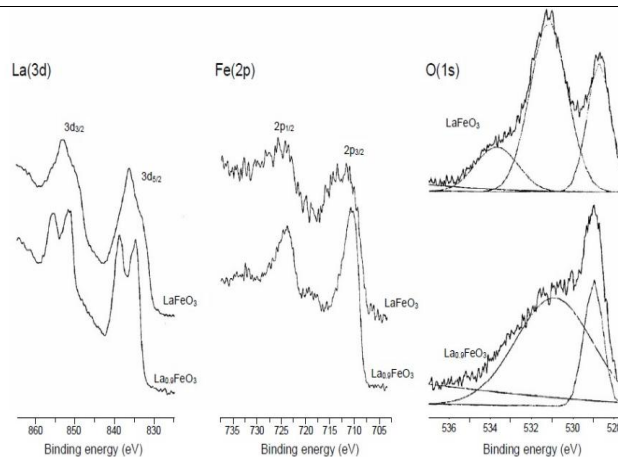In addition, HRTEM may demonstrate the various

morphologies and particle properties of several perovskites [111]. HRTEM images of LaNiO₃, LaCoO₃, LaFeO₃, and LaMnO₃ were prepared using the microwave-assisted citrate technique Fig (8). Images of LaFeO₃ taken using HRTEM revealed a crystallinity-rich orthorhombic phase, whereas LaNiO₃, LaCoO₃, and LaMnO₃ HRTEM photos revealed hexagonally deformed rhombohedral phases. For the different perovskites, the HRTEM diffraction patterns were similar to the XRD data [109].

c. FTIR

The perovskites' chemical bonding and chemical structure that has been generated may be investigated using FTIR. In the same way that XRD may provide structural evidence, the FTIR can as well. Biniwale et al. [113] synthesized $LaFeO_3$ using various methods, including sol-gel, combustion, and co-precipitation. In the FTIR of $LaFeO_3$, the stretching vibration mode of Fe-O was detected as an absorption band at 558cm^{-1} Fig (9). The O-Fe-O vibration mode was discovered to be linked to the 430cm^{-1} band. In $LaFeO_3$, which is connected to La-O in lanthanum oxide, co-precipitation produced a strong band at 3609 cm^{-1}. The band at 3600 cm^{-1} simply vanished in the other two methods, suggesting the production of a somewhat pure perovskite phase. Additional bands occurred at 1360 and 1480 cm^{-1} in the co-precipitation technique, indicating future stages. Consequently, the absorption peak of about 558 cm^{-1} was connected to metallic oxygen bond stretching modes, as described in the literature [109].

d. XPS

The surface compositions of the individual components of the developed perovskites may be examined utilizing XPS. Lee et al. [114] prepared $La_{0.9}FeO_3$ and $LaFeO_3$ samples and investigated their structural composition using XPS analysis. Fig (10) exhibits the XPS spectra of La (3d), Fe (2p), and O (1s) in $La_{0.9}FeO_3$ and $LaFeO_3$ samples. The binding energy of La (3d$_{5/2}$) in $LaFeO_3$ and $La_{0.9}FeO_3$ was 833.5eV and 833.8eV, respectively, corresponding to the La^{+3} ions in the oxide state. In both samples, however, the bandgap of Fe (2p$_{3/2}$) was 710.2 eV. This signifies Fe^{3+} ions in the form of oxide. The Fe (2p) XPS signal can't understand the difference between Fe^{3+} and

Fe^{4+}. The XPS signal of O (1s) was split into two peaks at 529.9 and 532.1 eV in the case of $La_{0.9}FeO_3$. The O (1s) XPS signal for $LaFeO_3$ contained three peaks: 529.4, 531.9, and 534.4 eV. The binding energies of O (1s) at lattice oxygen species are 529.9 and 529.4 eV in both experiments. The chemisorbed oxygen species OH or O are important for the peaks at 532.1 and 531.9 eV. The binding energy of chemisorbed oxygen species is 2.1– 2.5eV bigger than even lattice oxygen species. In the instance of $LaFeO_3$, the peak at 534.4eV was ascribed to adsorbed water species related to the particularly hygroscopic surface lanthanum oxide [114,109].

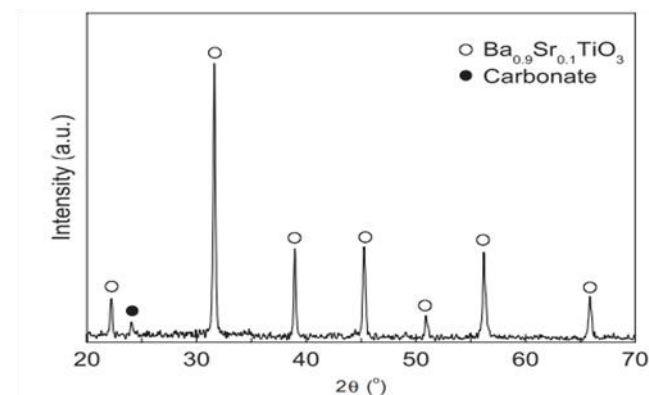**Fig. 11** ($Ba_{0.9}$ $Sr_{0.1}$) TiO_3 nanoparticles X-ray diffraction pattern. Reproduced with permission from ref. 115. Copyright 2005 Journal of solid state chemistry

Figure 12: Using field-emission scanning electron microscopy, BST nanoparticles were observed (FE-SEM). With permission from ref. 115. SThis article has been reprinted. Copyright 2005 Journal of solid state chemistry

7.2. [(Ba, Sr) TiO₃] Perovskite barium strontium titanate nanoparticles

a. XRD

Jian Quan qi et al. [115] used wet chemical synthesis at room temperature and ambient pressure to make perovskite barium strontium titanate nanoparticles (BST). Starting ingredients include titanium alkoxide and alkali earth hydroxides, which are processed using very simple methods. Changing the processing conditions can change particle size and crystallinity. The crystallinity and phase of the Ba, Sr) TiO₃ nanoparticles were determined using X-ray diffraction. Fig (11). In the XRD pattern, at 24⁰ barium carbonate appears as a peak. The interactions between carbon dioxide dissolved into solutions from the air and alkaline earth hydroxides are proposed to be the reason for barium carbonate manufacturing. Finishing the production in a protected climate (such as argon or nitrogen) or scrubbing the powders with dilute acids can prevent the impure phase [115].

b. SEM, TEM

The microstructure of as-produced particles of BST was further described using SEM and TEM shows a typical SEM picture of the synthesized BST nanoparticles. The particles are homogeneous in size and have a diameter of 50 nm as shown in Fig (12) [115].

The findings of the TEM observation are shown in (The size of the particle found by TEM was 50nm diameter, Fig (13a) which was consistent with the SEM findings. SAED pattern having atom planes of indexed shown in the inset of HR image of TEM is shown in Fig (13b). The image's darker half shows well-organized patterns, indicating that the particles under investigation have solidified. The structural information of the nanoparticles was obtained using a rapid Fourier transform technique, as illustrated in the Fast Fourier Transform (FFT). The material's lattice parameter was discovered to be 0.398 nm Fig (13c). In the FFT image, dislocations were also discovered in Fig (13 d). The production of dislocations is caused by the low synthesis temperature [115].

7.3. Perovskite oxide Baₓ Mn₁₋ₓ O₃

a. XRD

$Ba_xMn_{1-x}O_3$ oxide of perovskite is a good material for producing electrochemical instruments or devices because of its attractive chemical and physical features. Using a hydrothermal technique, Muhammad Rafique et al. [116] synthesized Ba-doped MnO₃ (BaMnO₃) with variable Ba concentrations. X-ray diffraction was used to characterize the generated material.

Fig (14) exhibits XRD patterns of $Ba_xMn_{1-x}O_3$ (x = 0.1, 0.15, 0.2), which reveal angles with miller indices of 27.20 (101), 31.60 (110), 380 (002), 41.280 (201), 50.450, 52.90 (211), 560 (300), 65.50 (220), 710 (203), 72.20 (311), and 78.90 (222), respectively. With a = 5.6720, b = 5.6720, and c = 4.7100, hexagonal crystalline structure is discovered. Peaks blue-shift as doping levels rise, with the full width at half maximum (FWHM) increasing and peak strength dropping, reflecting a drop in crystallinity. Doping raises the cell volume and material density to 131.23 cm⁻² and 6.06 g/cm⁻³, respectively, after doping. The EDX results reveal that the samples are pure because there are no impurity peaks in the spectra. The crystallite size was calculated using Debye Scherer's formula based on the obtained data [116].

$$D = \frac{K\lambda}{\beta Cos\Theta}$$

CLK = 0.15418nm is the X-ray wavelength, is the FWHM, and is the diffraction angle. K is the form factor (0.9 for hexagonal structures), D is the crystallite size, and CuK = 0.15418nm is the X-ray wavelength. The crystallite size in pure material is 22.1nm. However, when the number of dopants increases, it shrinks. The crystallite diameters of 10wt percent, 15wt percent, and 20wt percent doped BaMnO₃ are 21.2, 16.5, and 11.3 nm, respectively. Lattice distortion is caused by a mismatch in the radius of the matrix and the dopant element, which causes crystallite size to decrease. A stress or strain field is created due to this occurrence, disrupting crystal formation in one direction. Because the dopant (Ba) is big, compression forces produce blue shifting of the diffraction peaks. Tensile strains, on the other hand, move the peaks toward larger angles
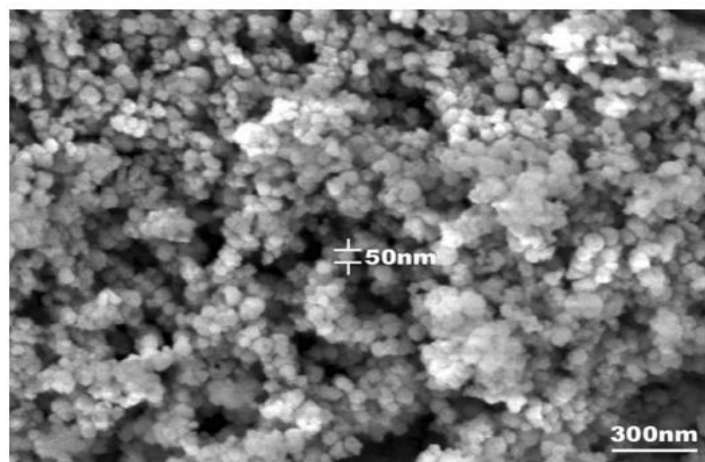
Figure 12: Using field-emission scanning electron microscopy, BST nanoparticles were observed (FE-SEM). With permission from ref. 115. This article has been reprinted. Copyright 2005 Journal of solid state chemistry

Fig. 13 TEM images of BST nanoparticles: **(a)** 'morphologies', **(b)** 'HR image', **(c)** This FFT graphic shows the link between the atom planes and **(d)** The dislocations in the nanostructure are seen in this FFT image. Reproduced with permission from ref.

115. Copyright 2005 Journal of solid state chemistry

Figure. 14 XRD of pure MnO_2 and 10, 15, and 20wt% doped $BaMnO_3$. Reprint with permission from ref. 116. Copyright 2021 International journal of energy research

Figure 15: SEM micrographs of Ba_xMnO_3 **(A)** At 10wt% doped $BaMnO_3$, **(B)** At 15wt% doped $BaMnO_3$, and **(C)** At 20wt% doped $BaMnO_3$. Reproduced with permission from ref. 116. Copyright 2021 International journal of energy research

Fig 16: EDX spectra of $BaMnO_3$. Reproduced with permission from ref. 116. Copyright 2021 International journal of energy research

Due to a large amount of dopant. Oxygen vacancies have an important role in structural mobility. A cation vacancy is created in this situation by giving dopants (Ba) an excess positive charge. When these vacancies come into contact with oxygen vacancies in MnO_2, they become less mobile [116].

b. SEM

SEM micrographs of Ba-doped MnO_3 at 10, 15, and 20% by weight are depicted in Fig (15A, B, C). The micrographs for 10, 15, and 20wt percent doped materials reveal nanorods with diameters of 90-110nm, 180-220nm, and 10 to 15nm, respectively [116].

The degree of doping in the nanorods determines their shape and diameter. This is owing to a difference in the materials' ionic radius, which induces bond constriction and, as a result, a size reduction. According to the findings, doping increased the material's surface area, which improved the electrochemical characteristics of MnO_2 by increasing the material's reactive sites [116].

c. EDX

The EDX spectrum of Ba-doped MnO_3 is shown in Fig (16). The spectrum reveals that the samples produced are fully contaminant-free. This spectrum contains only the elements Mn, Ba, and O, contributing 26.45, 7.13, and 65.76 percent by Weight, respectively. As a result, the hydrothermal technique synthesized morphological materials that were both pure and controlled [116].

7.4. NdNiO₃ Perovskite Nanoparticles

a. XRD

To create $NdNiO_3$ perovskite nanoparticles that were calcined at various temperatures, M. I.Maulana et al. [117] employed sodium hydroxide as a precipitating agent and polyethylene glycol as a surfactant in a co-precipitation approach.

$$Nd(NO_3)_3 + Ni(NO_3)_2 + 5NaOH \longrightarrow Nd(OH)_3 + Ni(OH)_2 + 5NaNO_3 (1)$$

$$2Nd(OH)_3 + 2Ni(OH)_2 \longrightarrow Nd_2O_3 + 2NiO + 5H_2O \text{ (heating stage 1) (2)}$$

$$Nd_2O_3 + 2NiO + \tfrac{1}{2} O_2 \longrightarrow 2NdNiO_3 \text{ (heating stage 2) (3)}$$

The products are identified using X-ray diffraction Fig (17).

Shows the material's pattern of XRD obtained during manufacturing, calcined at different temperatures. As calculations temperature grew, peaks strength changed. In products of as-synthesis calcined at about 900°C, a crystalline phase of $NdNiO_3$ nanoparticles, ABO_3 perovskite-type, was identified (P900). Peaks corresponding to novel oxide phases ascribed to extraordinarily strong peaks such as Nd_2O_3, NiO, and Nd_2NiO_4 were also discovered at that temperature.

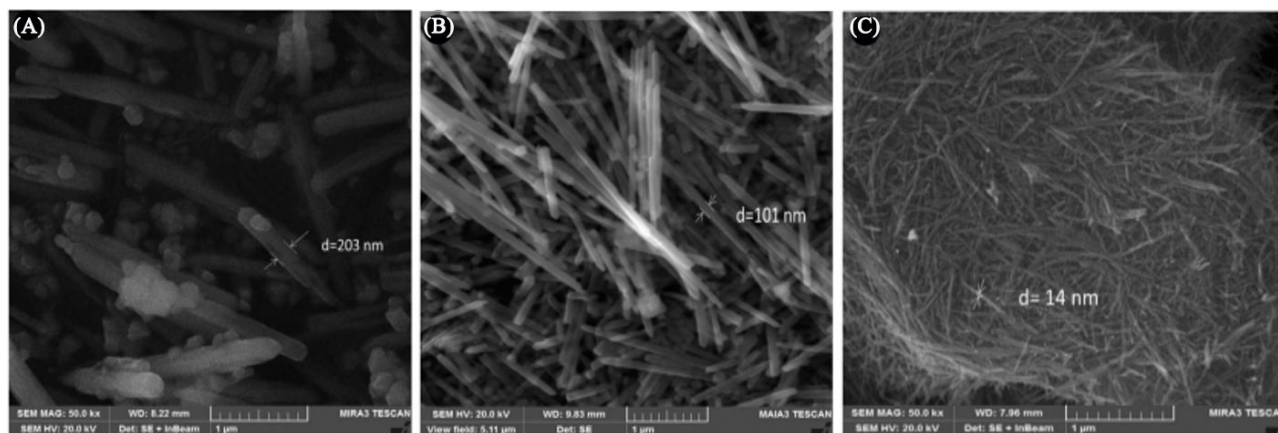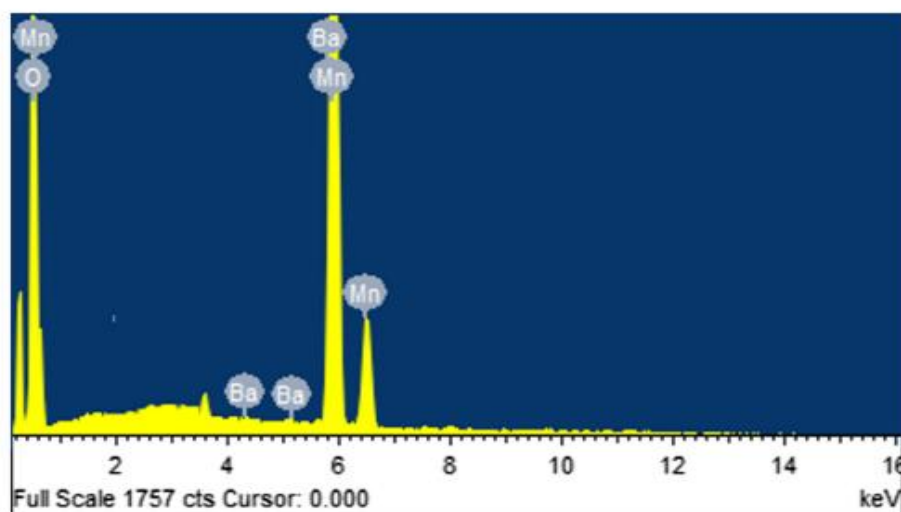The hydroxide and oxide phases coexist in the calcined synthesis result at 700°C, namely $Nd(OH)_3$, $Ni(OH)_2$, Nd_2O_3, NiO, and Nd_2NiO_4, were formed (P700). The metal hydroxides were expected to not entirely dissolve to oxides during heating step 1 (Reaction 2).However, they can be converted to perovskites at extremely high calcination temperatures. It might be because the first heating stage is just 1hour long. At temperatures below 900 °C, $NdNiO_3$ nanoparticles do not form. Analysis of XRD indicated that P900 was far superior to P700 due to the development of perovskite and the lack of hydroxide compounds [117].

b. FTIR

The materials were analyzed using FTIR before and after calcination. It was done to see if we successfully eliminated contaminants containing undesirable functional groups. It was also hoped that a wide range of vibrations of Ni–O stretching and O–Ni–O bending would be detected. After calcination, the functional groups OH, CH, and CO in the nitrate ion as precursors, NO, and O–H, C–H, and C–O as a surfactant in PEG 400, OH, CH, and CO, should evaporate. It was considered that the substance was pure since it lacked specific functional groups. The PEG structure is shown in Fig (18) [117].

The spectrum of FTIR of PEG 400 as well as the final product before and after calcination are shown in Fig. (19). The spectra of PEG were given peaks of absorption for OH wide stretched, CH stretched, CO stretched, and CO stretched (primary alcohol) vibrations at 3432, 2866, 1092, and 1066 cm^{-1}, respectively. As the temperature of calcination increased, the OH vibrations of stretching were slowly lost and may not be recognized in spectra of P900, & also as on CH stretched and CO stretched

vibrations [117].

Oxides of metals, like Ni–O, show absorption peaks under 800 cm^{-1} because of interatomic vibrations. Significant absorption is about 6–7 hundred cm^{-1} region because of Ni–O vibration stretching mode [118].

The peak of absorption at 634 cm^{-1} is also caused by the MO, OMO, and M-O-M (M=Ni, Co) [119] vibrations. According to the FTIR spectrum, Ni–O's stretching vibrations were identified before and after calcination in the product. The stretching Ni–O led Ni(OH)$_2$ to form in the precipitate of the product before calcination. O–H stretching was measured during that time, which supported the idea even more. P700 contains a trace quantity of Ni(OH)$_2$ as evidenced by mild stretching O–H and NiO$_2$ vibrations in Nd$_2$NiO$_4$ compounds. On the other hand, the O–Ni–O vibration in P900 created NdNiO$_3$ perovskite nanoparticles. XRD examination verified that all of these assignments gave NdNiO$_3$ [117].

7.5. LaNiO$_3$ perovskite oxides

a. XRD and SEM

Harikrishnan and co-presented a co-precipitation technique for synthesizing LaNiO$_3$ nanoparticles, which were then annealed at various temperatures. Materials' structure and morphology are studied using XRD Fig (20a) [120]. XRD is utilized to identify the structure of the crystal.

The sample that was annealed at 600 degrees had a less crystalline XRD pattern. The crystalline structure of the LaNiO$_3$ nanoparticles matches 34-1028 which corresponds to a rhombohedral perovskite structure when annealed at temperatures of 700, 800 and 900 degrees Celsius, as illustrated in (Fig. 21) (a) peaks at 23.42°, 33.02°, 40.88°, 41.33°, 47.04°, 53.72°, 54.04°, 58.96°, 59.72°, 68.8°, 69.17°, 74.52°, 78.67°, 79.49° correspond to (101), (110), (021), (003), (020), (211), (113), (122), (220), (029), (303), (312), (214). No other peaks at 700oC, 800oC, or 900oC could be attributed to La$_2$O$_3$ or other phases indicating the presence of single-phase LaNiO$_3$ in the sample. The powerful diffraction patterns confirm the material's crystalline structure [120]. The surface morphology of the generated different LaNiO$_3$ (LNO) nano-particles ie LNO1, LNO2, LNO3, LNO4, and LNO5 is

investigated using SEM. Fig. (20 b,c,d) depicts the SEM images of the samples. The diverse morphologies of the particles for various calcination temperatures are readily seen in these SEM images [120]. Typically, they appear as a clump of deformed sphere-like particles. Additionally, as the calcination temperature rises, so does the degree of particle agglomeration. Particle grain growth during high-temperature calcination might cause the degree of agglomeration [120].

8. Catalytic Applications of Perovskite Oxides

Oxides based on perovskite having common formula ABO$_3$ have effectively ammonia oxidation, methane combustion, catalyzed hydrogenation, and CO oxidation, among other reactions. We used examples from previous research that revealed catalytic behavior in numerous conversions to show case the catalytic applicability of perovskite oxides.

