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Research Article

Recovery Optimization of Plant Derived Antioxidants And Their Incorporation in Cosmetic Creams to Enhance Antioxidant Potential

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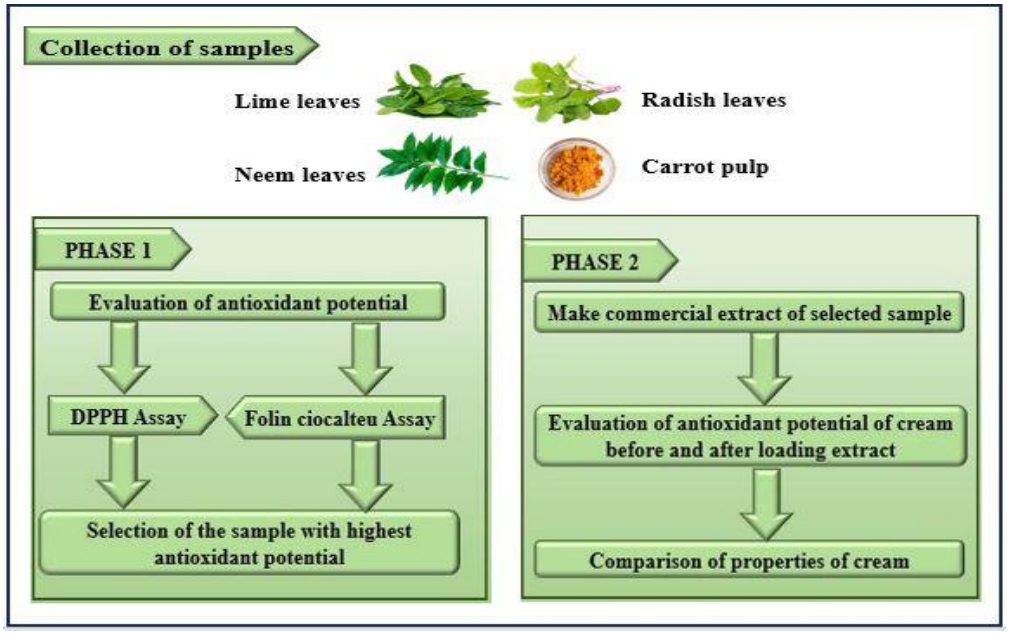
Abstract

Plants waste is enriched with valuable antioxidants. Extraction optimization, quantification of Total phenolic content (TPC) and evaluation of their anti-aging potential was the prime goal of this research study. Lime, Neem, Radish leaves, and carrot pulp were extracted with two types of solvents under various extraction conditions. The highest TPC were extracted through boiling water (2-3min) for all plant materials as follow: Neem leaves (112.3mg GAE/g), Lime leaves (108.5mg GAE/g), Radish leaves (76.02mg GAE/g) and Carrot pulp (65.84mg GAE/g); Highest free radical scavenging activities were achieved in Neem Leaves (92.82%), Lime leaves (92.34%), Radish leaves (87.4%) and Carrot Pulp (81.22%) under different extraction conditions. Water as extraction solvent yielded greater TPC values than ethanol. Upon the addition of phytoextracts, a substantial increase in the antioxidant activity of the cream samples was detected. Upon loading 2% lime extract, one cream sample exhibited a rise in TPC content from 1.8 to 54.05mg GAE/g and antioxidant activity from 19.34% to 95.35%. Cream samples infused with phytoextracts also showed notable antimicrobial activities. Conclusively, waste derived phytoextracts can be cost-effectively utilized in formulation of skin anti-aging creams.

Keywords: Antioxidant, natural anti-aging agents, plant bioactive compounds, polyphenols, DPPH assay

Graphical abstract



1. Introduction

Topical applications of antioxidants have become a common component in modern anti-aging skincare. Antioxidants are recognized for their ability to delay the aging process by scavenging harmful free radicals [1, 2]. Buildup of free radicals is the main culprit in the development of aging and age-related diseases [3]. All cells produce pro-oxidants like Reactive Nitrogen Species (RNS) and Reactive Oxygen species (ROS) during various physiological processes. However, when an excessive amount of these pro-oxidants is present, they interact with fundamental biomolecules and cause tissue damage [4].

Skin aging process is influenced by both intrinsic and extrinsic factors in environment. Internal factors include hormones, genetics and cellular metabolic processes. External factors include sun exposure, ionizing radiations, pollution, toxins and hazardous chemicals, etc. [5, 6]. With aging, the creation of harmful free radicals increases while the defensive mechanism that fights them internally weakens. This imbalance causes oxidative stress which leads to cellular structural damage and induces aging [7]. The most effective topical treatments utilize antioxidants, hormones, vitamins and minerals with potential to fight harmful free radicals involved in pre-mature skin aging. Antioxidants are widely recognized as effective anti-aging agents because of their ability to undergo oxidation on reaction with free radicals and protection of cellular biomolecules from oxidative stress [8], Antioxidants fight oxidative stress which aids in in the restoration of skin damage such as wrinkles, elasticity loss, premature aging. Previous studies have revealed that topical application of antioxidants can enhance the skin's self-repair process and shield it against prolonged environmental damages [9, 10].

Antioxidants can be synthetic or natural. Natural antioxidants include phenolic compounds, which can be substantially found in all plant parts, including fruits, vegetables, seeds, nuts, leaves, barks and roots [11]. The fundamental structure of phenolic compounds consists of a minimum of one phenol ring attached to one or more hydroxyl groups. Plants primarily contain groups of multiple phenol rings, therefore known as polyphenols. Polyphenols are among the second most common metabolites in plants, and they are widespread in nature. Currently, around 8000 distinct structural variations of polyphenols are found in plants [12].

Based on recent shift in consumers' demand