8.1. Comparison of Catalytic Activity of GdAlO$_3$, SrMnO$_3$, SrCoO$_3$, and MnFeO$_3$ Perovskite Oxides

Perovskite oxides, which appear to be potential catalysts, appear to enhance VOC combustion. During the combustion method of Propane, benzene, acetone, and gasoline (Pb-free), Nicolae Rezlescu et al. [121] compare the catalysis activity of several ordinary perovskites having variable cationic concentrations. Nanometer particles with nominal compositions of SrMnO$_3$, GdAlO$_3$, MnFeO$_3$, and SrCoO$_3$ were created using the self-combustion sol-gel process and subsequently heat treated in the air at 1000ºC. As for catalytic studies, the catalytic activity level varied significantly depending on the perovskite content. SrMnO$_3$ is the most active of the four perovskites when the weather is cold only in the conversion of acetone did catalysts MnFeO$_3$ and SrCoO$_{3-x}$ exhibit considerable activity as catalysts [121].

(a) Perovskites' Catalytic Activity

Perovskites catalytic capabilities in the combustion of investigated (distinct VOCs) at temperatures ranging from 20 to 550ºC. For each perovskite composition, Fig. (21) displays the gas conversion as a parameter of reactive temperature. The following considerations should be taken into account: [121]

● The catalytic activity of the perovskite catalyst is affected by the reaction temperature. Raising the reaction

temperature aids gas combustion.

- Perovskites of diverse compositions have dramatically different catalytic activity, which is consistent with Seyfi et al findings [122]. Strontium manganite catalyst has higher catalytic activity than gadolinium aluminate catalyst Fig. (21 a,b) The activity differential between the two samples cannot be explained by their different surface areas. The surface area of $GdAlO_3$ is 10 m^2/g, while the surface area of $SrMnO_3$ is 2.2 m^2/g.

- Gas combustion over the $SrMnO_3$ catalyst began at substantially lower temperatures (about100°C) than over other perovskites. The $SrMnO_3$ catalyst's increased VOC conversion activity reflects the existence of reactive oxygen species on the catalyst surface. Because of the high concentration of Mn^{4+} ions on the perovskite surface, oxygen may be less anchored and thus more available for VOC oxidation.

- An interesting result was found for manganese ferrate ($MnFeO_3$) Fig. (21c) and strontium cobaltite ($SrCoO_{3-x}$) Fig. (21d). Regardless of chemical composition, the two catalysts only demonstrated high catalytic activity for acetone conversion and low catalytic activity for propane, benzene, and gasoline catalytic combustion. The reasons for such a restricted catalytic activity are unknown. This behavior could be explained by a rearrangement of their lattice structure, which controls their catalytic properties, and, as a result, the active site configuration. It's worth noting that temperature significantly impacts acetone conversion over the $MnFeO_3$ catalyst. The acetone conversion started at a low temperature (150 °C), and when the temperature increased from 200 to 300 °C, the conversion rate increased substantially from 10% to 80%. (Fig.22c) This effect was not observed with the other perovskites. Unlike the $MnFeO_3$ catalyst, the $SrCoO_{3-x}$ catalyst started converting acetone at about 200°C and reached an acetone conversion rate of 80 percent around 450°C. The four gases were converted more efficiently using the $GdAlO_3$ and $SrMnO_3$ catalysts [121].

Table 2 shows the data gathered in flame-less combustion of VOC on the four perovskite catalysts. The conversion against temperature graph can be used to calculate T_{10} and T_{50}, which are the temperatures required to convert a gas by 10% and 50%, respectively. T_{50} is a common metric for assessing the catalytic activity of a catalyst [121].

The catalytic activity for complete gas oxidation is sufficient at T_{50} temperature, and there is substantial contact between the catalyst surface and the reactants. The catalyst is more active if this number is lower. $SrMnO_3$ appears to be more active than the other catalysts. It has much lower T_{10} and T_{50} temperatures than the other catalysts [121].

a   Reaction rate for VOC concentration at low conversion per unit surface area of catalyst.

b   Apparent activation energy for low conversions

The chemical composition of catalysts of perovskite, as well as the kind of used gas, have a very much impact on the performance of the catalyst in the combustion of VOC, as shown in Fig (22). On the other hand, $SrCoO_3$ and $MnFeO_3$ catalysts favor acetone oxidation alone. At 500 degrees Celsius, catalysts can convert 85% of acetone, but only 30 percent of the other gases. In light of the contrast, despite the little surface area, the most preferred catalyst among the four perovskite samples, $SrMnO_3$, has the greatest catalytic activity at low temperatures. This catalyst converted 95 percent of propane, 83 % lead-free gasoline, and 75 percent acetone at 500 Celsius. $SrMnO_3$ less specific area perovskite does not appear to play a role in the catalyst's greater activity. The greater activity of the catalyst to $SrMnO_3$ might be attributable to the increased oxygen mobility generated by vacancies of oxygen caused by the manganese ions' presence of varied valence. The different activity of catalysts of the four perovskites is not able to be described by distinct surfaces. No indication that activity and surface area were linked. Factors like structural flaws and oxygen mobility are likely to affect the catalytic efficacy of these perovskite catalysts [121].

8.2. Methane Oxidative coupling, ABO_3 perovskites are used

Yujin Sim et al. [123] investigated the active-sites behavior of catalysts of perovskite in the coupling that is oxidative

coupling/reaction of methane (OCM) utilizing 10 different ABO_3 types catalysts of perovskite) with different structural features based on their A and B site components. In addition to being the structure definite and simple having the stability of heat, these materials offer remarkable activity of the catalyst in a range of conversion of CH_4 activities. According to findings, the surface lattice of catalyst species of oxygen is required for targeted methane conversion. Oxygen species of the surface lattice with lesser binding energies were used to purposefully speed the generation of hydrocarbons of C_2 from the OCM. Oxygen-adsorbed of surface and mixed oxygen lattice species were used to fill oxygen surface gaps created by the interaction of oxygen lattice with CH_4. The oxygen ion conductivity of perovskites is significantly related to this oxygen cycle, which can be predicted using structural features tolerance factor and particular free volume are two examples. The simple oxygen cycle converted a considerable amount of CH_4 during this reaction. Finally, they discovered that the oxygen lattice characteristics and for the systematic design of effective catalysts OCM, the conductivity of ion of oxygen of perovskite catalysts is a major component that influences catalytic activity and must be carefully managed [123].

According to the researchers, the conductivity of oxygen-ion of catalysts of perovskite might be utilized to forecast CH_4 conversion during the OCM process. Higher binding energies lattice oxygen species aided CO generation. Furthermore, the oxygen surface adsorbed species formed by gas-phase oxygen adsorption changed CO_2 to CO. As a result, the electrical characteristics of the oxygen species lattice and the conductivity of oxygen ions are essential determinants in the determination of the OCM activity of the catalyst of perovskites. Under the specified reaction conditions, the catalyst $CaZrO_3$ having strong conversion CH_4 and C_2 selectivity produced the maximum yield of C_2 (14.2 percent) [123].

8.3. $BaCe_{1-x}Mn_xO_{3-\delta}$ perovskite for methane combustion

The Maganese doped in $BaCeO_3$ catalyst oxides of the series

composite were synthesized using a sol-gel methodology. According to Xihan Tan et al. [124] methane as catalyst activity of combustion of $BaCe_{1-x}Mn_xO_{3-\delta}$ catalytic oxide of the series composite was investigated on lesser temperatures using a reactor of fixed bed (200-600 °C). Physically catalyst parameters are described using SEM, size of particle analysis, XRD, and measurement of a surface-specific area, while sample conductivity is determined using the impedance of AC spectroscopy [124].

According to the findings, the catalyst oxide of the perovskite phase composite constructed using the sol-gel catalyst phase of perovskite created has excellent catalyst performance and great thermal stability. Catalyst catalytic performance is affected by its conduciveness, which has a proportion in connection. According to the findings, the catalyst oxide of the perovskite phase composite is effectively manufactured using the sol-gel method. The catalyst phase of perovskite generated has better performance and great thermal stability. Catalyst catalytic performance is affected by its conduciveness, and there is a proportional relationship between the two. When the temperature is below 650°C, Mn doping considerably increases the activity of catalysis of the catalyst $BaCeO_3$ for the combustion of methane. As the quantity of Mn-doped rises, the catalytic activity increases at first, then decreases [124].

They also observed that when the sintering temperature climbed, sample conductivity increased firstly then decreased, grew continuously while increasing temperature test, and rose first and then plummeted as the Mn percentage increased. A $BaCe_{0.6}Mn_{0.4}O_{3-\delta}$ catalyst sample sintered at 1250°C had the maximum conductivity at 800°C, $0.4975 scm^{-1}$. They observed that the conductivity change rule is precisely opposite that of the activation energy in the diagram of Arrhenius of Mn sample conductivity. The activation energy is lowest when the catalyst sample reaches its maximum conductivity. The result of the activity of catalytic oxidation of CH_4 tests explains adding a catalyst considerably enhances methane catalytic oxidation efficiency when compared to doing so without one, and that

CH$_4$ after doping has a better catalytic oxidation efficiency than BaCeO$_3$. When the element Mn=0.4 is doped, sample catalytic activity peaks of CH$_4$. The initial temperature of methane is 249.7 degrees Celsius, which is 400.3 degrees Celsius lesser than the experiment of blank, 95.3 degrees Celsius lesser to BaCeO$_3$ [124].

8.4. Co$_x$Fe$_{1-x}$O$_y$ catalyst for CO$_2$ hydrogenation

The study of CoO$_x$–FeO$_x$ catalysts reveals a lot about how multi-transition metal oxide catalysts are made and used. Minshan Meng et al. [125] stated that an efficient and solid-state mechanochemical redox process for obtaining Co$_x$Fe$_{1-x}$O$_y$ from CoCl$_2$.6H$_2$O and KMnO$_4$ was established with just two rounds of ball milling (BM). The transition metal oxide

Co$_x$Fe$_{1-x}$O$_y$ generated in the RWGS method may be used as a high-activity catalyst as well as a CO selectivity catalyst. Over the whole temperature range, selectivity is greater than 80%. At 500 degrees Celsius, it converted CO$_2$ at a rate of 43 percent, compared to 15.6 percent for Co$_x$Fe$_{1-x}$O$_y$ -CP and 15.8 percent for Co$_x$Fe$_{1-x}$O$_y$-SG [125].

During 120 hours of high ambient temperature, the conversion rate of Co$_x$Fe$_{1-x}$O$_y$–BM increased, suggesting that adding Fe to the Co element improved thermal stability. In the future, such a breakthrough is predicted to be the road to a greater and more efficient system of industries for synthesizing multi-transition metal oxides that are solvent-free and have outstanding catalytic performance [125].

8.5. $La_{1-x}Sr_xCuO$, CO_2 hydrogenation to methanol catalysts

Because it is a cost-effective solution to the environmental greenhouse gas problem, CO_2 catalytic hydrogenation of the methanol process has become a popular CO_2 consumption technique. Structured materials of perovskite have come as very attractive other possible standard catalysts supports for this process because of their mobility of oxygen feature and feasibility of structural increasing adsorption CO_2 capacity by easy having metal oxides of alkali doping. The impact of the adsorption strength of CO_2 on hydrogenation of CO_2 on the activity of methanol in $La_xSr_{1-x}Cu_{1.o}O$ materials with a perovskite structure was investigated and published by Antonius Jeffry Poerjoto et al. [126] $La_{0.9}Sr_{0.1}CuO$ beat all other Sr-modified catalysts in terms of conversion of CO_2 (8.59%), selectivity of methanol on 300°C, 3.0MPa pressure (49 percent). Moreover, $La_{0.9}Sr_{0.1}CuO$ demonstrated sustained catalytic activity over 24 hours with no carbon generation throughout the CO_2 hydrogenation cycle. XRD analysis revealed the forming structures of perovskite in catalysts of calcined. According to XPS research, $La_{0.9}Sr_{0.1}CuO$ has a larger number of lattice oxygen species than the others. Furthermore, because there is a relationship between lattice oxygen concentration and methanol yields, lattice oxygen species are significant for improving methanol selectivity during CO_2 hydrogenation. $La_{1-x}Sr_xCuO$ catalysts of perovskite having catalytic concentration $La_{0.9}$ $Sr_{0.1}CuO$ outperformed another catalysis for hydrogenation of CO_2 in methanol [126].

Fig. 18: Polyethylene glycol's chemical structure

8.6. Metal Catalysis on Perovskite-Type Oxides

Rojas Cervantes and colleagues investigated the use of catalysts of perovskite oxide-based oxidation for wastewater treatment using various oxidants and so UV-visible irradiation producing photocatalysis [127]. The researchers investigate the physicochemical aspects of perovskite oxides like lattice oxygen vacancy and mobility formation as well as maintaining distinct states of oxidation of component elements which may be used to increase AOP employing radicals [128].

Using X-ray absorption spectroscopy, Kim et al. [129] show how Fe drives the oxygen evolution process (OER) in the $PrBaCo_{2\,(1-x)}Fe_{2x}O_{6-\delta}$ layer double perovskite used as a catalyst under alkaline circumstances. In $PrBaCo_2O_{6-\delta}$, Fe leads Cobalt to enter a lesser oxidation state that allows for charge compensation. In reaction circumstances, it prevents Cobalt and the layer doubled perovskites from melting, enabling active surface Co oxy-hydroxide layer to form [128].

Guo et al. [130] show that employing $LaCo_yGa_{1-y}O_3$ mixed oxides as catalysts, alcohols (mostly methanol/ethanol) may be synthesized from syngas. The precursor of segregated cobalt nanoparticles in the LaGaO composite oxide is $La_{1-x}K_xCo_{0.65}Ga_{0.35}O_3$, which boosts its stability in a reactive environment. The impact of K on boosting the dispersion (atomic) of Cobalt and improving the coking composites resistance catalysts is identified by synthesizing La_2O_3 [128].

Steiger et al. [131] examine sulfur tolerance in solid oxide fuel cells and employ segregation reversible Ni to be an element active for the aqueous gas shift process and the second metal transition. Compared to Mn, Mo, Cr, and Fe, only Fe enhances the sulfur tolerance of $La_{0.3}Sr_{0.55}Ti_{0.95}Ni_{0.05}O_3$. Segregation simultaneously of iron and nickel in higher temperatures has little effect on the to and fro segregation reintegration of a couple of metals inside the mixed oxide of perovskite during oxidations, enabling greater time high-temperature uses [128].

Wark and colleagues [132] explain how $LaFeO_3$ works as a photocatalyst in the breakdown of Rhodamine-B and look at the best circumstances for getting the best results. Photoelectrochemical analysis was employed in addition to textural evaluation to explain variations in behavior as a function of calcination temperature. The results indicated lower temperatures boosted separation efficiency with photo-induced

charge carrier transfer [128].

Fig 19: FTIR spectrum of **(a)** 'PEG 400', **(b)** 'product before it is calcined', **(c)** 'P700', and **(d)** 'P900'. Reproduced with permission from ref. [117]. Copyright 2020 Nano Express

With DFT research by Glisenti et al. [133], replacing La with Sr at the A-site of $LaCoO_3$ decreases the energy required to create oxygen vacancies, which is helpful in three-way catalysts for CO oxidation. Similar results can be produced by switching Co for Cu at the B-site. Effects of substitution appear as higher in $SrTiO_3$ [128].

The impact of some Ti substituting $BaTiO_3$ utilized as lean NOx catalyst trap on NOx storage capacity is investigated by Aldridge et al. [134] Cu is beneficial because it helps to separate Ba_2TiO_4 storage from NOx storage. Among highly active noble metal-based catalysts, $BaTi_{0.8}Cu_{0.2}O_3$ has the most oxygen vacancies and the maximum storage capacity [128].

Fig. 20(a) 'XRD spectrum images of LNO1, LNO2, LNO3, LNO4, and LNO5' **(b)** 'Images showing SEM analysis of LNO3', **(c)** 'LNO4' and **(d)** 'LNO5' respectively. Reproduced with permission from ref. 120. Copyright 2019 In AIP

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Roning and colleagues looked at various $LaCo_{1-x}Mn_xO_3$ and $LaCo_{1-y}Ni_yO_3$ catalysts for NO oxidation to NO_2. [138] While $LaCoO_3$ has the maximum activity among un-doped perovskites, $LaCo_{0.75}Ni_{0.25}O_3$ and $LaCo_{0.75}Ni_{0.25}O_3$ have the highest activity in substituted catalysts, suggesting that perovskites could be used as NO oxidation catalysts in the industry [128].

Heidinger et al. [136] used 3 stages of crushing: a reactive approach that includes high-energy wet ball milling, solid-state synthesis, and low-energy wet ball milling, all of which were subsequently calcined at 400°C. The catalytic efficiency for toluene oxidation improves in both cases after each synthesis phase, per the gain in surface-specific area, which marks about nineteen m^2g^{-1} to $LaFeO_3$ [128].

9. Conclusions and Future Perspective

This review has covered how to manufacture various morphologies of perovskite materials, define perovskite oxides, and employ perovskite oxides in catalysis. Because the sum of oxide needs a higher temperature and a large period of calcination, perovskite oxides are composed of some simple oxides and have a very small surface area. The area and properties of the surface of perovskite oxides must be increased to be used in heterogeneous catalysis or surface reactions. This study demonstrates how to make perovskite oxides in a variety of bulk, nanoscaled, supported, porous, and hollow morphologies, to maximize surface area and surface properties while adhering to reaction conditions. Several methods have been developed to understand perovskite oxides better to analyze their structure and physicochemical characteristics. Perovskite oxides' catalytic properties have been examined regarding their usage in catalysis.

We've already discussed nanoporous perovskite oxides, which can be made using soft and hard templates and colloidal crystal templates. Similarly, template-free and template-assisted synthesis of 1D perovskite oxide nanostructures, as well as thin sheets or multilayers of perovskite oxide and PLD of 2D perovskite oxide nanostructures, have been reported. Chemical solution deposition, CVD and MOCVD, & MBE are also

included in PLD (MBE). Top-down and bottom-up methods can be used to make planar perovskite oxide nanostructures in two dimensions. The synthesis of a 3D perovskite oxide nanostructure is also briefly explored.

In characterization, we have reported the characterization of various perovskite oxides namely $SrPdO_3$ by XRD, SEM, TEM, FTIR, XPS, and $[(Ba, Sr) TiO_3]$ by XRD, SEM, TEM, and $Ba_xMn_{1-x}O_3$ by XRD, SEM, EDX and $NdNiO_3$ by XRD, FTIR and $LaNiO_3$ by XRD, SEM respectively.

We compared the catalytic activity of $GdAlO_3$, $SrMnO_3$, $SrCoO_3$, and $MnFeO_3$ to overview the catalytic uses of

perovskite oxides. $SrMnO_3$ at low temperatures that appearing as a catalyst that is active in most of the four perovskites, according to the findings. Only in the conversion of acetone did $MnFeO_3$ and $SrCoO_{3-x}$ catalysts exhibit strong catalytic activity. We have also reported ABO_3 perovskite in oxidative coupling of methane, $BaCe_{1-x}Mn_xO_{3-\delta}$ perovskite for methane combustion, $Co_xFe_{1-x}O_y$ catalyst for carbon dioxide hydrogenation, $La_{1-x}Sr_xCuO$ catalyst for carbon dioxide hydrogenation to methanol, and metal catalysis on perovskite oxides from recent studies.

Fig 21: Temperature vs. benzene, propane, acetone, and gasoline of Pb-free conversion by catalytic flameless combustion **(a)** "$GdAlO_3$", **(b)** "$SrMnO_3$", **(c)** "$MnFeO_3$" and **(d)** "$SrCoO_{3-x}$" perovskites. Reproduced with permission from ref. [121]. Copyright 2014 Composites Part B: Engineering

Table 2. T_{10} T_{50} and Kinetic parameters (reaction and rate[a] and activation energy[b]) for perovskite catalysts: Reproduced with permission from ref. [121]

VOCs	GdAlO$_3$				SrMnO$_3$			
	T_{10} ($^{\circ}$C)	T_{50} ($^{\circ}$C)	Reaction Rate (μmol s^{-1} m^{-2})	Activation energy (KJ / mol)	T_{10} ($^{\circ}$C)	T_{50} ($^{\circ}$C)	Reaction Rate (μmol s^{-1} m^{-2})	Activation energy (KJ / mol)
Acetone	295	390	6.7×10^{-2}	89	130	200	140×10^{-2}	37
Propane	330	440	7.8×10^{-2}	71	180	240	9.8×10^{-2}	31
Benzene	260	350	6.5×10^{-2}	68	175	325	26×10^{-2}	35
Pb free gasoline	390	-	4.2×10^{-1}	62	160	240	55×10^{-2}	36

	MnFeO$_3$				SrCoO$_3$			
	T_{10} ($^{\circ}$C)	T_{50} ($^{\circ}$C)	Reaction Rate (μmol s^{-1} m^{-2})	Activation energy (kJ/ mol)	T_{10} ($^{\circ}$C)	T_{50} ($^{\circ}$C)	Reaction Rate (μmol s^{-1} m^{-2})	Activation energy (KJ /mol)
Acetone	200	230	65×10^{-2}	98	250	325	16×10^{-2}	41
Propane	400	-	6.1×10^{-2}	80	270	-	3.8×10^{-2}	48
Benzene	255	-	17×10^{-2}	45	190	-	56×10^{-2}	44
Pb free gasoline	340	-	12×10^{-2}	47	190	-	11×10^{-2}	40

Despite major academic advances over the years, perovskite oxides have failed to find commercial applications as a catalyst. Their limited catalytic efficacy and sensitivity to pollutants like sulfur dioxide could be part of the problem. To commercialize these materials for industrial use, researchers should continue to work on developing a more efficient catalyst and learn more about how toxins interact with the surface of perovskite oxides.

Fig 22: The effect of perovskite catalyst chemical composition on gas conversion at 500°C. Reproduced with permission from ref. [121]. Copyright 2014 Composites Part B: Engineering

Acknowledgment

Conflict of interest

The authors reported no potential conflict of interest.

Authors Contribution

F. A and S.Y.B convinced the main idea and wrote the manuscript. K.e.K, S.F revised the manuscript and prepared figures and references.

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ARTICLE

Fractionation and Characterization of the Bioactive Compounds of the Extracts of Buds of *Syzygium aromaticum*

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Abstract

This research focused on the separation and identification of bioactive components of the methanolic extracts of the buds of *Syzygium aromaticum*. A bioassay and phytochemical screening were performed on the various solvent fractions, and the most active fraction was subjected to spectroscopic analysis using infrared, mass spectroscopy, and nuclear magnetic resonance to determine the structure of the active compounds present. The methodology involved extracting the flower buds of *Syzygium aromaticum* using methanol, fractionating the plant extract using three solvents—n-hexane, ethylacetate, and methanol, and performing a bio Using the agar well diffusion method, the antibacterial properties of the three solvent fractions were ascertained. The analysis' findings revealed that each of the three solvent fractions had tannins, alkaloids, flavonoids, and reducing sugars; the only fraction to contain saponins was the ethylacetate fraction, and the only fraction to have glycosides. The results of the study further showed that the ethylacetate fraction had the strongest antimicrobial activity against the test organisms, inhibiting the growth of bacteria such as *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis,* and *Candida albicans* at concentrations of 200 mg/mL. The structural elucidation of the active compounds responsible for the antimicrobial was done through spectroscopic analysis using infrared, nuclear magnetic resonance and mass spectroscopy. The antimicrobial activities of this plant highlighted the significance of the extracts in traditional drug preparations, according to the study's findings, which suggested that the antimicrobial properties of the flower buds of Syzygium aromaticum may be due to the synergetic or individual effects of the phytoconstituents found.

Keywords: Antimicrobial, characterization, fractionation, screening, *syzygium aromaticum*

1. Introduction

Numerous chemical substances that plants produce have therapeutic benefits in the treatment of ailments. Different plant parts with beneficial chemical constituents have been extracted and biologically tested to demonstrate their therapeutic potentials [1]. Additionally, the modern medications utilized in conventional treatment have been derived from plants [2]. Therefore, it is not surprising that medicinal plants are widely used in the treatment of a variety of illnesses, such as measles, hepatitis, arthritis, rheumatism, burns, scalds, abdominal colic, peptic ulcer, diarrhea, and dysentery [3]. Proper bioassays need to be conducted to establish the biological activity shown by plant extracts as simple isolation and elucidation of chemical structures of plant extracts may not be enough to identify the medicinal

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importance of the plants [4].

Because of its benefits to healthcare, medicinal plants have been the subject of scientific study in many nations. Exploring methods for collecting the necessary plant materials and analyzing their constituents has been sparked by the ongoing interest and quest for natural plant products that can be used as medications [5]. Many of the commercially successful medications used in contemporary medicine were first employed in undeveloped forms in conventional or folk medicine, or for other uses that revealed potential biological utility. The main advantages of employing plant-derived medicines are that they are generally safer than synthetic equivalents, provide significant therapeutic advantages, and are more cost-effective than other forms of therapy.

Syzygium aromaticum or cloves, is a plant in the Myrtaceae family that are indigenous to Indonesia's Maluku Islands. An evergreen tree with broad leaves and terminal clusters of crimson flowers, S. aromaticum can reach heights of 8 to 12 meters. The color of the flower buds changes from pale to gradually green to vivid red when they are ready for harvest. Cloves are picked when they are 1.5 to 2.0 cm long and are made up of four unopened petals that create a compact central ball and a long calyx that ends in four spreading sepals. Traditional healers have employed the plant *Syzygium aromaticum* to treat a variety of illnesses like toothache, a burning sensation in the tissue, pains in the body, and used to improve peristalsis [6].

Clove has been utilized in modern aromatherapy to treat illnesses like anxiety, sadness, sexual dysfunction, and weariness. It also works to balance hormones and tone the nervous system [7]. It is also employed in the flavoring of food and pharmaceutical products [8], as well as an antiseptic [9]. This study is consequently embarked upon with the intention of carrying out fractionation, antimicrobial screening, and characterisation of the bio-active chemicals on the methanolic extract of buds of *Syzygium aromaticum* in order to advance research on the pharmacological significance of the clove plant.

1.1 Statement of the problem

The use of different plant parts to cure illnesses is a global phenomenon that has been more prevalent in recent years as scientific evidence of herbal medicines' efficacy has become more publicly available [10]. In order to defend themselves from antimicrobial treatments, bacterial and fungal pathogens have developed a variety of defensive mechanisms. As a result, resistance to both old and new medications is increasing. The majority of medications, including antibiotics, are no longer effective against the intended pathogens. As a result, we see the development of bacteria that are resistant to antibiotics. The majority of conventional medications also cost a lot of money and have many negative side effects for the consumers. The cost of managing patients is very high, particularly in underdeveloped nations. Discovering and identifying new safe drugs without severe side effects has become an important goal of research in biomedical science.

The plant *Syzygium aromaticum* has been recognized as a potential treatment for human illnesses like toothaches, hormonal imbalances, sexual dysfunction, and exhaustion [7]. It has also been recognized as a significant source for the discovery of novel pharmacologically active compounds, from which many drugs can be derived either directly or indirectly [1]. In order to find the compound(s) responsible for the plant's numerous pharmacological and medicinal activities, it is therefore essential to advance the research on the plant by conducting spectroscopic analyses on the plant with reference to its methanolic extract, which is the motivation behind this study.

1.2 Aim and objectives of the study

The study's aim include methanolic extract fractionation, antimicrobial screening, and characterisation of the bioactive components from *Syzygium aromaticum* buds. The study's objectives are as follows:

1. To extract the bioactive components of *Syzygium aromaticum* using methanol
2. To fractionate the plant extract using n-hexane, ethylacetate and methanol
3. To determine the phytochemicals present in the different solvent fractions and to conduct the

antimicrobial screening of the fractions

4. To subject the pure compound of the most active fraction to spectroscopic analysis using infrared, mass spectroscopy and nuclear magnetic resonance with a view to ascertaining the structure of the compounds present.

1.2.1   Scope of the study

1. The fractionation of the methanolic plant extract of *Syzygium aromaticum* using n-hexane, ethylacetate and methanol.

2. The phytochemical screening of the different fractions of the flower buds of *Syzygium aromaticum* to determine the presence of alkaloids, tannin, glycosides, saponins, flavonoids and reducing sugars using standard analytical procedures.

3. Antibacterial screening of the solvent fractions using gram positive *Staphylococcus aureus* and *Bacillus subtilis,* and gram negative *Escherichia coli* and *Salmonella typhi*

4. Antifungal screening of the fungi *Aspergillus nigger* and *Candida albicans*

5. Spectroscopic analysis of the pure compound of the most active fraction using Infrared (IR), Mass Spectroscopy and Nuclear Magnetic Resonance (NMR).

6. Identification of the active chemical compounds from the flower buds of *Syzygium aromaticum* that exhibit highest bioassay actions against micro-organisms.

2. Methods

2.1.   Plant material:

The *Syzygium aromaticum* flower buds were acquired from the Orie Igbo-Eze Market in the Udenu Local Government Area of Enugu State, Nigeria. A taxonomist from the Department of Plant Science and Biotechnology at the University of Nigeria, Nsukka, Mr. Onyukwu authenticated the flower buds, and the voucher specimen was kept for reference in the departmental herbarium.

2.2   Preparation of plant sample

Syzygium aromaticum flower buds were cleaned before air dried for a week at room temperature. They were ground with a hand grinder. After that, the powder was kept in an airtight container for the remainder of the project.

2.3   Solvent extraction

Methanol was employed in the extraction process. In a 2L sterile bucket, 500g of the powdered plant material was steeped in 1000mL of methanol. Stirred, covered, and left to stand for 48 hours at room temperature. Whatmann No. 1 filter paper was used to filter the mixture, and the filtrate was concentrated using a rotary evaporator before being kept in a refrigerator at 40°C until needed. After extraction, 168.4g of stock extract were obtained.

2.3   Determination of extractive value (percentage yield) of the crude methanol extract

The value of the extract produced by evaporating a solution of the dried flower buds is known as the extractive value of *Syzygium aromaticum* flower buds. To do this, the methanol extract was evaporated in a conical flask with a specified weight, and the difference was measured after the evaporation. The crude methanol extract's extractive value was 33.68%.

2.4   Fractionation of the methanol extract using separating funnel

The methanol extract of the flower buds of *A. Syzygium* was fractionated using ethylacetate, n-hexane, and methanol. The solvents were utilized for the fractionation using separating funnel in the following order of increasing polarity: n-hexane, ethylacetate, and methanol. Following fractionation, the various solvent fractions were gently dried using a rotary evaporator and kept in a refrigerator at 40 degrees Celsius until needed. The stock fractions obtained were 4.93g for the ethylacetate fraction, 5.52g for the n-hexane fraction, and 6.08g for the methanolic fraction.

2.5   Preparation of stock solutions of the different solvent fractions

The stock solvent fractions were used to create the stock solutions for the three solvent fractions, which included 200 mg/mL concentrations of the methanolic, ethylacetate, and n-

hexane fractions. This was accomplished by dissolving 2g of each fraction in 10ml of sterile distilled water. They were clearly labeled and kept at 40°C until needed.

2.6 Preparation of test organisms

The Department of Microbiology, University of Nigeria Nsukka, provided the stock bacterial and fungi isolates that were used in the experiment. The isolate cultures obtained on agar slants were used to create new plates of the test organisms. The isolates were sub-cultured into new, sterile nutritional broth for bacteria and Saboraud Dextrose Agar SDA for fungus, and incubated for 24 hours at 37°C for bacteria and 25°C for fungi, respectively. By comparing the turbidity to the 0.5 McFarland turbidity standard, they were standardised. To regulate the turbidity of the microbial solution so that the quantity of microorganisms will be within a specific range, the McFarland standard was employed as a guide. The 0.5 McFarland standard was made by continuously swirling 9.95 mL of 0.18M H_2SO_4 with 0.05ml of barium chloride ($BaCl_2$) (1.17% w/v $BaCl_2.2H_2O$). The McFarland standard tube was kept in storage with a tight seal to prevent evaporation loss [11, 12, 13].

Colonies of the various isolated bacterial fresh cultures were selected using sterile wire loop, and they were then suspended in 5 mL of nutritional broth in sterile 10 mL bijou bottles with clear labels. They were incubated for 24 hours at 37^0C.

2.7. Determination of phytochemical constituents of the fractions

Chemical investigations of the various solvent fractions were conducted for the qualitative assessment of phytochemical contents as described by [14] and [15].

2.8. Determination of saponins

1 mL of the stock solutions (200 mg/mL) of each of the solvent fractions was added to five milliliters of distilled water before being heated. The mixture's soluble portion was heated up and then decanted into a test tube. The solution was used for the following tests:

2.9. Emulsion test

A test tube containing 1mL of the decanted solutions and two drops of olive oil was thoroughly shaken to see the emulsion.

2.10. Frothing test

A test tube containing 1mL of the filtrates and 3mL of distilled water was violently shaken to observe the presence of stable foam.

2.11. Determination of tannins

Acid test: Into a test tube, 3mL from the stock solution (200mg/mL) of methanol fraction was added to 2mL of 1% hydrogen chloride. The solution was observed for reddish brown ppt. The procedure was repeated for ethylacetate and n-hexane fractions.

Bromine water test: Into a test tube, 2mL from the stock solution (200mg/mL) of methanol fraction was added to 2mL of bromine water. The solution was observed for greenish-red colour. The procedure was repeated for ethylacetate and n-hexane fractions also.

Determination of alkaloids: 5mL of 2% hydrogen chloride acid were added to the methanol fraction (1mL) that was obtained from the stock solution of 200 mg/mL in the test tube. Whatmann No. 1 filter paper was used to filter the combinations after they had been heated in a water bath at 40^0C for 10 minutes. The following tests were performed on the filtrate:

Wagner's test: 1mL of Wagner's reagent was added to each filtrate (1mL) in a test tube. The mixture was thoroughly shaken, and a reddish-brown ppt color change revealed the presence of an alkaloid.

Meyer's test: 1 mL of Meyer's reagent was added to 1 mL of each filtrate in a test tube. After thoroughly shaking the combination, an assessment was done for the presence of an alkaloid-indicating cream color. The methanol fraction's alkaloid identification process was repeated for the ethylacetate and n-hexane fractions, respectively.

2.12 Determination of flavonoids

FeCl₃ test: To 1mL of methanol fraction got from 200mg/mL of the stock solution was added 1mL of 10% ferric chloride. The solution was mixed thoroughly and observed for colour change (green/black colour). This procedure was repeated for ethylacetate and n-hexane fractions respectively.

Lead acetate test: To 1mL of methanol fraction (from the stock solution of 200mg/mL) in a test tube was added 1mL of 10% lead acetate. It was mixed thoroughly. The mixture was observed for black colour or ppt, and the procedure repeated for n-hexane and ethylacetate fractions.

2.13 Determination of glycosides

Glycosides were measured by covering the powdered plant bud (1g) with adequate water in a 250mL conical flask. Picrate paper was suspended in the flask by a thread. The flask was heated for one hour at 40^0C in a water bath. It was noted that the picrate paper's color changed from yellow to brick-red.

2.14. Determination of reducing sugar

The amount of reducing sugar was determined by adding 7.5mL of Fehling's solution to 1g of powdered plant bud in a test tube. The mixture was heated in a water bath for 5 minutes at 40^0C while being watched for a change in color to brick-red.

2.15. Determination of preliminary antimicrobial activity of the different solvent fractions

Using the agar well diffusion technique, the antibacterial activity of the n-hexane, ethylacetate, and methanol fractions was assessed [16]. *Escherichia coli* and *Salmonella typhi* for gram positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis* for gram negative bacteria; *Candida albicans* (yeast) and *Apergillus nigger* (mold) for fungi; and a panel of organisms representing the different classes of microorganisms were used to test the fractions. Each of the bacterial isolates was seeded onto a nutrient agar plate with 0.1 mL of an overnight broth culture, whereas each of the fungal strains was seeded onto a sabouraud dextrose agar plate with a comparable amount. The seeded plates were given time to set before being dried. Eight uniform wells of 8mm in diameter were drilled into the agar's surface using a sterile cork borer.

To each of the agar wells, 0.1mL of each of the solvent fractions of the stock solution (200mg/mL) were added. For the bacterial strains' positive and negative controls, the 7th and 8th wells were filled with 0.1 mL of chloramphenicol (25 mg/mL), while the fungal strains' positive and negative controls were fluconazole (50 mg/mL) and sterile distilled water. For the pre-diffusion step, the plates were left on the bench for 40 minutes. Then, for bacterial isolates, an overnight incubation at 37°C and for fungi isolates, an overnight incubation at 25°C were performed. Each solvent fraction's level of antibacterial activity was quantified by measuring the inhibition zone diameter in millimeters. In triplicates, the sensitivity test was conducted. The zone of inhibition for the specific bacterial and fungal isolates at the given concentration was determined to be the average of the three values.

2.16. Purification of the fraction with the highest anti-microbial activity

Gradient-elution chromatography was used to further purify the solvent fraction with the best anti-microbial activity. For the chromatography, various n-hexane and ethylacetate solvent systems were employed. At the end of use of each solvent system, a thin layer chromatography (TLC) also at a solvent system of 80% n-hexane and 20% ethylacetate was carried out on the eluates to evaluate the presence or lack of compound(s) under a UV light. The Gradient-Elution chromatography process at different solvent systems was carried out until there was no further elution, which was confirmed by subjecting the eluate to UV lamp of the TLC. The eluates that showed identical peaks under the UV lamp were combined together and labelled.

2.17. Spectroscopic analysis of the compound

Spectroscopic analysis was carried out on the fraction with the highest antimicrobial activity to determine the structure of its active component(s). The spectroscopic analysis carried out were Infrared Spectroscopy (IR), Mass Spectroscopy, and Nuclear Magnetic Resonance (NMR).

3. Results and Discussions

3.1. Percentage yield and macroscopic characteristics

Table 1 below shows the extractive yield and macroscopic characteristics of the crude extract of the flower buds of *Syzygium aromaticum*. The percentage yield of the crude extract of the plant was 33.68%, while its macroscopic characteristics showed that it was a solid substance, brown and

sticky in texture.

Table 1. Extraction yield and macroscopic characteristics of the crude methanolic extract

Extract	Percentage yield (%)	Macroscopic characteristics
Crude methanolic extract	33.68	A brown sticky substance

Table 2 below shows the extractive yield and macroscopic characteristics of the three solvent fractions. The % yield of the n-hexane fraction was 32.70%, had a dark green colour with sticky mass texture. The % yield of the methanol fraction was 33.56%. It had a dark brown colour with molten mass texture, while the ethylacetate fraction has a percentage yield of 24.90%. It was an oily mass substance, with greenish colour.

According to the results of the various solvent fractions, the methanol extract of *Syzygium aromaticum* flower buds had the highest extractive value (33.56%), was followed by the n-hexane fraction (32.70%), and had the lowest yield (24.90%). This finding demonstrates that methanol, which is used as the solvent in the fractionation process, has greater extrinsic and fractionating power than n-hexane and ethylacetate.

Table 2. Extraction yield and macroscopic characteristics of the three solvent (n-hexane, methanol and ethylacetate) fractions

Extract	Percentage yield (%)	Macroscopic characteristics
n-hexane fraction	32.70	A dark green colour with sticky mass texture
Methanol fraction	33.56	A dark brown substance with molten mass texture
Ethylacetate fraction	24.90	An oily mass substance, greenish in colour

3.2. Phytochemical screening

The findings of the phytochemical screening of the various fractions of the methanolic extract of *A. syzygium* were displayed in Tables 3, 4, and 5 above. The findings demonstrated that tannins, alkaloids, flavonoids, and reducing sugars were present in all of the fractions. Saponins were only present in the ethylacetate fraction, and glycosides were only found in the n-hexane fraction. Since they extracted all phytochemicals except for one—glycosides for ethylacetate and saponins for n-hexane—these solvents were discovered to be effective for extracting phytochemicals. Saponins and glycosides were not extracted using methanol.

The chemical makeup of the *Syzygium aromaticum* flower buds' three solvent fractions is revealed by phytochemical screening, which can also be used to look for bioactive substances that could be used to create very beneficial medications [17]. According to the study's phytochemical analysis of *Syzygium aromaticum's* flower buds, tannins, flavonoids, glycosides, saponins, alkaloids, and reducing sugars are all present. It demonstrates that the solvent fractions of this plant all include tannins, alkaloids, flavonoids, and reducing sugars, whereas only the ethylacetate fraction and n-hexane contain saponins and glycosides, respectively.

The findings, which excluded saponins from the phytochemical content of the methanol and n-hexane fractions, did not accord with the findings of [18], but they did with those of [19]. The geographical locations of the plant may be responsible for this variation. Different phytochemicals have the ability to inhibit microbial growth in various ways; for instance, tannins can work by robbing microbial cells of essential proteins like enzymes, hydrogen bonding, or iron [20, 21].

Tannins are widely known for their diuretic, calming, anti-inflammatory, and anti-microbial activities, as well as their antioxidant and anti-microbial capabilities [22]. Tannin-containing plants are astringent in nature and are used to treat gastrointestinal conditions like diarrhea and dysentery [23]. This could also explain why *Syzygium aromaticum* is used as a traditional treatment for typhoid and digestive problems [24].

Only the ethylacetate fraction, which has the most anti-microbial activity, tested positive for saponins, which are responsible for several pharmacological activities [25]. The majority of the biological effects that have been seen are attributed to saponins, which are regarded as a vital component of traditional Chinese medicine [26]. They reduce cholesterol and have anti-diabetic, anti-carcinogenic, and anti-cancer activities [27]. Additionally, saponins have expectorant, antitussive, and hemolytic properties [15, 28].

The most revered phytochemicals, alkaloids, are thought to be pharmacologically active; they are believed to affect the autonomic nervous system, blood vessels, respiratory system, gastrointestinal tract, uterus, malignant illnesses, infections, and malaria [27]. Alkaloids also have antibacterial, analgesic, and antispasmodic properties [22]. All solvent fractions of plant material contain alkaloids, which have a characteristic poisonous nature that boosts their biological capabilities and their activities against cells of foreign species [21]. Additionally, flavonoids are said to be a key antibacterial component [29, 30], and are potent polyphenolic antioxidants [19].

Table 3. Phytochemical properties of methanolic fraction of flower buds of *Syzygium aromaticum*.

	Phytochemical test	Observation	Methanolic fraction
1.	**Tannins**		
	Acid test	Reddish brown precipitate observed	+
	Bromine water test	Greenish red colour observed	+
2.	**Saponins**		
	Frothing test	No thick persistent froth observed	-
	Emulsion test	No emulsion observed	-
3.	**Alkaloids**		
	Wagner's test	Reddish-brown precipitate observed	+
	Meyer's test	Cream colour precipitate observed	+
4.	**Flavonoids**		
	Lead acetate test	Black colour or precipitate observed	+
	$FeCl_2$ test	Green/black colour observed	+
5.	**Glycosides**		
	Picrate paper test	No brick red colour observed	-
6.	**Reducing sugars**		
	Fehling's test	Brick-red precipitate observed	+

+ = Present
- = Absent

Table 4. Phytochemical properties of n-hexane fraction of flower buds of *Syzygium aromaticum*.

	Phytochemical test	Observation	n-hexane fraction
1.	**Tannins**		
	Acid test	Reddish brown precipitate observed	+
	Bromine water test	Greenish red colour observed	+
2.	**Saponins**		
	Frothing test	No thick persistent froth observed	-
	Emulsion test	No emulsion observed	-
3.	**Alkaloids**		
	Wagner's test	Reddish-brown precipitate observed	+
	Meyer's test	Cream colour precipitate observed	+
4.	**Flavonoids**		
	Lead acetate test	Black colour or precipitate observed	+
	$FeCl_2$ test	Green/black colour observed	+
5.	**Glycosides**		
	Picrate paper test	Brick red colour observed	+
6.	**Reducing sugars**		
	Fehling's test	Brick-red precipitate observed	+

+ = Present
- = Absent

Table 5. Phytochemical properties of ethylacetate fraction of flower buds of *Syzygium aromaticum*.

	Phytochemical test	Observation	Ethylacetate fraction
1.	**Tannins**		
	Acid test	Reddish brown precipitate observed	+
	Bromine water test	Greenish red colour observed	
2.	**Saponins**		
	Frothing test	Thick persistent froth observed	+
	Emulsion test	Emulsion observed	
3.	**Alkaloids**		
	Wagner's test	Reddish-brown precipitate observed	+
	Meyer's test	Cream colour precipitate observed	
4.	**Flavonoids**		
	Lead acetate test	Black colour or precipitate observed	+
	$FeCl_2$ test	Green/black colour observed	
5.	**Glycosides**		
	Picrate paper test	No brick red colour observed	-
6.	**Reducing sugars**		
	Fehling's test	Brick-red precipitate observed	+

+ = Present
-   = Absent

Table 6. Sensitivity analysis of the solvent fractions at concentration of 200mg/mL.

	Test organisms	Inhibition Zone Diameter, IZD (mm)			Standards	
		Methanolic fraction	n-hexane fraction	Ethylacetate fraction	Fluconazole (50mg/mL)	Chloramphenicole (25mg/mL)
1.	*Escherichia coli*	--	7	17		27
2.	*Salmonella typhi*	--	7	15		21
3.	*Staphylococcus aureus*	--	--	--		19
4.	*Bacillus subtilis*	11	15	7		13
5.	*Candida albicans*	--	20	24	22	
6.	*Aspergillus nigger*	--	--	--	19	

Key: **--** = No inhibition

Flavonoids have been proven to prevent the peroxidation of polyunsaturated fatty acids in cell membranes by studies of [31, 32]. Additionally, research has demonstrated that flavonoids from the *Syzygium* genus prevent the production of hydroxyl radicals and superoxide ions, two potent peroxidation agents [33]. While heated caramels made of reducing sugars have astringent and poisonous effects, glycoside works on the heart muscles and increases renal flow (diuresis) [34].

3.3   Anti-microbial screening of the fractions

The findings of the preliminary/sensitivity study of the fractions at concentrations of 200 mg/mL were displayed in Table 6 below. The outcome demonstrated that only *Bacillus subtilis*, with an inhibition zone diameter (IZD) of 11mm, was sensitive to the methanolic fraction. With IZDs of 7 mm, 7 mm, 15 mm, and 20 mm, respectively, the n-hexane fraction was sensitive to *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Candida albicans*. Further analysis revealed that the ethylacetate fraction had IZDs of 17 mm, 15 mm, 7 mm, and 24 mm for Escherichia coli, *Salmonella typhi*, *Bacillus subtilis*, and *Candida albicans*, respectively. The findings indicated that none of the fractions were sensitive to the development of the bacterium *Staphylococcus aureus* and the fungus *Aspergillus nigger*.

Comparing the results of the antibacterial activities of the different solvent fractions to that of the standards (Fluconazole for fungi and Chloramphenicole for bacteria), it could be observed that methanolic (11mm) and n-hexane (15mm) fractions exhibited antibacterial activities against *Bacillus subtilis* that could be related to the antibacterial activity of Chloramphenicole (13mm) against *Bacillus subtilis*, while n-hexane (20mm) and ethylacetate (24mm) fractions exhibited

anti-fungal activities similar to Fluconazole (22mm) against *Candida albicans*. From the above result, the three solvent fractions could be compared to the standards (Chloramphenicole and Fluconazole) as their inhibition zone diameter (in mm) is almost the same as that of the standards.

3.4. Antimicrobial activity of the ethylacetate fraction

The ethylacetate fraction's antibacterial activity was displayed in Table 7 below at concentrations ranging from 200 mg/mL to 6.25 mg/mL. According to the results, the fraction exhibited antimicrobial effects on *Escherichia coli* at doses of 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, respectively. The inhibition zone diameters, or IZDs, of the fraction were 17 mm, 14 mm, 10 mm, 6 mm, and 4 mm. Additionally, the fraction inhibits *Salmonella typhi* at 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL, respectively, with IZDs of 15 mm, 11 mm, 9 mm, and 3 mm. Additionally, the ethylacetate fraction inhibited the growth of *Bacillus subtilis*, but only at high concentrations of 200 mg/mL and 100 mg/mL with IZDs of 7 mm and 5 mm, respectively. Conversely, the ethylacetate fraction had the highest inhibition against *Candida albicans*, with IZDs of 24 mm, 20 mm, 18 mm, 11 mm, 7 mm, and 5 mm at concentrations of 200mg/mL.

3.5. Antimicrobial activity of the n-hexane fraction

The antibacterial performance of the n-hexane fraction at concentrations ranging from 20 mg/ml to 6.25 mg/mL is shown in Table 8 below. The results revealed that the fraction had inhibitory effects against *Salmonella typhi* with IZDs of 7mm and 3mm at concentrations of 200mg/mL and 100mg/mL, respectively, but only at high concentrations of 200mg/mL and 100mg/mL with IZDs of 7mm and 4mm.

With IZDs of 15mm, 11mm, 9mm, 5mm, and 3mm at concentrations of 200mg/mL, 100mg/mL, 50mg/mL, 25mg/mL, and 12.5mg/mL, respectively, the n-hexane fraction likewise shown a significant inhibitory activity against *Bacillus subtilis*. With IZDs of 20mm, 17mm, 11mm, 9mm, 5mm, and 4mm at doses of 200mg/mL, 100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL, and 6.25mg/mL, respectively, the fraction showed the most inhibitory activity against *Candida albicans*.

3.6. Antimicrobial activity of the methanolic fraction

Table 9 below showed the antimicrobial result of the methanolic fraction. The result showed that the fraction had the lowest inhibition activity against the microorganisms with IZDs of 11mm, 8mm and 3mm at concentrations of 200mg/mL, 100mg/mL and 50mg/mL respectively against *Bacillus subtilis* only. From these results, it could be ascertained that the ethylacetate fraction had the highest antimicrobial activity, followed by n-hexane fraction and the least was methanolic fraction.

The ethylacetate fraction which has the highest anti-microbial activity was further purified using solvent system of 80% n-hexane and 20% ethylacetate. The resulting fraction was again tested against the test microorganisms and the result is shown in table 10 below.

3.7. Antimicrobial activity of the purified ethylacetate fraction

Table 10 below showed the antimicrobial activity of the purified ethylacetate fraction at 100mg/mL to 6.25mg/mL. The outcome demonstrates that the unpurified fraction and the purified fraction almost had the same activity. The antimicrobial results revealed that the fraction had inhibitory activities against *Salmonella typhi* with IZDs of 12mm, 11mm, and 4mm at concentrations of 100mg/mL, 50mg/mL, and 12.5mg/mL, respectively, while inhibiting the growth of *Escherichia coli* with IZDs of 15mm, 12mm, 8mm, and 4mm at concentrations of 100mg/mL, 50mg/mL, The purified ethylacetate fraction demonstrated significant inhibitory activity against *Bacillus subtilis* with IZDs of 9 mm, 7 mm, and 3 mm at concentrations of 100 mg/mL, 50 mg/mL, and 25 mg/mL, respectively. Conversely, it demonstrated its greatest inhibitory activity against *Candida albicans* with IZDs of 22 mm, 19 mm, 13 mm, 10 mm, and 5 mm at concentrations of 100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL respectively.

The n-hexane and ethylacetate fractions of the *Syzygium aromaticum* flower buds evaluated for antibacterial activity against disease-causing organisms showed substantial activity,

but the methanol fraction showed very little activity and exclusively against *Bacillus subtillis*.

Of the three solvent fractions, result showed that both n-hexane and ethylacetate fractions of *Syzygium aromaticum* had antimicrobial activity against all the microorganisms except *Aspergillus nigger* while the methanol fraction exhibited very little activity, and only against *Bacillus subtillis*.

The Syzygium aromaticum flower buds of n-hexane and ethylacetate fractions had little effect on *Aspergillus nigger* but had the strongest effect on *Candida albicans*, with zones of inhibition at 20 and 24 mm, respectively. The zone of inhibition for the methanol fraction's activity against *Bacillus subtillis* was barely 10 mm, and it had no effect on other microbes. This outcome relates to research on the effectiveness of *Syzygium aromaticum* flower buds against yeast microorganisms [35].

From the antimicrobial activity conducted, the ethylacetate fraction had the most inhibitory activity against the microorganisms used for the study, and thus the spectroscopic analysis and Gas Chromatography-Mass Spectroscopy (GC-MS) were carried out on it to determine the active compounds present.

3.8. Spectroscopic analysis of the purified ethyl acetate fraction

The result of the spectroscopic analysis of the ethylacetate fraction was interpreted as follows:

3.8.1. Infrared analysis

The IR result (Table 11) suggested that the isolated compound contains a carbonyl of ketone or aldehyde; a hydroxyl (OH) group band, an NH_2 band probably of an amide given the appearance of C = O band of amide at 1638cm-[1]. The fraction also contains an aromatic ring and aliphatic chains.

3.8.2. Proton Nuclear Magnetic Resonance (^{1}H NMR) analysis

Table 12 showed the results of the ^{1}HNMR of the isolated compounds. From the spectra obtained, signal at 1.29ppm indicated the presence of one hydrogen (1H, CH) singlet, while signal at 2.32ppm showed 3H of CH_3 singlet. Also,

signals at 3.40-3.33ppm showed 6H of CH_3 multiplet; 3.84ppm showed 3H of CH_3 singlet; 3.88ppm showed 4H of $2CH_2$ singlet which were hydrogen of cycloalkanes, while 5.15-5.06ppm depicted 12H of $4CH_3$ multiplet.

Signals at 5.58ppm showed hydrogen of NH_3 which is singlet and broad, while signals at 5.92 – 6.02ppm showed 5H multiplet which could be aromatic hydrogen; peaks at 6.72 – 6.07ppm depicted 2H multiplet of aromatic hydrogen and peaks at 6.81 – 6.77ppm and 6.88 – 6.86ppm showed 1H multiplet and 2H multiplet respectively, both of which were aromatic hydrogen, while signals at 6.98 – 6.96ppm depicted 1H doublet which could be aromatic hydrogen.

Furthermore, the Correlation Spectroscopy (COSY) result showed that the protons at 3.40 – 3.83ppm were coupled to protons at 6.72 – 6.70ppm, 6.02 – 5.92ppm, and 5.15 – 5.06ppm, while the protons at 6.72 – 6.70ppm were coupled to protons at 3.83ppm. Also, the protons at 6.02 – 5.92ppm were coupled to the protons at 5.15 – 5.06ppm, while the rest of the protons were not coupled.

3.8.3. ^{13}C - Nuclear Magnetic Resonance (^{13}C – NMR) analysis

Table 13 below showed the results of ^{13}C-NMR of the isolated compound. From the spectra obtained, peaks at 169.01 showed the presence of carbonyl (C=O) group; peaks at 151.20, 146.46, 143.91, 139.02, 137.84, 137.05, 131.91, 122.52, 121.18, 120.67, 116.15, 115.51, 114.29, 112.73, 111.14 showed the presence of 15 aromatic, alkenyl or quaternary carbons, signals at 77.39 – 76.76 indicated the solvent peak, while signals at 55.85, 40.09, 39.89, and 20.67 showed the presence of four aliphatic carbons.

3.8.4. Gas Chromatography - Mass Spectroscopy (GC-MS)

The GCMS data revealed compounds which were identified following their fragmentation patterns. The fragmentation pattern of 2-methoxy-4-(prop-2-enyl) phenyl ethanoate is shown in figure 1.

The DEPT result further simplified the ^{13}C-NMR result as it showed that there were six quaternary carbons with signals at 169.01, 151.20, 146.46, 143.91, 139.02, and 131.91. The result further showed that there were eight C-H carbons with signals at 137.84, 137.05, 122.52, 121.18, 120.67, 114.29, 112.73,

111.14, while signals at 116.15, 115.51, 40.09, 39.89 indicated the presence of four CH_2 carbons and signals at 55.83 and 20.67 indicated the presence of two CH_3 carbons.

3.8.5. Structure Elucidation

Investigation of the pure ethylacetate fraction was done by the purification of the ethylacetate fraction over polyamide column and elution with solvent system of 80% n-hexane and 20% ethylacetate. The structure of the compounds present in the purified ethylacetate fraction were confirmed by interpretation and comparison of their spectral data.

Also, the fragmentation pattern of 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol is shown in figure 2.

Table 7. Antimicrobial activity of ethylacetate fraction.

Microorganisms	Inhibition Zone Diameters(mm)					
	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
Escherichia coli	17	14	10	6	4	--
Salmonella typhi	15	11	9	3	--	--
Bacillus subtillis	7	5	--	--	--	--
Candida albicans	24	20	18	11	7	5

Key: --   = No inhibition

Table 8. Antimicrobial activity of n-hexane fraction.

Microorganisms	Inhibition Zone Diameters(mm)					
	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
Escherichia coli	7	4	--	--	--	--
Salmonella typhi	7	3	--	--	--	--
Bacillus subtillis	15	11	9	5	3	--
Candida albicans	20	17	14	11	8	6

Key: --   = No inhibition

Table 9. Antimicrobial activity of methanolic fraction.

Microorganisms	Inhibition Zone Diameters(mm)					
	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
Bacillus subtillis	11	8	3	3	--	--

Key: --   = No inhibition

Table 10. Antimicrobial activity of the purified ethylacetate fraction (at 100mg/mL concentration).

Microorganisms	Inhibition Zone Diameters (mm)				
	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
Escherichia coli	15	12	8	4	--
Salmonella typhi	12	11	4	--	--
Bacillus subtillis	9	7	3	--	--
Candida albicans	22	19	13	10	5

Key: --   = No inhibition

Table 11. Infrared spectroscopy interpretation of the purified ethylacetate fraction.

Infrared Spectroscopy bands (cm-1)	Possible functional groups
3516, 3455	OH, NH_2
3177, 3077, 3003	C-H of aromatic ring
2842	C-H of aliphatic ring
1764	C=O of a ketone or aldehyde
1638	C=O of amide
1231, 1196, 1148, 1120, 1032	C-N, C-O

Table 12. Proton NMR (^{1}H NMR) (δ or ppm) interpretation of isolated compound.

1H NMR (δ or ppm)	Interpretations
1.29	s, 1H, CH
2.32	s, 3H, CH_3
3.40 – 3.33	m, 6H, CH_3
3.84	s, 3H, CH_3
3.88	s, 4H, $2CH_2$ of cyclo alkanes
5.15 – 5.06	m, 12H, $4CH_3$
5.58	s, broad, NH_3
6.02 – 5.92	m, 5H, ArH
6.72 – 6.70	m, 2H, ArH
6.81 – 6.77	m, 1H, ArH
6.88 – 6.86	m, 2H, ArH
6.98 – 6.96	d, J = 8.0 H_2, IH, ArH

Table 13: ^{13}C - NMR interpretation of the isolated compound.

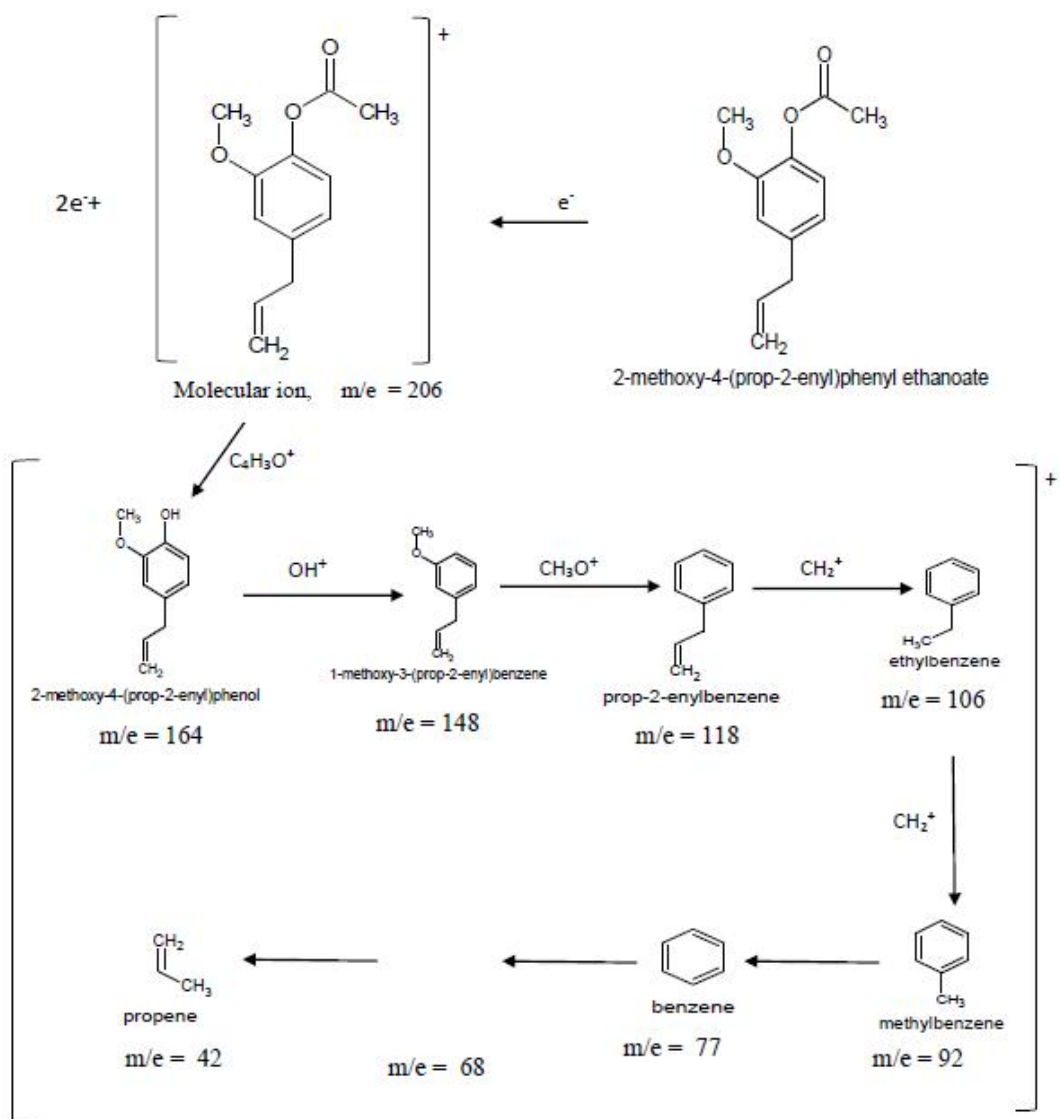| ^{13}C – NMR	Interpretations
169.01	C = O
151.20, 146.46, 143.91, 139.02, 137.84, 137.05, 131.91, 122.52, 121.18, 120.67, 116.15, 115.51, 114.29, 112.73, 111.14	15 Aromatic, alkenyl or quaternary carbons
77.39 – 76.76	Solvent peak ($CDCl_3$)
55.85, 40.09, 39.89, 20.67	Four aliphatic carbons
DEPT Interpretation	
169.01, 151.20, 146.46, 143.91, 139.02, 131.91	Six Quaternary carbons
137.84, 137.05, 122.52, 121.18, 120.67, 114.29, 112.73, 111.14	Eight C – H carbons
116.15, 115.51, 40.09, 39.89	Four CH_2 carbons
55.83 and 20.67	Two CH_3 carbons

Figure 1. The GCMS fragmentation pattern of 2-methoxy-4-(prop-2-enyl) phenyl ethanoate
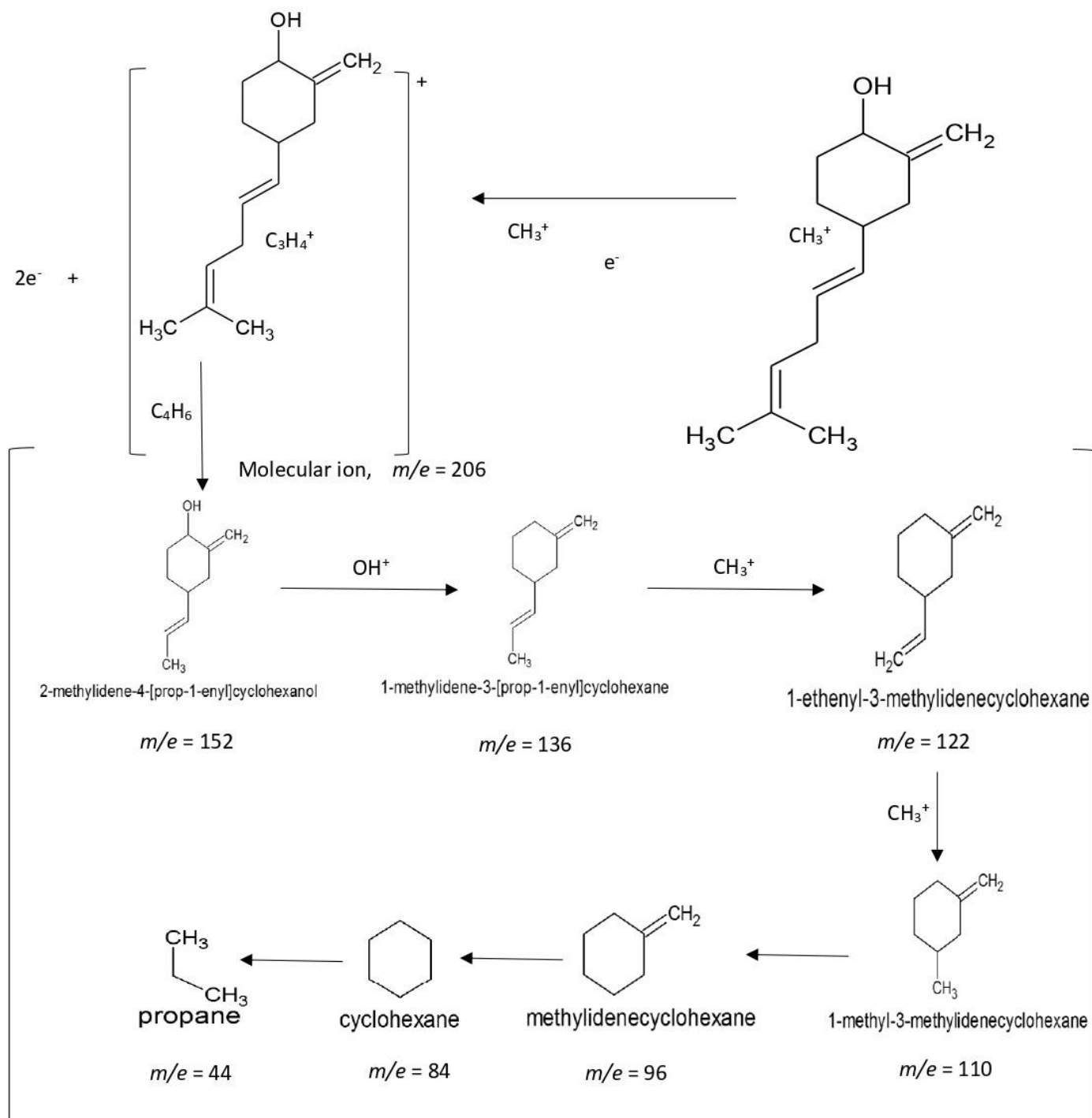
Figure 2. The GCMS fragmentation pattern of 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol

After analyzing the spectral data of the purified ethylacetate fraction, the compounds present were Aceteugenol with IUPAC name of 2-methoxy-4-(prop-2-enyl)phenylethanoate and 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol. These compounds were authenticated using their fragmentation pattern which agrees with their structures as was revealed by the Gas-Chromatography-Mass Spectroscopy carried out on them. Based on the total spectral analyses done, the structures of the compound are as shown in figure 3.
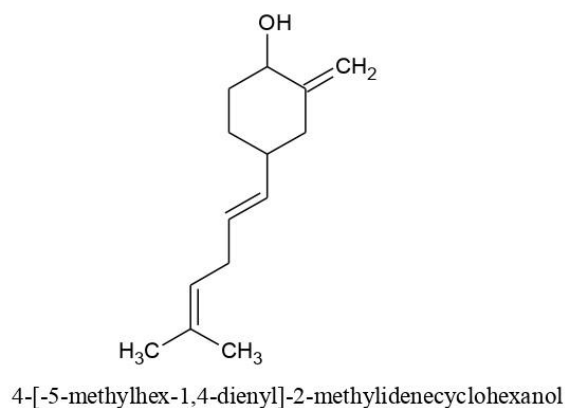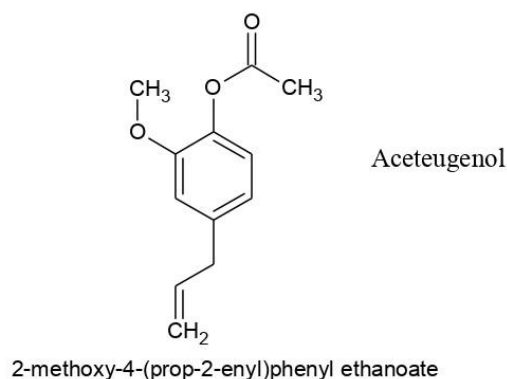

Aceteugenol

2-methoxy-4-(prop-2-enyl)phenyl ethanoate

4-[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol

Figure 3. Structures of 2-methoxy-4-(prop-2-enyl)phenyl ethanoate and 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol

The discovery of these compounds, 2-methoxy-4-(prop-2-enyl)phenyl ethanoate and 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol, as well as their inhibitory activities against microorganisms, support previous research on the benefits of Syzygium aromaticum cloves for oral health by

authors like [36]. Their research showed that Syzygium aromaticum extracts had analgesic, anti-inflammatory, and biocidal properties against A. albopictus (tiger mosquitos), which aid in the prevention and treatment of malaria. The study's findings also agree with those of [6], which claimed that eugenol, which is present in clove oil from Syzygium aromaticum and has antioxidant properties, prevents cancer.

The study's finding is also in line with the study on the antibacterial properties of spices and herbs done by [37]. The research showed that *S. aureus*, *L. monocytogenes,* and *C. albicans* are all inhibited by Syzygium aromaticum extract, particularly clove oil. Additionally, eugenol, which has antibacterial, antifungal, anti-inflammatory, insecticidal, and antioxidant properties, is highly concentrated in *Syzygium aromaticum* flower buds, according to a study by [38] on the constituents of the essential oil from the plant's leaves and buds, and it is traditionally used as a flavoring agent and an antimicrobial.

This study therefore supports and justifies the traditional uses of Syzygium aromaticum flower buds for treating a variety of diseases. This is in line with the finding made by [38] who investigated the antibacterial activity of *Syzygium aromaticum* flower buds and came to the conclusion that the main ingredient in clove oil, eugenol, is widely used in folk medicine as an analgesic, anti-vomiting, antispasmodic, kidney-enhancer, antiseptic, diuretic, and aromatic agent [39].

4. Conclusion

The natural world contains herbs in abundance. Natural elements found in plants have the potential to improve health. According to the results of the current investigations, the antimicrobial properties of Syzygium aromaticum flower buds may result from the combined or individual effects of the phytoconstituents identified. This conclusion is further supported by extensive studies, the findings of which revealed that the phytochemicals present in the various solvent fractions of the plant include tannins, alkaloids, flavonoids, reducing sugars, saponins, and glycosides. As it inhibited the growth *of*

Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, and *Candida albicans* at a concentration of 200 mg/mL, the plant's ethylacetate fraction exhibited the highest antimicrobial activity against the test organisms. Its spectroscopic analysis using infrared spectroscopy, nuclear magnetic resonance, and mass spectroscopy for structural elucidation of the active compounds responsible for the antimicrobial inhibition revealed the presence of two compounds; 2‑methoxy‑4‑(prop‑2‑enyl) phenylethanoate and

4[‑5‑methylhex‑1,4‑dienyl]‑2‑methylidenecyclohexanol.

The antibacterial properties of this plant brought to light the significance of the extracts in conventional medication formulations. Further investigation should be done to determine how the two compounds obtained from the ethylacetate fractions can be produced in large quantities without incurring much cost for their efficient integration into precursors used for drug production. This is because they inhibited the growth of some microorganisms.

Conflict of Interest

The authors declare no conflict of interest.

Authors' credit statement

Agu, C.L. conducted the research work, wrote the manuscript and has the main idea. Omeje N.O. revised the manuscript and provided suggestions.

Acknowledgement

Profound gratitude goes to Professor I.O. Okerulu of the Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria for providing guidance and intellectual instructions needed to carry out this research.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Journal of Chemistry and Environment

Evaluation of Heavy Metals in Drinking Water of Tribal Districts Ex-FATA Pakistan

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Abstract

This study was conducted to evaluate the heavy metals such as zinc (Zn), iron (Fe), copper (Cu), nickel (Ni), chromium (Cr), and cadmium (Cd) in seven water samples which were collected from Ex-FATA Pakistan (Bajaur, Mohmand, Khyber, Orakzai, Kurram, South Waziristan and North Waziristan). All samples were digested using the wet digestion method and the digested samples were analyzed for heavy metals using an atomic absorption spectrophotometer. The results of water samples from seven districts were compared to the recommended standard value from the World Health Organization and the Environmental Protection Agency. The results obtained from the analysis for nickel (Ni) showed that the highest concentration (0.093 mg/l) was reported in the water of the Khyber district, while the lowest concentration (0.011 mg/l) was found in the water of the Orakzai district. Iron (Fe) highest concentration (0.86 mg/l) was found in the water of the district Mohmand which was slightly high than the WHO recommended value. The highest concentration (0.19 mg/L) of chromium (Cr) was reported in the water of the Orakzai district. furthermore, the result showed that the highest concentration (0.87 mg/l) of zinc (Zn) was in the Orakzai district, the highest concentration (1.92 mg/l) of copper (Cu) in Khyber and Mohmand districts (1.92 mg/l), while the highest concentration (0.0029 mg/l) of cadmium (Cd) was measured in the water of Orakzai district. After the comparison of all these values to WHO and EPA standard values, this study shows that the water of all these districts is safe for drinking water purposes

Keywords: Drinking water, water quality, heavy metals, human health, environment

1. Introduction

Water is one of the naturally available resources that play a key role in the sustainability of life[1]. Pollutant-free water is a basic need for healthy human life. Toxic heavy metals present in water are very harmful to human health and all living organisms. Some of the heavy metals are required by the human body in small amounts, while their excess leads to dangerous effects on human life. The presence of some heavy metals is very toxic even in very small amounts[2]. Increasing

environmental pollution from pollutants is a major concern for local users. Numerous pollutants are regularly introduced into the aquatic environment, mainly as a result of increasing industrialization, technological advances, population growth, resource depletion, and runoff of household and agricultural waste[3]. Increasing environmental pollution from pollutants is a major concern for local users. Numerous pollutants are regularly introduced into the aquatic environment, mainly as a result of increasing industrialization, technological advances, population growth, resource depletion, and runoff of household

and agricultural waste[4]. Increasing environmental pollution from pollutants is a major concern for local users. Numerous pollutants are regularly introduced into the aquatic environment, mainly as a result of increasing industrialization, technological advances, population growth, resource depletion, and runoff of household and agricultural waste[5].

Historically, there have been reports of heavy metals in drinking water covering their types and amounts, as well as origins, human exposure, hazard, and distance. Despite enormous progress, research is still needed to obtain clean drinking water[6]. Due to their limited economic capacity, many low- and middle-income countries are particularly concerned about the problem of reducing small numbers of heavy metals below the proposed limits[7]. A region's medical problems can be found by routinely evaluating the drinking water quality. Because heavy metals are toxic, persistent, and bio accumulative, heavy metal contamination of drinking water and food has become a major concern for environmental professionals worldwide[8].

The drinking water contaminated with heavy metals has not yet been investigated in the study region. To determine heavy metal concentrations in drinking water from seven EX-FATA districts, this study was designed with population, geology, and anthropogenic inputs in mind, the heavy metal concentrations were examined for potential health risks.

2. Materials and Methods

2.1 Samples Collection

Drinking water samples were collected from seven districts of the Ex-FATA. Two water samples were collected at different points from each district and were mixed. The samples were stored in polythene bottles. The samples were collected in the districts showing in figure 1 and table 1.

2.2 Preparation Sample

The samples were taken to the laboratory where they were later digested. 10 ml of the sample, 5 ml of concentrated HNO3 and 5 ml of concentrated HCl were used for the digestion. This combination was left at room temperature for almost an hour after being gently stirred and covered with a watch glass. Then the samples were heated on a hot plate until yellow fumes were generated, and the solution became clear.

After cooling, a Millipore filter (0.4μ) was used to filter the acid solution and deionized water was used to bring the volume to 50 mL[9].
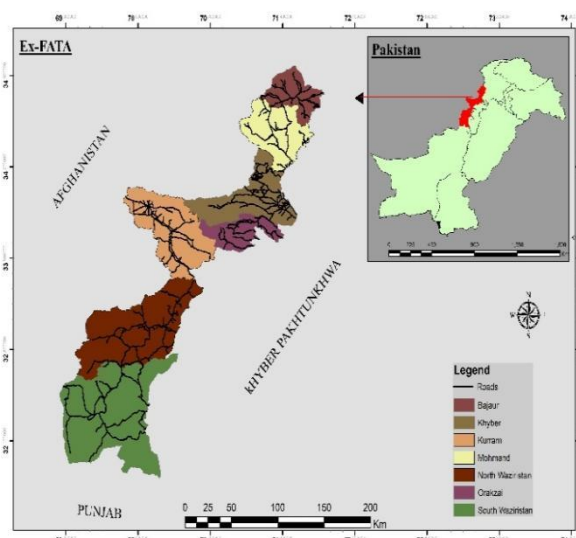

Figure 1. Map representing the districts where samples were collected.

Table 1. Districts and Locations of collected samples.

S.No	District	Location
1	Bajaur	Tehsil Salarzai
2	Mohmand	Tehsil Safi
3	Orakzai	Lower Orakzai
4	North Waziristan	Tehsil Miranshah
5	Khybar	Tehsil Bara
6	Kurram	Lower Kurram
7	South Waziristan	Tehsil Wana

2.3 Preparation of standards

Deionized water was used to dilute several prepared standards of each element (0.1g/100ml; Fisher Chemicals, U.K). Ultra-pure chemicals were used for the analytical analysis. Overall process of research are presented in figure 2.

2.4 Analysis of Heavy metals

Atomic absorption spectrometer (PerkinAnalyst 800 JAPAN) was used for the analysis of heavy metals such as copper (Cu), Iron (Fe), Cadmium (Cd), Chromium (Cr) , Zinc (Zn), and Nickle (Ni).
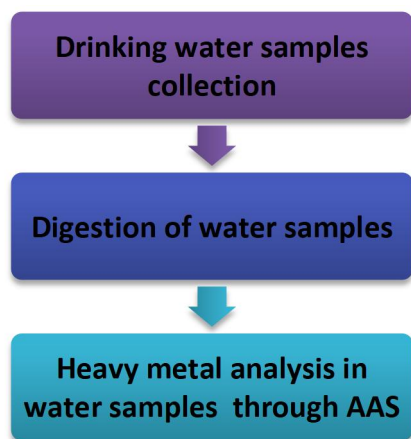
Figure 2. Overall process of research.

3.   Results and Discussion

3.1 Concentration of Nickel (Ni):

Nickel is a heavy metal found in the environment such as water, air, and soil. The main sources that generate nickel are the various industries, municipalities, fuels, and industrial effluents[10]. Excessive nickel consumption can cause various diseases in humans such as pulmonary fibrosis, lung cancer, cardiovascular disease, and kidney disease[11]. Figure 3 shows the total concentration of nickel in each water sample, ranging from 0.011 to 0.093 mg/L. The water sample from Khyber district showed the highest concentration with 0.093 mg/L, followed by the water from North Waziristan district and the water from South Waziristan with 0.09 and 0.07 mg/L nickel, which were below that from the WHO recommended value (0.1 mg/L) The lowest concentration of 0.011 mg/L was found in the water of Orakzai district, followed by Bajaur, Mohmand, and Kurram with 0.017, 0.019 and 0.049 mg/L.
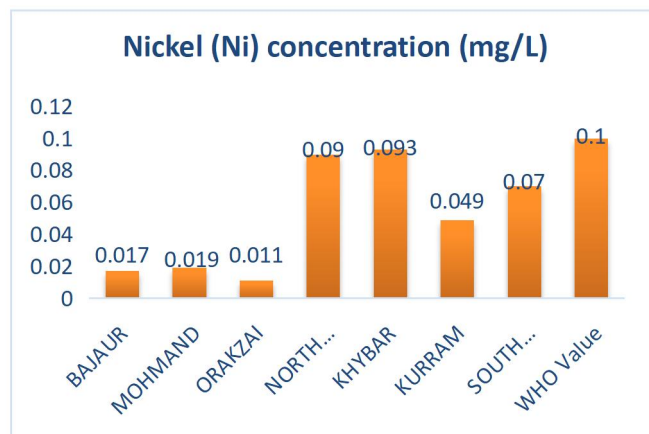

Figure 3. Concentration of Nickel (Ni) in each district water

samples.

3.2 Concentration of Iron (Fe):

In the human body, iron is one of the main elements that play a very important role in various reactions in the human body, such as intracellular and oxygen transport[13]. Iron deficiency can cause various types of diseases in humans, including anemia or iron deficiency, although the high levels of iron in water also have adverse effects on humans, leading to heart problems, liver disease, and diabetes[14]. The availability of iron in the soil is also important for plants as it plays an important role in the process of photosynthesis and chlorophyll[15], while the excess/lower iron content in the soil can cause direct damage to plants, including low-fat or protein content, disruption of root viability and cell damage[16]. The level of iron (Fe) in drinking water recommended by the World Health Organization is 0.3 mg/l[17].The results of Fig. 4 shows that all fifteen samples have Fe values ranging from 0.07 mg/L to 0.86 mg/L. The highest concentration, above the WHO recommended level (0.3 mg/L), was found in the drinking water of the Mohmand district (0.86 mg/L) and Orakzai district at 0.32 mg/L, while the lowest concentrations were found in the water of the district Khyber (0.07 mg/L).
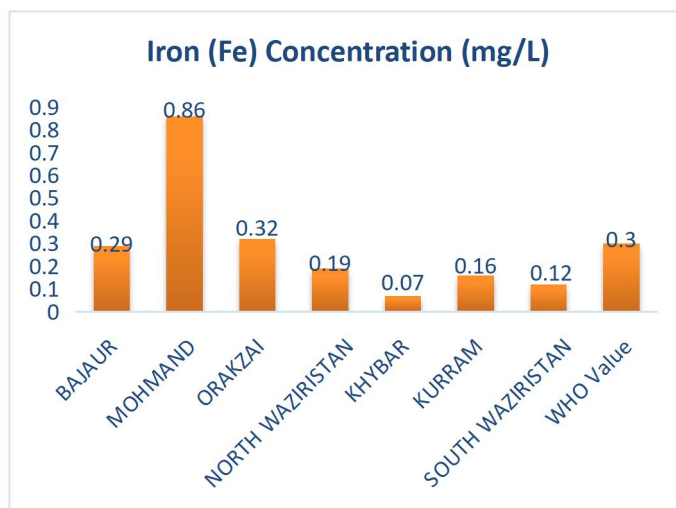

Figure 4. Concentration of Iron (Fe) in each district water samples.

3. 3 Concentration of Chromium (Cr)

Chromium is one of the important heavy metals that play an important role in enhancing the action of insulin and lowering the level of glucose in the human body[18], although its excess intake of chromium can lead to irregular heartbeats, headaches,

allergic reactions, kidney and liver damage[19]. The excess amount of chromium in the soil/water also has adverse effects on plant growth and other important metabolic processes that make plants toxic such as oxidative stress[20]. The WHO recommended level of chromium in water is 0.1 mg/L[21]. The results in Fig. 5 show that all of the water samples from the Orakzai district have a high value of 0.19 mg/l, followed by the water sample from the Mohmand district with 0.16 mg/l, which is slightly above the recommended standard value (0. 1 mg/l). The lowest concentration was recorded in Khyber District (0.07 mg/L), South Waziristan Water District (0.08 mg/L), Bajaur District (0.09 mg/L), and Kurram District (0.1 mg /L) detected.
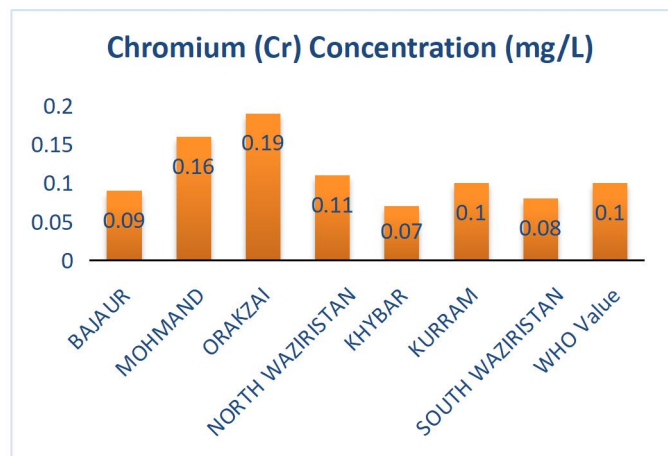

Figure 5. Concentration of chromium (Cr) in each district water samples.

3.4 Concentration of Zinc (Zn):

Zinc is one of the vital heavy metals that play a key role in both humans and plants[22]. Zinc is most abundant in foods that are primarily of animal origin[23]. Low zinc intake can lead to various types of diseases such as wound healing disorders and hypogonadal dwarfism. After taking zinc, it accumulates very quickly in different parts of the body[24]. Excessive intake of zinc has negative effects on humans and plants[25]. The recommended standard value for zinc is 3 mg/L, this standard value is the same for both surface water and groundwater[26]. The results in Fig. 6 show that the concentration value in all water samples is below the recommended standard value, which is between 0.018 mg/L and 0.087 mg/L. The lowest concentration was found in the water of the Bajaur district (0.018 mg/l), while the highest concentration was reported in the water of the Orakzai district

(0.087 mg/l).

Figure 6. Concentration of Zinc (Zn) in each district water samples.

3.5 Concentration of Copper (Cu):

Copper is one of the most important essential trace elements that play a vital role in the human body[27]. In humans, copper plays an important role in the absorption of iron, the formation of red blood cells, and the maintenance of the immune system and nerve cells[28], while too high or too low levels of copper can cause severe damage to the brain, heart, and kidneys in humans[29]. According to the WHO, the recommended standard range for copper water is 2 mg/L. The result in Fig. 7 shows that the copper concentration in all water samples was below the recommended standard value.
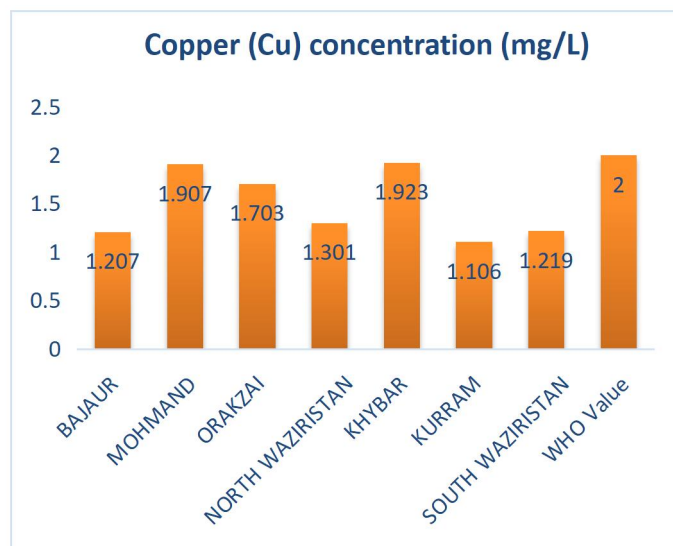

Figure 7. Concentration of Copper (Cu) in each district water samples.

3.6 Concentration of Cadmium (Cd):

Cadmium is one of the toxic heavy metals that are toxic to humans in the long and short term[30]. Intake of cadmium in food and water can cause severe harmful effects on humans like intestinal diseases and kidney damage [31]. According to the world health organization, the recommended safe level of cadmium in water is 0.003 mg/L [32]. The results in Fig. 8 show that the cadmium levels in all water samples are below the WHO-recommended standard level of safe drinking water.
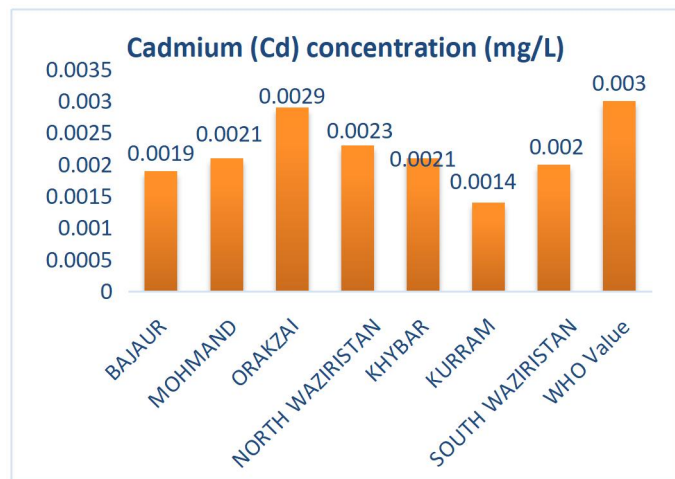

Figure 8. Concentration of Cadmium (Cd) in each district water samples.

4. Conclusion

This study was conducted to evaluate the concentration of six heavy metals in drinking water from Ex-FATA seven districts (Bajaur, Mohmand, Orakzai, Kurram, Khyber, South Waziristan, and North Waziristan). Drinking water samples from the groundwater of the tested sites, after careful examination and comparison with the recommended WHO standard values, the results of this study show that the water in these areas is not carcinogenic and is suitable for both drinking and agricultural use.

Authors Contribution

R.U and M.S supervised the research work, wrote the Manuscript, and has the main idea and NN, AN, KA and KB helps in lab work and samples collection. HF, ZAS, MNA, YA, revised the manuscript and provided suggestions

Conflicts of Interest

There are no conflicts of interest reported by the writers.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

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Journal of Chemistry and Environment

Advancement and Future Perspectives of Prostate Cancer Treatment by Using Plant Bio-actives: A Review

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Abstract

Prostate cancer (PCa) is the world's second most lethal and hateful disease in people. Even while chemotherapy medications have made considerable progress against cancer disease, the body still has to deal with their toxic side effects. In order to produce anticancer medicines with the lowest cost and treatment time, mostly people are using mechanistic techniques. In addition to chemotherapy advanced treatment techniques are also used in clinical practices, and they have an excellent healing results by enhancing patient survival rates. The social care net faces serious challenges because of the lack and high cost of modern therapeutic techniques. The side effects of chemotherapeutic drugs and expensive advance techniques triggered the patient interest towards phytochemicals drugs which indicate that nature always attracts human to fulfill their medical needs at very low cost.

The pharmaceutical industries are showing strong interest in recent research, which has led to the addition of a quite large number of phyto-medicines in PCa therapeutic practices. Currently, several experimental epidemiological and clinical research reports confirmed that plant bio-actives play a significant role in PCa prevention by using different mechanistic ways such as suppressing adhesion, anti-angiogenesis, pro-apoptosis, anti-proliferation, invasion and migration. This review systematically highlighted various strategies to treat PCa and advances in research by using different bioactive plant extracts and isolated components that have been tested for PCa therapy along with corresponding clinical and epidemiological studies.

Keywords: Prostate Cancer, Phytochemicals, Bio-actives, Treatment, Radiotherapy.

1.   Introduction

The prostate is the glandular organ composed of epithelial cells found under the bladder in a fibro-muscular network [1]. Globally, prostate cancer (PCa) is a common fatal disease in men, and the second lethal disease in western countries. It may be asymptotic in the early stages of PCa [2, 3]. It is a complex and heterogeneous type of malignancy that can be aggressive or non-aggressive early-onset or indolent, high or low grade PCa [4]. However, in Asian countries the relative ratio of PCa is less than the western countries, but its occurrence and mortality rate is rapidly increasing by adopting the western life style and the exchange in the socio-cultural life [5]. The PCa treatment includes many different methods such as chemotherapy, radiotherapy and surgery. The chemotherapy is more effective at the early stage of tumor development which induces side effects. When the tumor is spread from their original site to the other body parts, all the treatment is ineffective and form some metastatic tumors in the body. The metastasis stage is highly resistant to treatment tactics as a result death ratio increases among

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the patients [6, 7]. However, PCa can be treated via radiotherapy define as the treatment through x-ray's or similar form of radiations and can be applied either external or internal, but ensure that the method is complex and highly expensive [8]. Although, advancement in the radiotherapy leads to many of the targeted radionuclide such as Lutetium 177 (Lu) labelled PSMA peptides (a molecular biomarker) that exhibits the high tolerance in men with prostate-cancer and low toxicity profile [9-11]. Meanwhile, surgery is the only effective method when PCa tumor is localized in early diagnosis.

Epidemiological studies show that cancer can be controlled by intake of more fruits and vegetables. Several reports highlighted that the chances of death occur due to cancer is reduced up to 75% by taking nutrients rich food and drinks (Figure 1). Why fruits and vegetables is essential for the control and treatment of cancer? The fruits and vegetables consists of several biomolecules known as phytochemicals (i.e. polyphenolics, terpenes, carotenoids, alkaloids, anthocyanin's etc), they shows simultaneous targeting multiple neoplastic eventuality by preventing the damage of DNA, inhibiting proliferation of malignant cells, modulation inflammation, so that reducing the overall risk of cancer [6, 12]. Scientific community are now moving towards the herbal or natural treatment strategies for the development of safe and effective treating ways of malignancy, due to the adverse side-effects of the chemotherapy and the other treating methods. Ancient literature studies and current epidemiological review are directed the researchers to focus on the treating ways by using phytochemicals, because these are natural, easily acceptable and have minimum side effects [13]. Further, it is estimated that over 60% anti-cancer agents currently used are extracted from natural sources [14]. The increasing attention for chemoprevention by phytochemicals is just because that they have chemical diversity, essential biological activity, easily available,

affordable, and less toxic effects [15]. Meanwhile, with the development of technology it is estimated that many novel natural components from the medicinal plants will be recognized and developed as anti-cancer agents [16, 17]. This review highlights the advances in PCa treatment by using plant bio-actives as the chemo-preventive and anti-inflammatory agents. We mainly focus and analyzed the past ten years' work that was reported on the treatment of the PCa by bioactive components (i.e. tanshinones, biochanin-A, oleuropein, anthocyanins) from different medicinal plants.

Figure1: Estimation of poor diet associated with cancer deaths. Intake of natural plant based diet (fruits, vegetables etc.) has been proved to significantly reduce the cancer risk. Reproduced with permission: Copyright 2019, MDPI [6].

2. Prostate Cancer (PCa) Treatment

Prostate cancer is the most commonly occurring cancer in men. It is the most lethal malignant disease in people whose symptoms started at the base of the bladder in prostate gland. It surround the proximal part of the urethra that brings the urine from bladder [18]. Geographically, the disease does not show the same symptoms and affects in people across the world, but it shows variation among patients based on their regions or continents. For example,

Asian cancer effected person having less incidence rate of PCa than white and black of Americans. In America and Africa it is reported that 37% of the death occur due to cancer in 2013 [19]. According to UK researchers, approximately and above 40,000 people are effected with this lethal disease every year. National Institute for Health and Care Excellence (NICE) describes PCa as slow growing disease, and hence many of the person may not die due to this disease in their lifespan. In spite of the slow growing localized PCa that is not prove to be fatal, metastatic PCa after multimodal therapy largely remains incurable. About 80% of the newly diagnosed PCa cases are localized, while remaining are the adversely metastatic or advanced. There are various treatment strategies are applying for the treatment of PCa including active surveillance, surgery, radiation therapy, high intensity focused ultrasound, immunotherapy, vaccine treatment, targeted therapy and bone directed treatments etc. [20-23].

2.1 Risk factors of PCa

As PCa is a heterogeneous malignant disease that's why its causality varied from patient to patient even when they have the same type of tumor [24]. Main risk factors that may become the reason of PCa occurrence and associated with treatment, mortality or survival are mainly divided into two categories that is adaptable and non-adaptable risk factors [25]. Some of the main non adaptable risk factors for the PCa to be occur are age, race, ethnicity, geography, positive family history (for example one's first degree relatives) and genetics. Meanwhile, several of other adaptable risk factors such as obesity, smoking, may not the reason of its incidence but may become the booster of PCa mortality. Epigenetic changes i.e. life style ascribe approximately 90%, while somatic or epigenetic changes may attribute 10% or less for the cause of PCa. Similarly, inflammation is the evident process that associates cancer with the main risk factors [26-29]. Physical activities, infectious diseases, external and occupational exposure, endogenous hormones are also

some of the adaptable PCa risk factors[30].

2.1.1 Age

PCa possibly occurs among the older men with the age of about 66 years old. Probability of PCa occurrence is increasing with the age, 5% men with age 60-69 years and 7.69% men with age ≥70 years are diagnosed. PCa is the third leading cause of death in men with the age 60-79, and at second with the men age ≥80 years [31, 32]. It has been observed that over the age of 50 years risk factors increases in white men with no PCa family history, and in black men over the age of 50 having PCa family history [33].

2.1.2 Race, Ethnicity and Geography

Globally, the occurrence of PCa is vary on the basis of geography. In developed countries of the world such as Western and North Europe, North America and Australia, the rate of PCa is more than the rest of the world. The developed counties have the facilities for its early detection, screening and medical cares. In contrast death rate associated with PCa is highest in ancient African. Based on population it was reported i.e., GLOBOCAN, 2012, Sub-Saharan African (SSA) and Afro-Caribbean (AC) people are suffered from the world highest death rate due to PCa with the rate 18.7-29.3 deaths per 100,000 [29]. Main reason of these differences of PCa between the countries has not clear entirely. It may have attributed to prostate specific antigen (PSA) testing the main reason of the worldwide variations of PCa incidence. Recent research demonstrated that approximately 20-40% of the PCa cases in Europe and USA may because of the over diagnosis via extensive PSA testing [3].

However, race is actually not associated with the survival after local treatment in metastatic PCa patients. The radiotherapy treated patients of Caucasian race are more associated with the higher cancer-specific mortality and overall mortality than that of the African American race. In the study 2004-2014, people with newly diagnosed metastatic PCa 408 (77.2%) Caucasians and 121 (22.8%) Africa-American's were treated with local-therapy: radiotherapy (n=357) or (n=172). When local therapy

includes radical prostatectomy then the Caucasian race patients demonstrates comparatively higher survival vs. African American's: cancer specific mortality free survival 123 vs. 63 months (p=0.004) and overall mortality free survival were 108 vs 46 months (p=0.002). Thus, it is evident from the foregoing details that when radical prostatectomy is used as local therapy, there will be no racial differences in cancer-specific mortality or total mortality [34].

2.1.3 Family history and Genetics

Family history of PCa is strongly associated with the increased risk of PCa incidence and also the higher mortality rate. A recent analytical study reveals the relationship between the PCa incidence and that of the positive family history of PCa are highly associated with the increased risk of PCa in overall cohort of PCa patients and also the PCa risk in non-screened sub-cohort. From the total 74,781participants, 5281 participants were having the first degree relative (FDR) positive family history and 69,500 participants was without any positive family history. From the participants having no family history of PCa in FDR, PCa were to be diagnosed in the total of 7540 participants' (10.5%). However, the patients having the family history of PCa in FDR are 889(16.5%) from the total are diagnosed [35].

Mutations play a major role in PCa carcinogenesis, as family history and race are linked to PCa incidence and mortality. Family history helps identify PCa-prone genes. Many of studies have identified some of the susceptible genes that are RNASEL, ELAC2, MSR1 and HOXB13. Among them HOXB13 found to be the gene play significant role in the PCa development that is actually the homeo-box transcription factor gene [36]. Increased risk factors for PCa has been notably demonstrated by the BRCA2 and HOXB13 mutations and observed more commonly in early onset PCa diagnosis among the patients. It has been shown that HOXB13 recurrent mutation leads to the hereditary PCa [37].

2.1.4 Obesity

Weight gain or obesity is highly associated with the increased risk of fatal and advanced level of PCa. Metabolic changes that are concerned with obesity or weight gain may become the reason for PCa development [38]. Obesity may play its role as a factor that leads to the less likely early stage PCa diagnosis in the obese patients. Physical examination, laboratory test and imaging process may hindered due to the adiposity [39]. A quantitative study on the relationship between obesity and PCa had done. In cohort studies total of the 3,569,926 individuals were selected in 17 studies takes up to the result that obesity was not as such associated with the PCa incidence. However, further analysis provides with the evidences that obesity were significantly associated with the high risk of PCa aggressiveness [40, 41].

2.1.5 Smoking

Smoking increases PCa incidence and death. Cigarettes' mutagens may induce prostate tumorigenesis [42]. Smokers PCa risk factors rise, leading to PCa-related death. Many studies have linked aggressive-violent PCa to smoking at diagnosis [43]. A quantitative cohort study elaborated the PCa specific mortality associated with smoking, leading to the results that PCa patients with current smoking are at high risk of PCa specific mortality compared to non-smokers, and this increased risk was also partially attributable to tumor characteristics [44]. Recent studies also provides with the evidence that smoking at the time of diagnosis enhance the risk for PCa specific and all-cause mortality [45].

3. PCa therapeutic strategies: A brief summary

PCa treatment includes many different methods such as chemotherapy, radiotherapy, immunotherapy, hormone therapy, and surgery. In the following sections we briefly explain all these types of clinically practiced strategies.

3.1 Chemotherapy

Chemotherapy kills cancerous cells by stopping their replication. Chemotherapeutic agents may cause cell death by producing reactive oxygen species, necrosis or apoptosis of malignant cells, or influencing cell proliferation enzymes. Chemotherapeutic agents are

categorized by their chemical structure, mode of action, and interaction with other drugs, such as alkylating, cross-linking, intercalating, DNA cleaving, and anti-tubulin agents. Treatment by chemotherapy has been used by targeting the escalation potential and metastatic ability of the cancerous tumor cells. Present use of chemotherapy involves DNA interactive agents (doxorubicin, cisplatin), anti-tubulin agents (taxanes), antimetabolite (i.e. methotrexate), molecular targeting agents and hormones [46, 47].

In an analytical study exhibits the results that N-acetylated α-linked acidic dipeptides (NAALADase) activity of PSMA expressing cells can be inhibited by the nanoparticle conjugate forming a dendrimer G5 PAMAM while they bind specifically to these cells. In nanoparticle conjugate the drug methotrexate and a small targeting agent molecule glutamate urea conjugated through the serum stable amide links to a dendrimer G5 PAMAM. In vitro study of this target showed the more cytotoxic behavior towards LNCap cells as compared to PC-3 cells. Maximum of the inhibition of cell growth was reached approximately at 300nM that was about 50% for the conjugate and 70% for the free methotrexate [48]. Despite progress in developing strong chemotherapeutic drugs, their toxic effect on normal body cells (such as hair loss) and adverse concomitant in multiple organ systems (i.e. gastrointestinal lesions, neurological dysfunction, bone-marrow suppression) are major impediments to their successful clinical use. Cytotoxic agents decrease the life of cancer patients, resulting in dire complaints and long-term effects on survivors. Toxicity limits the usefulness of anti-cancer agents, which is why patients stop treatment [13, 47].

3.2 Radiotherapy

Radiotherapy is a physical process in which ionizing radiations are used for the destruction of cancerous cells. Energy from the ionizing radiations passing through the cancerous cells in turn alters the genetic structure and hence blocking their ability to proliferate. In radiotherapy the main goal is to maximally destroy the cancerous cells by the high energy radiation without affecting the adjacent normal cells. Despite of all the development in radiation therapy, the main drawback of it that normal cells are also damaged along with the cancerous cells. Prostate cancerous cells may develop the radiation resistance because of some unknown factors. Hence making radiotherapy an ineffective strategy and causing cancer metastasis. Many of the combination procedures are going through the clinical trials for finding new ways for the effective radiotherapy. Radiotherapy mainly exercised by the two ways describes below.

3.2.1 External beam radiation therapy (EBRT)

External radiotherapy is the grade level strategy which can be opted for the localized PCa. It is the process in which high energy radiation are externally aimed at the location of cancer and destroy them by different way of actions such as apoptosis, autophagy, necrosis or mitotic cell death. Many of the technological advancements in external beam radiation therapy such as 3D conformal radiotherapy, Intensity modulated radiation therapy, and Image guided radiotherapy, stereotactic body radiation therapy are developing in order to decrease the toxic effects of EBRT and making them more effective against PCa [49-51]. External radiation therapy varies in effect as different radiations/particles (photon or proton) and protocols are uses accordingly for the treatment of PCa. Patients recovered from PCa by external beam radiation therapy have 72% increased risk of acquiring second primary bladder cancer than that of the surgical strategy [52].

3.2.2 Internal Radiotherapy

Internal radiotherapy is described as the brachytherapy in which sealed radionuclide (a source of radiation) is introducing into tumor or next to it for treating it directly or by means of catheters. These radionuclides emit a range of radiations including auger electrons, alpha, beta, gamma and x-rays. Most commonly used radionuclides (Yttrium-90, Lutetium-177, and Iodine-131) emits beta rays while others are less common [53-55]. Brachytherapy provides

with a significant good option treatment for the PCa and more advancement in this therapy provides with the marvelous oncological outcomes, limited toxicity rate and also increased life expectancy of PCa patients [56].

Internal radiotherapy is actually the targeted treatment strategy, a lot of development and new radionuclides are formed specifically for the treatment of PCa. An alpha-emitter radionuclide ^{225}Ac complexed with PSMA forms the ligand ^{225}Ac-PSMA-617 for the PCa treatment although still in clinical trials however also demonstrating the strong potential toward the advanced stage PCa. The other most efficient therapeutic radionuclide that are also in practice to label PSMA is β-emitter Lu-177 – which required one or two more cycles of therapy to eradicate the PCa as compared to 225Ac-PSMA [57, 58]. ^{177}Lu labelled PSMA which demonstrating strong efficacy in short period of time due to which patient quickly recover from the PCa [59]. Two main factor are big barrier to adopt this therapy; one is the high cost and second is the establishment of 225Ac/177Lu-PSMA therapy.

3.3 Immunotherapy

Immunotherapy plays a consequential role in recent years for the PCa treatment by triggering the immune system of patient's to kill the PCa [22]. A noteworthy reduction in T-cells (immune responsive lymphocytes) are detected in the high risk PCa as compared to the benign nodular hyperplasia of the prostate. Several of the immunologic therapies for the castrateresistant PCa includes antibodies, adoptive T cell therapy, vaccines or targeting the immune function by chemical compounds and the most successful immunotherapy from all of them is the vaccinated immunotherapy while others exhibits less activity comparatively. One of the FDA (Food and drug administration) approved vaccine Sipuleucel-T as the first successful vaccine for PCa immune therapy [60-62]. Despite of the extensive research towards the immunotherapeutic treatment of PCa as monotherapy are not exhibiting the satisfying results. Much more of the further researches are needed in this regard as not all PCa

tumors exhibiting the immune-sensitive behavior. For the purpose of more improvements, Combination immunotherapies (immunotherapy with other therapies such as chemotherapy, radiotherapy, hormonal therapy or surgery) shows some positive response, hence paving the way for immunotherapy as a revolutionized PCa treatment strategy [63, 64].

3.4 Hormonal therapy

Hormonal therapy is also regarded as the androgen deprivation therapy. Androgen receptors are the steroidal transcriptional factor for dihydro-testosterone and testosterone PCa growth is triggered by the increased level of androgens, hence provided the basis for androgen deprivation therapy. Androgen deprivation therapy suppresses the androgens and in turn suppresses the growth of PCa. This therapy may also result in the high risk PCa to castrate resistant PCa [65, 66]. Androgen deprivation therapy may also leads to many other diseases involving cerebrovascular disease, cardiovascular disease, Alzheimer's disease and osteoporosis [67, 68].

3.5 Surgery

Surgery is not considered as the only treatment option for the PCa treatment, instead it's the part of multimodal approaches for treatment. Surgery is mostly suggested for the treatment of localized PCa in which tumor is surgically removed from the body and in turn provides with the survival benefits. Surgery may become the protocol for localized PCa treatment and more favorable than watchful waiting of impermanence, risk of metastatic and localized proliferation. Most commonly applicable surgery types for the PCa are pelvic lymphadenectomy and radical prostatectomy (RP). However, RP is generally not preferred for the high risk PCa due to the adverse side effects such as metastasis of lymph nodes, elevated positive surgical margins and PSA recurrence. RP increases the life expectancy of patient with high risk of PCa. While patients having intermediate risk disease gained high benefits by RP, with 24% reduction in PCa specific death, 15% reduction in overall death and 20%

reduction in metastatic disease development. RP may lead to the poorer sexual and urinary function, while leading to the much better execution in bowel domain. However, surgery is not the only and complete treatment option for cancer, as it's just the first mechanistic approach to remove maximum tumor cells/tissues hence surgery is followed by the other treatment options such as radiation therapy or chemotherapy to dead the remaining cancerous cells [69-71].

4. PCa treatment by natural product

Various plant bioactive chemo-preventive compounds target signaling pathways and cellular molecules. These include reactive oxygen species (ROS) formation and signaling, cyclooxygenase-2 (COX-2), xenobiotic metabolizing enzyme (XME), lipo-oxygenase pathways, cell cycle proteins, transcription factors, apoptosis, angiogenesis, invasion, and epigenetic enzyme alterations [15]. PCa treatment with a plant-based extract containing bioactive components reduces the incidence rate of this malignant disease and kills prostate cells through many different pathways (cell cycle arrest, inhibiting cancer cell growth, inducing apoptosis, anti-angiogenesis or anti-proliferation activity, etc.).

In the next sections, we describe all these criteria to update our knowledge of PCa origination, primary risk factors, natural product therapy processes, and screening the remainder of the flora for PCa treatment. Different prostate cell lines (i.e. LNCaP, DU145 or PC-3 cell lines) are used by in vivo, in vitro, or animal model to find the exact pathways of treating PCa by the specific bioactive in specific efficacious concentration from the specific plants, which may be traditional herbal medicine plants or dietary plants etc. These natural chemicals originating from plants are bioactives exhibit their potential anti-cancerous activity within days or months. For better treatment of PCa by plant extracts, more clinical trials and research are needed to obtain viable, less costly, and easily available cancer treatment procedures.

4.1 Plant derived bioactive phytochemicals and PCa

Commonly, it was believed that the chemo-preventive drugs from the last 40 years are used for the treatment of cancer a natural products extracted from plant roots. These natural products are many different types of bioactive components that are present in different parts of the plant i.e. leaves, flowers, fruits, seeds, bark, and roots etc. These bioactive components may have anti-cancer effects used for multiple purposes such as by inducing apoptosis, altering the cell cycle, or inhibiting cancer cell growth and by scavenging free radicals etc. [16].

Researchers have found many plants derivative bioactive components that show the anti-PCa activities in vivo or in vitro, such as *Psoralea corylifolia,* Danshen, American cranberry, olive oil,*Salvia miltiorrhiza* Bunge, black pepper, *O. gratissimum* etc.and many of other plants are describe below **(Table 1)**.

Table 1: List of plants from which phytochemicals have been extracted and are used to treat prostate cancer.

Plant	Used phytochemicals	Efficacy	Prostate target cell lines	Analysis time (Hours, days)	Model of PCa	Result of the used model	References
Psoralea corylifolia	Neobava-isoflavone and psoralidin	Cause apoptosis in the PCa cells.	LNCaP cells	48-h	In vivo & In vitro	Causes increased Percentage apoptosis $77.5 \pm 0.5\%$ or $64.4 \pm 0.5\%$,	[2]
Danshen (*Salvia miltiorrhiza Bunge*)	Diterpene compounds (Cryptotanshinone (CT), Tanshinone IIA (T2A) and Tanshinone I (T1))	Anti-angiogenesis activity, anti-growth, anti-invasion	PC-3, LNCaP, and DU145 cell lines(in vitro) Bax and Bcl-2 proteins(in vivo)	7 days (In vivo), 5h (In vitro),	In vivo (in mice) & in vitro	T1 shows IC_{50}'s around 3-6.5 μM, IC_{50}'s of CT and T2A are around 10–25 μM and 8–15 μM, respectively	[72]
American cranberry (*Vaccinium macrocarpon*)	Anthocyanin glycosides (cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-galactoside, and peonidin-3-arabinoside)	Cell cycle arrest	DU145 human PCa cells.	6-h	In vitro (human PCa cells)	Viability decreased by 26%, 32% and 46% at 10, 25 and 50 mgml_1 of WCE	[73]
Olive leaves	Oleuropein	Antiangiogenic	LNCaP & DU145 PCa cell lines & on BPH-1 non-malignant cells	72 h	*In vivo* and *in vitro* studies	100-500 μM oleuropein causes significant reduction in cell viability (Particularly in LNCaP & DU145 cells)	[74]
Chinese medicinal herbs(*Salvia miltiorrhiza Bunge)*	Tanshinones	Inhibitory potency to the PCa cell lines.	PCa cells	24 h	In vitro, in vivo(mice)	For androgen-dependent LNCaP cells, Shows strong inhibitory potency having order of TIIA≈cryptotanshinone>tanshinone I	[75]
Tomato Powder and Soy Germ	Lycopene (tomatoes) & Parent Isoflavones ,isoflavone metabolites(soy germ)	Reduces prostate carcinogenesis	mouse prostate (TRAMP)model	14 week	In mice	Mice consuming TP (61%, $P < 0.001$), SG (66%, $P < 0.001$), and TPþSG (45%, $P < 0.001$) Having lower incidences of PCa.	[76]
Black pepper	Piperine	Reduces tumor growth, anti-migratory effects.	LNCaP, PC-3 and DU-145 PCa cells	24 h, 48h,& 72 h.	In vivo(in mice) and in vitro	LNCaP (AD), PC3, 22Rv1 & DU-145 (AI) cell lines shows reduction of proliferation with an IC_{50} value of 60μm, 75μm, 110μm and 160μm	[77]

						respectively.	
O. gratissimum leaf	Ethanolic extracts (anti-oxidants)	Anti-inflammatory and anti-angiogenesis properties	PC3•AR cells	24, 48, & 72 hours.	In-vitro	The three extracts P2,P3-2 and PS/PT1 in a dose dependent way(P2>P3–2>PS/PT1), inhibits proliferation of PC3•AR cells	[78]
Turmeric and Chinese goldthread	Curcumin and *ar*-turmerone, berberine and coptisine	Induce cell-cycle arrest, inhibition of cell invasion ,cellular apoptosis and metastasis	CWR22Rv1 and HEK293 cells	24h	In-vitro	IC$_{50}$ value of bioactives (combined phytochemical), inhibited the cell proliferation in PCa cell lines that are studied.	[79]
Dried ginger (*Zingiber officinale* Roscoe)	6-Shogaol, 6-paradol (6-PAR) & 6-GIN.	Cause apoptosis	DU145, LNCaP, and PC3 (human) & mouse (HMVP2) PCa cell lines.	24h (human prostate cells) , 32 days (mice)	In vitro (human cultured cells), in vivo (in mouse)	6-SHO was the most chmopreventive than other two bioactives in inhibiting PCa cells (in vivo & in vitro) by apoptosis.	[80]
Cucumber	Cucurbitacin B	Inhibits cell growth, induces apoptosis.	LNCaP and PC-3	24h(in vitro), 30 days (in vivo)	In-vitro , in vivo(mouse)	IC$_{50}$ of CuB results in inhibiting cell viability of prostate cncer cells; also (0.1 µmol/day of CuB) inhibits the growth in athymic mice PC-3 xenografts.	[81]
Alnus japonica (bark)	Hirsutenone	Induce apoptosis	PC3 and LNCaP PCa cells	72h	in silico, in vitro (human PCa cells)	Hirsutenone induces apoptosis by targeting Akt1 and 2 in human PCa cells.	[82]
Cruciferous vegetables	Erucin (isothiocyanates)	induce a proliferation- arrest state	prostate adenocarcinoma cells (PC3)	24 h	In vitro	ER concentration up to 15 µM caused a significant decrease in proliferation of PC3 cell at 25 µM($P ≤0.01$)	[83]
Arctium lappa (seed), green tea,Curcuma longa (root)	Arctigenin (Arctium lappa), epigallocatechin gallate(green tea), curcumin	Cell cycle arrest, apoptosis	LNCaP PCa	48 h	In vitro	IC$_{50}$ values of EGCG, curcumin, arctigen induces antiproliferative effect in combination &individually induces apoptosis LNCaP cells.	[84]
Salvia triloba (Lamiaceae)	1,8- cineole, β –pinene, β-caryophyllene , camphor	Induced cytotoxicity, apoptosis, angiogensis	PC-3, DU-145 & HUVEC cells	72 h	In vitro	IC$_{50}$ values of extract was 287±8 & 456±15 µg/ml in PC-3& DU-145 cells shows that it induces apoptosis to them and no cyto-toxic affect to normal cells.	[85]

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Hedyotis diffusa (Leaves & root)	Diffusa cyclotide 1 to 3 (DC1-3)	Induces cytotoxicity, hepatoprotective, neuroprotective activities inhibited cell migration	PC3, LNCap and DU145(in vitro) Mouse xenograft model (in vivo)	72 h	In vitro and In vivo	IC_{50} value of bioactives is shown at the level below 10 µM for three cancer cell lines. These bioactives shows anticancer affects in vivo and in vitro.	[86]
Milk thistle	Silibinin	Induces autophagy	PC-3 cells	48 h.	In vitro	Silibin induces autophagy (apoptosis) in PC-3 cells by the production of Reactive Oxygen Species.	[87]
Eurycoma longifolia	Quassinoids	Induced cytotoxicity, anti-tumorigenic activity, inhibition of LNCaP cells,	LNCaP cancer cells.	72 h(in vitro, in vitro (6 week)	In vitro(human), In vivo (mice)	IC_{50} value of SQ40 at level of 5.97 µg/mL inhibits growth of LNCaP while the injecton of 5 and 10 mg/kg of SQ40 inhibits LNCaP tumor growth in mice xenograft.	[88]
Carica Papaya	Crude flavnoid extract (CFE), ELE & MLE	Anti-cancer activity, Induces inhibition of cancer cell growth.	DU-145	48-72h	In vitro	Growth inhibition of DU-145 cancer cells induced at IC_{50} of CFE, ELE and MLE at the level of 2.2 µg/ml, 2.4 µg/ml & 2.6 µg/ml respectively.	[89]
Pumpkin (seed)	Cucurbitin	Inhibiting cell growth	DU145 (androgen insensitive) & LNCaP (androgen sensitive)		In vitro	The considered curcurbitin is not the component that causes the Inhibition of cancerous cells. Although the seed extract are considered safe for the PCa.	[90]
Luobuma (leaves)	Sterols (Sitgmasterol, sitosterol) , Triterpenoid (Lupeol) , Flavonoids (Kaempferol, Isorhamnetin)	Inhibits cell proliferation, induce apoptosis, augment cell cytotoxicity	PC3 cells	24h	In vitro	Lupeol accounted for (w/w) 19.3% of F8 and inhibits proliferation of androgen-insensitive-prostate-cancer cells. Other bioactives also exerts anti-cancer effects by different mechanisms.	[91]
Ginger	6-shogaol, 10-shogaol 6-gingerol, & 10-gingerol,	Inhibit proliferation, induce apoptosis	PC3R, PC3 cells	24h	in vitro	6-shogaol, 10-shogaol 6-gingerol, & 10-gingerol at 100µM inhibits proliferation in PC3R, while same results also observe in PC3 by 6-gingerol, 6-shogaol and 10-shogaol.	[92]

Salvia miltiorrhiza Bunge (Danshen)	dihydroisotanshinone I (DT)	Increases survival rate of patients, inhibits cancer migration.	PC3, DU145 ,22Rv1 cells	15year (in vivo), 24h (in vitro)	in vivo (human), In vitro,	Danshen induces in vivo protective effect on the patients that survival rate increases. While in-vitro DT inhibits the migration ability of PCa by different mechanisms.	[93]
Soybean (seed)	Bioactive peptides	Inhibits cancerous cells	PC-3 cells	48–60 h	In vitro	Different soybean lines shows different inhibitory percentage .S03-543CR soybean line shows highest (63%) inhibitory effect to PCa cells.	[94]
Scutellaria altissima L.	Scutellarin (flavon)	Induces G2/M arrest, apoptosis, inhibits proliferation	PC-3 cells	24h	In vitro	Scutellarin induces apoptosis, G2/M arrest, and inhibits the proliferation of PCa. Sensitized the PC-3 cells to chemotherapy.	[95]
Solanum nigrum L.	Solanine	Reduces tumor growth, induce apoptosis	DU145 cells(human ,in vitro), DU145 cell (mouse xenograft, in vivo)	30 days(In vivo), 24h (invitro)	In vivo, in vitro	Solanine in vivo and in vitro regulated the cell cycle proteins i.e. Cyclin E1, P21, Cyclin D1, CDK4, CDK2, CDK6. IC$_{50}$ of solaline at level32.18 μmol/L causes apotosis. Decrease in tumor in mouse xenograft was observed.	[96, 97]
Citrus aurantium L. (stem bark)	acridone alkaloids (citrusinine-I, citracridone-I, 5-hydroxynoracronycin, natsucitrine-I, glycofolinine, citracridone-III)	Induces cyto-toxicity	PC3 cells	72 h	In vitro	IC$_{50}$ of Citracridone-I shows more antiproliferative activity at the level of 12.5 – 14.8 μM than the other alkaloids.	[98]
Plagiochila disticha (Plagiochilaceae)	plagiochiline A	Induces Cell cycle arrest &cancer cell death	DU145 cell	24 h	In vitro	1.75 μg/mL of plagiochiline A causes cell cycle arrest and cell death in DU145 cell.	[99]
Erythrina Excels (stem bark)	Excelsanone	Induces cyto-toxicity , inhibits cell growth	PC3 & DU145 cell lines	24, 48 and 72 h	In vitro	IC$_{50}$ of 1.31mg/ml of Excelsanone together with 6, 8-diprenylgenistein has moderate potential toward cyto-toxicity & also Inhibits cell growth in DU145 cell lines.	[100]
Punica granatum (juice and peel extract)	Ellagic acid and its derivatives, α & β-punicalagin(juice), punicalagin, ellagic acid and its derivatives (peel extract)	Inhibits migration, proliferation and colony formation	PC3 & DU145 cell lines	24 h or 48 h	In vitro	Pomegranate juice and peel extract both shows the antiproliferative effect for PCa cells. But peel extract show more robust result towards cancer than juice at similar concentration.	[101]

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Silybum marianum	Silychristine, silibinin & Silymarin-enriched extract (SEE)	G2/M blockade, inhibits anti-proliferative effect	PC-3 cells	24 ,48 and 72h	In vitro	Silychristine, silibinin IC$_{50}$ value of 3–120 µg/mL and SEE have IC50 of 44–52 µg/mL inhibits proliferation in dose dependent way. DXR-SEE co-treatment enhances the cell death than the SEE alone.	[102]
Rooibos (*Aspalathus linearis*)	Flavonoid (aspalathin)	G2/M cell cycle arrest, apoptosis	LNCaP 104-R1 cells xenogafted in mice.	96h	In vivo (mouse xenograft)	GRT extract aspalathin majorly inhibits the proliferation and suppressed the CRPC cells. Causes apoptosis in LNCaP 104-R1 xenografts in mice.	[103]
Paederia foetida (leaf extract)	Lupeol, β-sitosterol and MEPL.	Induces cyto-toxicity,	PC-3 and DU-145, THP-1 cells	24h	In vitro	Lupeol, β-sitosterol and MEPL at their respective IC$_{30}$ values exhibits cyto-toxicity, apoptosis & inhibits proliferation.	[104]
Citrus sinensis L. (peel extract)	Citric acid, narirutin & hesperidin	Suppresses DNA synthesis rate in PC cells,induce apoptosis, inhibits cell cycle re-entry	LNCaP, RWPE-1, GM3348 & PC-3	24h & 48h	In vitro	Narirutin & hesperidin was not the bioactives responsible for inhibiting cell cycle re-entry but it was the citric acid. So, citric acid along other bioactives can be act as chemopreventive.	[105]
Green tea	Epigallocatechin-3-gallate (EGCG)	Inhibits migration and invasiveness of cancer, upregulateTIMP-3 level, decreases class I EZH2 & HDACs.	LNCaP & DUPRO cells	6week (in vivo), 48h (in vitro)	In vivo (human), In vitro	EGCG/GTP inhibits migration & invasive capability of cancer cells. In addition, EGCG in patients increases TIMP-3 levels by balancing MMP: TIMP suppresses PCa.	[106]
Glycyrrhiza glabra	GGE (Glyccyrhiza glabra Extract)	Inhibits proliferation, induce apoptosis	PC-3 cells, WI-38 cells	96h	In vitro	GGE treatment causes proliferation at IC$_{50}$ value of PC-3 and WI-38 cells are at the level of 35.7 ± 2.0 lg/m and 96 ± 1.6 lg/ml. while ADR+GGE causes proliferation at IC50 11.6 ± 0.6nM.	[107]
Rosa canina	Phenolics (ascorbic acid, p-coumaric acid, gallic acid, quercetin, 3, 4- dihydroxy benzoic acid, rutin hydrates and chlorogenic acid).	Induces cyto-toxicity, apoptosis,cell cycle arrest, increases caspase activity.	PC-3 cells	72h	In vitro	Significant increase in M phase cells at observed at IC$_{90}$ of 257 and 378 mg/mL of *Rosa canina* extract. Apoptosis, cell cycle arrest and increase in caspase activity also observed that may be due to phelonic content of extract.	[108]

Cymbopogon citrates (lemon grass)	Citral	Induce apoptosis, inhibits colonogenic formation and proliferation of PCa cells.	PC3 and PC-3M cells	72h	In silico , in vitro	Citral significantly reduces the colonogenic potential, proliferation, cell viability, changes the morphology and inhibits the lipogensis of cancerous cells.	[109]
Rhizoma Curcuma	Germacrone	Reduces the viability, induces apoptosis and autophagy.	PC-3 & 22RV1 human PCa cells	48h	In vitro	Germacrone in dose dependent manner with IC_{50} have the value for PC-3 were 259 μM and for 22RV1 were 396.9 μM induces apoptosis and inhibits cells proliferation.	[110]
Orobanche crenata	Orobanche crenata methalonic extract	Inhibits PCa cells,	PC-3 PCa cells	24h	In vitro	O. crenata extract exhibits the anticancer, cytotoxic and antiproliferative activity due to the bioactive methalonic components present in it.	[111]
Moringa oleifera (Leaves)	Methalonic extract	Induces G0/G1 cell cycle arrest, anti-cancer potential and ROS-mediated apoptosis.	PC-3 cells	24h	In vitro	Moringa oleifera methalonic leaves extract demonstrates ROS-mediated apoptosis and reduction in proliferation by suppressing deregulated Hedgehog signaling in PCa.	[112]
Paris forrestii	Total saponins (polyphillin D, paris saponin Tg).	Induces apoptosis,PCT3 treatment changes mRNA and lncRNA.	LNCAP, DU145, PC3, RWPE	24h	In vitro	PCT3 exhibits the anticancer activity on PCa and also reveals some crucial mRNAs and lncRNAs that take part in the anticancer activity of PCa.	[113]

5. Multi-targeted Chemo-prevention

Assembling evidences describes that a lot of phytochemicals are acting via multiple mechanisms involving modulating pathways of signal transduction, interconnections with receptors and the genes includes in the control of apoptosis, cell cycle, cell proliferation and regulating transcription by exerting their chemotherapeutic and antitumor effects. Some of the potential phytochemicals are described under that are proven to be having the chemo-preventive effects towards the PCa [15].

5.1 Tanshinones

Tanshinones are abietane diterpene compounds found in Salvia miltiorrhiza. They include tanshinone-I "T1" (Compound 1), tanshinone-IIA "T2A" (Compound 2), and cryptotanshinone "CT" (Compound 3). (a traditional Chinese medicine). These bioactive elements are used to cure disorders and display anti-cancerous action by anti-angiogenesis, anti-proliferation, pro-apoptosis, reducing adhesion, metastasis, invasion, and migration, and inducing differentiation, with no negative effects on normal cells. [114, 115].

Tanshinones showed the anti-cancerous results while doing invitro study in the dose dependent manner by inducing apoptosis and cell cycle arrest. Tanshinone-I exhibits the potential activity with IC_{50} value around 3–6 μM. Aurora A kinase were identified as the probable target of these phytochemicals actions (tanshinones), as in the cell lines of PCa. Aurora "A" were over-expressed and hence decreases the PCa cell growth. Its expression was significantly downregulated by tanshinones. Tanshinones especially "T1" exhibits the anti-angiogensis effects in-vivo and in-vitro suggesting that these are the safe anti-cancerous and therapeutic agents against PCa[72] . T1 also increases TRAIL mediated apoptosis through the miR135a-3p arbitrated DR5 up-regulation in the PCa cells as an effective TRAIL sensitizer [116]. In another study tanshinone IIA isolated from Salviae Miltiorrhizae (root extract) exhibits the antiproliferative

effects to PCa in a dose dependent way. Tan- IIA causes the cell death and cell cycle arrest in LNCaP cells at G0/G1 phase and correlating it with the enhanced CDK inhibitors levels. These tanshinones induces ER stress, induces the cell death with the blockage of the expression GADD153/CHOP via siRNA reduced tanshinone IIA, increases the expression of down-stream of different molecules and suppresses the tumor growth and reduces tumor volume to 86.4% in xenograft model within the 13 days of treatment. In PC-3, LNCaP cells the IC_{50} value for the bioactives Tanshinone IIA were 2.54μg/mL and 5.77μg/mL[117].Tanshinone IIA also exhibits the inhibitory effects on the proliferation and growth of the LNCaP cells via BrdU incorporation assays and colony formation respectively and induces the cell cycle arrest at G1 phase by the activation of p53 signaling, also the down regulating Cyclin D1, CDK2 and CDK4 and inhibiting androgen receptor (AR) in LNCaP cells [118].
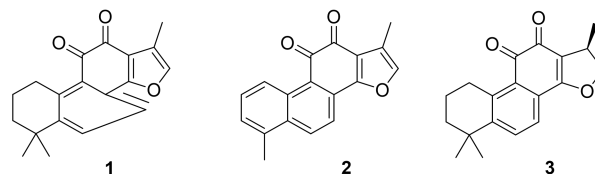

Figure 2: Molecular structures of tanshinone-I (1), tanshinone-IIA (2) and cryptotanshinone (3). Reproduced with permission: Copyright 2015, MDPI [119]

5.2 Biochanin-A

Iso-**flavones** are the most common plant-based bioactive chemicals. Biochanin-A (compound 4) in red clover and soy has chemo-preventive, anti-proliferative, and anti-cancerous activities. Biochanin-A significantly enhances the Trail mediated apoptosis and cytotoxicity in LNCaP and DU145 PCa cell lines. TRAIL is actually the innate potent anti-cancerous agent that inequitably induces the apoptosis in the malignant cells and cause no damage or toxicity to normal cells. In vitro study shows TRAIL cytotoxicity at 50-100ng/mL for 48h was 2.8% -1.4% to 6.2% -1.7% for DU145 and 8.3% -1.2% to 19.6%-1.1% for LNCap. Biochanin-A sensitized the LNCaP cells that are TRAIL-resistant by inhibiting the activity of transcription

factor NF-κB (p65). This compound enhanced the expression of TRAIL-R2 (DR5) a death receptor and disrupted the potential of mitochondrial membrane. Apoptosis is enhanced by inducing the potential of sensitized TRAIL and TRAIL resistant PCa. This isoflavone regulated NF-κB activity and also affected the intrinsic and extrinsic apoptotic pathways [120-122].
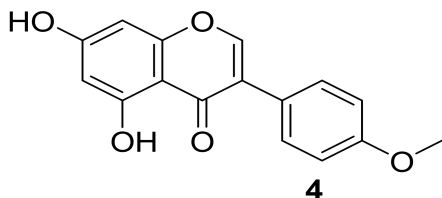

Figure 3: Molecular structure of Biochanin-A (4). Reproduced with permission: Copyright 2014, PLOS [123]

5.3 Oleuropein

Olive leaves, fruits, and oil contribute to the Mediterranean diet's health benefits. Oleuropein (Compound 5) is an anti-tumor polyphenol found in olive leaves. Olive leaves and oil contain polyphenols. Many of the invitro studies demonstrated the apoptotic and anti-proliferative effects in many of the cancer cell lines. In a study using MMT test to assess cell proliferation, 100-500M oleuropein treated over 72 hours on LNCaP, DU145, and non-malignant BPH-1 cells suppressed cell viability, especially in DU145 and LNCap cells. Hydrolysis of oleuropein produces the compounds such as hydroxytyrosol and eleonolic acid that are also the bioactive compounds. This polyphenol induces the decrease in cell viability and modification in the thiol group, ROS (reactive oxygen species), γ-glutamylcysteine synthetase, heme oxygenase-1 and pAkt. Oleuropein induces the antioxidant effect while exposing cell culture on the BPH-1 cells, a non-malignant cell line. Oleuropein proves to be an adjuvant agent while treating the prostasis, so as for preventing the modification of hypertrophic to malignant (cancerous) cell [74, 124-126].
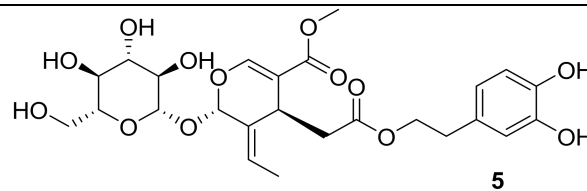

Figure 4: Molecular structure of Oleuropein (5). Reproduced with permission: Copyright 2015, Scientific Research Publishing, Inc. [127]

5.4 Anthocyanins and phenolic acid

Anthocyanins are phenolic pigments found in berries and grapes. Anthocyanins are also found in cereals, tubers, and other plants. These bioactive components have proven to be beneficial for health [128]. Sweet potato leaves contain anthocyanins and phenolic acid. Polyphenol rich sweet potato green extract (SPGE) stimulates anti-proliferative activity in prostate epithelial cells without injuring normal cells, modifies the cell cycle, lowers colonogenic survival, and induces apoptosis in human PCa "PC-3 cell line" in-vivo and in-vitro. Alternations in apoptosis regulatory constituents e.g. Bcl2 inactivation, BAX upregulation, release of cytochrome and the activation of the down-stream apoptotic signaling were observed by the action of SPGE. SPGE also cause the degradation of DNA as obvious via TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-nick-end labeling) staining of enhanced 3′-DNA ends concentration. In-vivo 400 mg/kg SPGE oral administration show the reticent progression and growth approximately 69% in prostate tumor xenograft model of mice and exhibits that normal tissues are also not affected[129].
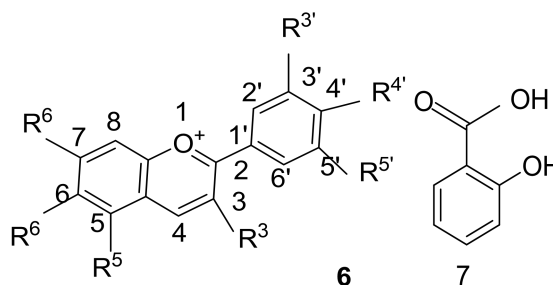

Figure 5: Molecular structure of Anthocyanins (6) and phenolic acid (7). Reproduced with permission: Copyright 2017, MDPI [130]
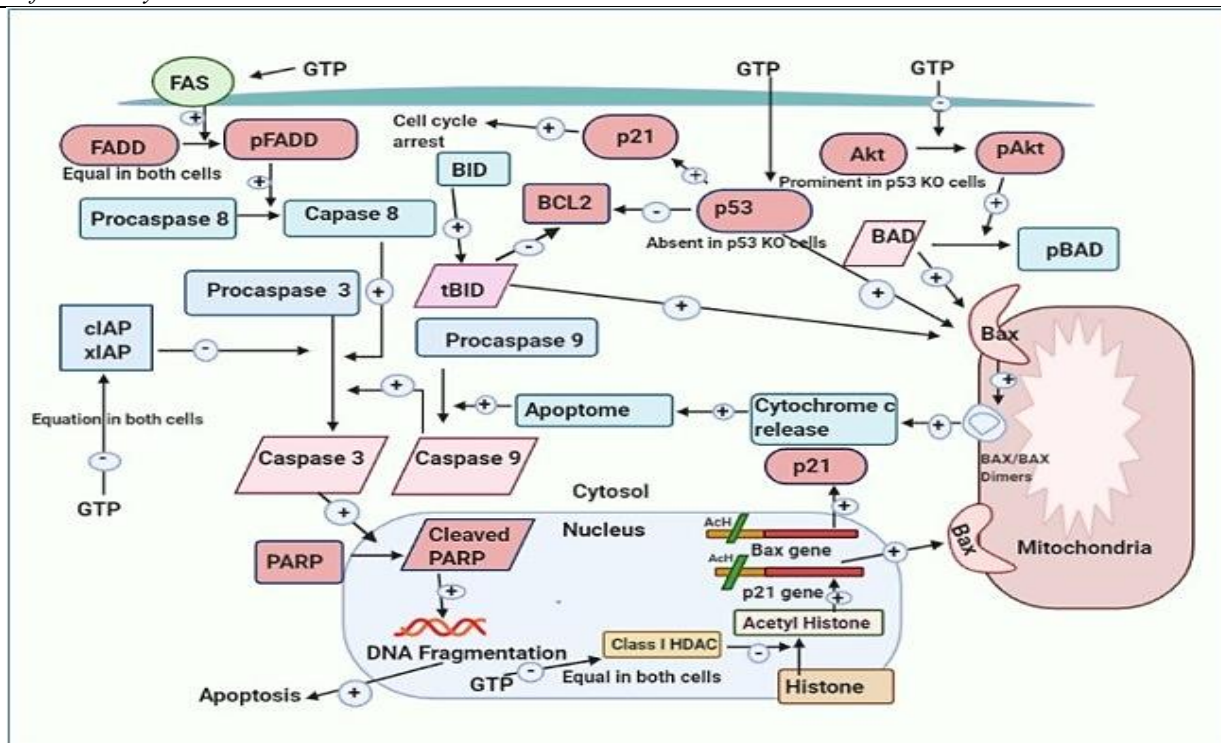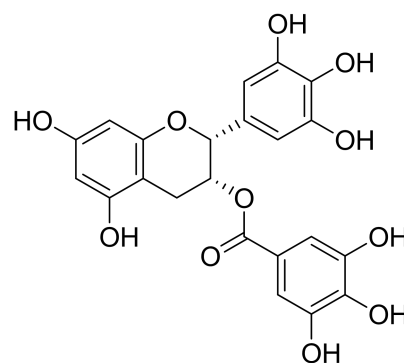
Figure 6: Schematic model illustration of the green tea polyphenol induced activation of intrinsic (death cascade of mitochondria) and extrinsic (death receptor pathways) molecular pathways in the presence and absence of p53. Reproduced with permission: Copyright 2012, PLOS [131].

5.5 Epigallocatechic-3-gallate (EGCG)

Epigallocatechic-3-gallate (EGCG) is the major green tea polyphenol. Green tea polyphenol induced P53 activation and stabilization of down-stream targets Bax and p21/waf1are induced by the GTP treatment in a dose dependent manner especially in LNCaP cells. These polyphenols promotes the apoptosis in cancerous cells effectively both in absence and presence of p53 function via agitation in signaling pathways involving the extrinsic FAS-FADD death receptor and constancy pathways that intersects in the induction of apoptosis via mitochondrial death cascade **(Figure 6)** [132].

An analytical study reported that EGCG in combination with quercetin induces apoptosis, cell cycle arrest and inhibits the cell proliferation in PCa in vitro by enhancing the EGCG intracellular concentration and lessen the EGCG methylation. As compared to the sum rate of inhibition of EGCG and quercetin individually their combination with 10μM or 20μM respectively enhances the reduction in PC-3 cell proliferation at 24h and 48h by

15% and 20%, or 21% and 19%, respectively. The combined effect of these agents based on the certainty that quercetin decreases the quantity of catechol-*O*-methyl transferase (COMT) activity while EGCG inhibits activity of catechol-*O*-methyl transferase (COMT). EGCG and quercetin leads to the additive effects by executing the strong anti-proliferative effects in LNCaP cells. Combination of these two bioactive molecules proved to be much effective in order to induce chemotherapeutic and chemo-preventive effects towards PCa[133].

8

Figure 7: Molecular structure of Epigallocatechic-3-gallate

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(8). Reproduced with permission: Copyright 2006, Elsevier [134]

5.6 Resveratrol

Resveratrol is a natural polyphenol found in peanuts, berries, and grapes. Many pre-clinical investigations suggest resveratrol is a promising natural bioactive anticancer drug [135]. These research showed that resveratrol (Compound-9) shields various biological systems, especially in cancer[136]. By altering androgen receptor signaling in PCa, resveratrol inhibits androgen-regulated gene expression and cell proliferation. Anti-androgenic resveratrol analogues were extracted from plants or semi-synthesized. LNCaP prostate cell lines were seeded in the luciferase assay along with MMTV-luc reporter plasmid for the measurement of androgen dependent (AR) activity. 4′-O-methylresveratrol (3, 5-dihydroxy-49-methoxystilbene) resveratrol analog were proved to be most potent abstractor of AR transcriptional activity as IC_{50} value for the resveratrol and its analogs were to be 5μM and 2μMrespectively. The hydroxyl (OH) in the ring of resveratrol plays the major role in the anti-androgenic effects via modulation of androgen dependent (AR) activity [137]. Peanut stem extract (PSE) contains high content of resveratrol that augments its radio-sensitization affects in PCa which is likely to be mediated through the apoptotic pathway activation, DSB (DNA double-strand break) repair attenuation and the arrest of cell cycle in G2/M phase. Resveratrol and PSE inhibits proliferation in LAPCD-KD cells for 48h treatment with IC_{50} value 25 and 500μg/mL, respectively. In addition, co-administration of PSE or resveratrol and radiation, induced the apoptosis in radio-resistant PCa cells. Radiation therapy were enhances effectively by exploration of PSE and resveratrol in the shDAB2IP PCa mouse xenograft model [138].
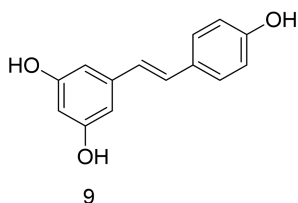

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Figure 8: Molecular structure of resveratrol (9). Reproduced with permission: Copyright 2019, MDPI [139]

5.7 Piperine

Black pepper contains the medicinal alkaloid piperine (*Piper nigrum* Linn.). Pepper has wide-ranging pharmacological effects and is used to treat numerous diseases. Piperine is anti-asthmatic, immune-modulatory, anti-ulcer, anti-oxidant, anti-inflammatory, and anti-carcinogenic, according to human research [140-143]. A study provide evidence that piperine exerts the antitumor activities towards the PCa, in-vivo and in-vitro. Piperine was pre-treated with DU145 cells for 48h and then their viability was examined by the CCK-8 assay. It clearly caused apoptosis and inhibited cell migration and proliferation in DU145 prostate cell lines by downregulating MMP-9 and Akt/mTOR signaling, also Akt/mTOR signalling appears to be MMP-9 protein upstream regulator (**Figure 9**) [144].

Piperine in the dose dependent manner suppresses different metastatic behavior and proliferation of PCa by inducing the G°/G1 phase arrest. The bioactive component piperine shows the IC_{50} value for the PC-3, DU145 and androgen-dependent LNCap cells were 111μM, 226.6μM and 74.4μM respectively106. In another in-vivo and in-vitro studiesthe effectiveness of piperene were examined. It induces apoptosis and inhibits proliferation in both the androgen independent PC-3, 22RV1 and DU145 and the androgen dependent LNCap cells in vitro. PC-3, 22RV1 and DU145 PCa cell line while treating with piperine results in the lessened expression of nuclear factor-kB (NF-kB) and phosphorylated STAT-3 transcription factors. In-vivo study shows that xeno-transplanted model with PCa in mice results in the reduction of androgen dependent (AD) and androgen independent (AI) tumor growth. Significant reduction in viability and proliferation of PC-3 (AI) and LNCaP (AD) cells with piperine were exhibits while assessing as MMT assay with the IC_{50} values of 75μM and 60μM respectively in a dose dependent way [77].
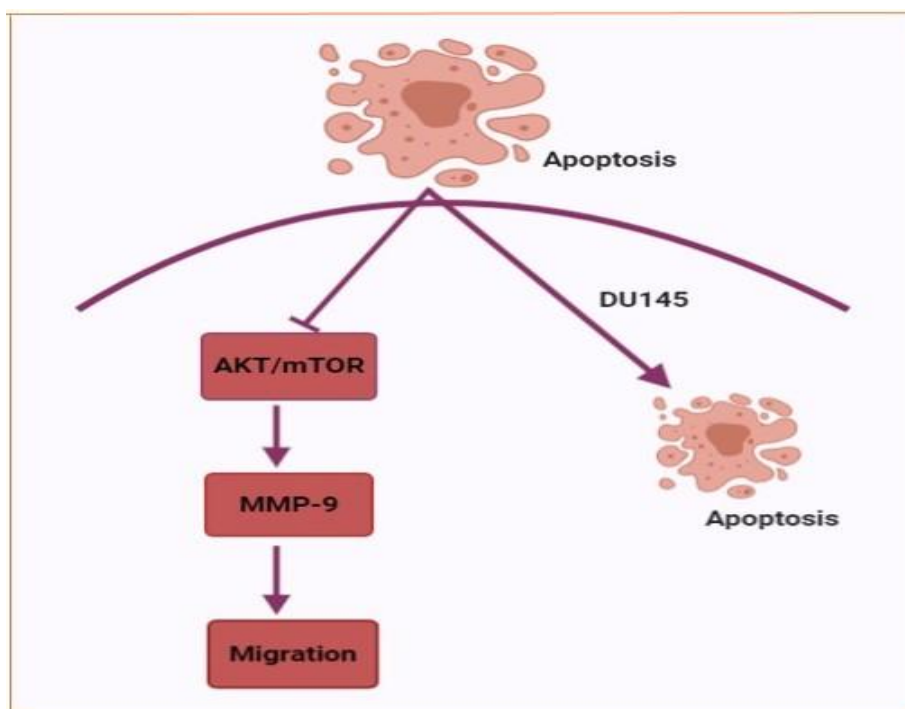
Figure 9: Piperine inhibits the continuation of migration via downregulating signaling pathway Akt/mTOR/MMP-9 in DU145 PCa cells. Reproduced with permission: Copyright 2018, Spandidos publications [145].
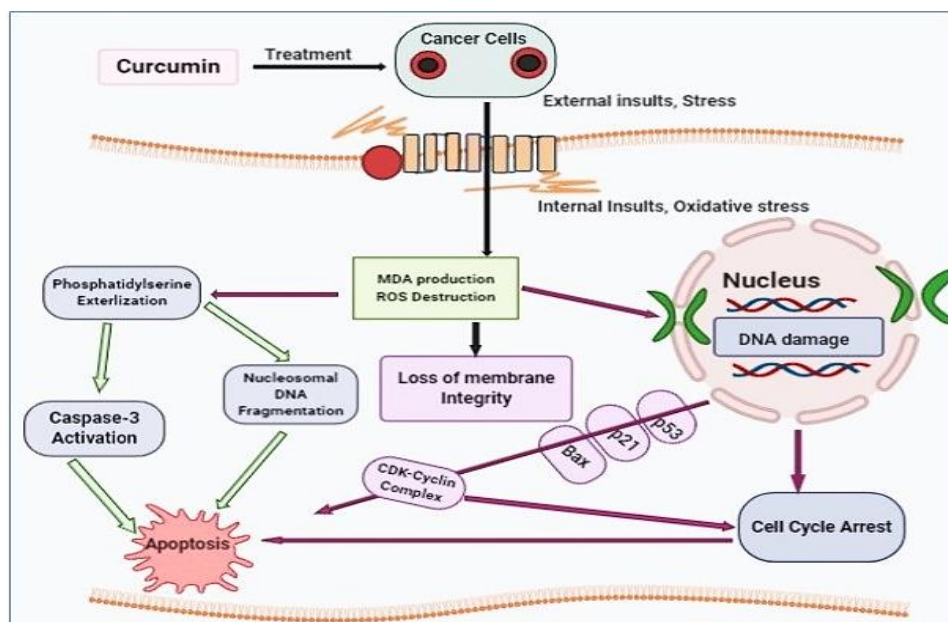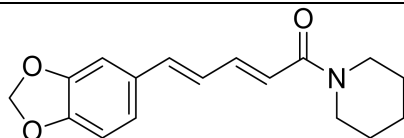

Figure 10: Schematic model representation of curcumin molecular mechanism, in malignancies management as a therapeutic agent. Reproduced with permission: Copyright 2019, MDPI [146].
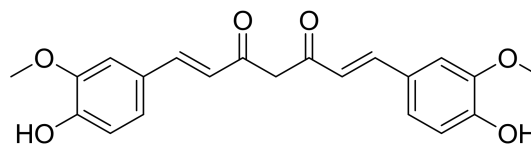
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Figure 11: Molecular structure of piperine (9)Reproduced with permission: Copyright 2022, Springer [147].

5.8 Curcumin

Curcumin a pleiotropic curcuma longa constituent is a polyphenol with therapeutic properties. Curcumin has anti-arthritic, antioxidant, anticancer, and anti-inflammatory properties, according to in-vivo and in-vitro studies. Due to its regulation and efficacy towards multiple targets, as well as its tolerability, safety, and non-toxicity for human use, curcumin is a potential therapeutic agent for the treatment and/or prevention of chronic diseases [148-151]. Curcumin (Curcuma longa) is a potential chemo-preventive for early-stage PCa. It affects cell proliferation by reducing the expression of ß-catenin-targeted genes, which links cell death with autophagy in androgen-dependent prostate cells. Curcumin cell viability were assessed by tryphan-blue exclusion test that reveals that curcumin exhibits cytotoxic effect for the concentration >75µM, both in androgen dependent and independent PCa. Androgen dependent PCa cells were more sensitive for the natural compound curcumin (IC_{50} value were 44 and 48µM for the cells 22rv1 and LNCaP, respectively) than in androgen independent PCa cells (IC_{50} value were 115 and 170µM for PC-3 and DU145 cells respectively) [152]. Curcumin inhibited the cell growth and induces cell growth arrest via DNA damage, cell cycle arrest, stress genes (Bax, p21, p53 and CDK Cyclin complex) expression, and induces apoptosis through the phosphatidylserine externalization modulation, fragmentation of nucleo-somal DNA and caspase-3 activation **(Figure 10)**[146].

In PCa metastasis liable phenotype can be induce as a result of chronic inflammation via sustaining pro-metastatic and a positive pro-inflammatory feedback loop betwixt CXCL1/-2 and NFκB. This feedback loop disrupted from curcumin by suppression of NFκB signaling that leads to the lessened in-vivo metastasis formation [153]. Curcumin's anticancer effects were studied in nude mice. PC-3 cells were subcutaneously injected to the nude mice for establishing the tumor model. Nude mice were divided into different groups, group B (6% polyethylene glycol and 6% anhydrous ethanol, group C (normal saline), and group H, M, L (100 mg/kg, 50 mg/kg, and 25 mg/kg curcumin). Tumor growth were measured every 6th day and after 30 days they were killed to weight the tumor. Cell apoptosis were determined by TUNEL assay. In group H, M, L the volume and weight of tumor was lower remarkably than that of the control groups (C, B) (P<0.05), and as the dose for curcumin increases the inhibitor rate were also increases. While comparing with that of the control group, in H, M,L group Bcl-2 gradually decreased and Bax protein expression were enhanced (p<0.05). So, this study leads to the result that curcumin have the ability to inhibit PC-3 cells growth, reduces the weight and volume of tumor and also induces apoptosis under the nude mice skin by up regulating Bax and down regulating Bcl-2 [154]. Curcumin repudiate cancer associated fibroblasts (CAF) induced capture/invasion and epithelial to mesenchymal-transition and suppresses CXCR4,IL-6 receptor expression and Reactive oxygen species(ROS)in the PCa cells via suppressing HIF 1α/mTOR/MAOA signaling so that exhibiting the potential therapeutic effects of curcumin in PCa[155].

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Figure 12: Molecular structure of Curcumin (9)Reproduced with permission: Copyright 2018, Frontiers [156].

6. Future Prospective

Health claims are highly concerns with the nutrition

regulation in order to protect and inform the public about the fallacious health declarations. Although it's challenging but human intrusion studies are important in supporting the plant derived bio-actives, for the validated scientific findings [157-159]. However, with the advancement in cancer studies, characterization of neoplastic transformations due to the aberration in several signaling pathways arise the need for the identification of chemo-preventives that must be target specific towards the carcinogenesis. Hence, unraveling the bio-actives synergistic interactions and their potential impact on humans would considerably help in attaining success in the chemo-prevention via the plant bio-actives [15]. Pure plant derive bioactive compounds individually are needed for determining their potential chemo-preventive effects from the dietary supplements. However, isolation of the pure bioactive compound is sometime become difficult and challenging because of the stability issue, and missing their potential or in some of the cases unknown constituents may also demonstrate bioactivity either synergistically or additively from the same plant of interest[160].

Nutraceuticals and dietary supplements exert chemo-preventive effects that vary within the populations and also largely rely on microbiota gastro-intestinal composition, which in turn affect the level of anticancer compounds and bio-available nutrients. So, studies that exhibit the distinction in microbiome betwixt high and low risk populations, or in-between patients and healthy person with metastatic or dormant and androgen insensitive disease may lead to the valuable insight into the microbiome role in the progression and development of PCa. Human microbiome modulation may exert beneficial effects in chemoprevention of PCa [161]. Even though unique chemotherapeutic constituents would be more and more efficacious against the cancerous cells, their drug resistance as well as toxicity to normal tissues remain the extensive obstacle for its clinical use. Using various plant derived bio-actives for the personalized approach provides with the new dimensions for the

standard cancerous growth therapy for the betterment of its outcome in complementary and complex way [47].

Despite, a lot of the data involvement for the carcinogenic studies and its treatment, only arbitrary controlled trial is able to assess adequate evidences for creating the universal guidelines. Additionally, only a few of the plant derive bio-actives have been taken for the reasonable clinical trials to evaluate their potential anti-PCa activity [162]. Many more efforts are needed in clinical, epidemiological research, as well as in Phyto-biology that must be addressed, before the potential health effect of these phytochemicals on human[163].

7. Conclusion

Finally, many cancer treatment strategies are still in clinical trials, and many are on the way to improvement despite the enormous amount of work that has already gone into them. Because of their proven effectiveness against a wide range of acute diseases, low cost, and widespread availability, the scientific community is also making headway in treating malignant PCa with natural plant sources. Several useful plants, shrubs, and herbs have been shown to have chemo-preventive effects against PCa malignancies due to the presence of novel bioactive components. Being the active constituent bio-actives of the plants act as an agent that distressing the signaling of the prostate cells in several of the pathways, causes blockage of cell cycle at various phases, induces alternations and killing of the PCa cells with the less chances of drug resistance. Numerous in-vivo and in-vitro clinical studies have been conducted to better understand the precise protocol pathway of these bioactives in their interaction with and effect on PCa cells. However, much more of the research and clinical trials are needed for the detection of chemo-preventive effects of different plants bio-actives, while watching towards the future prospective.

Conflict of interest

The authors declare no conflicts of interest.

Authors Contribution

Hira Zulfiqar convinced the main idea and wrote the

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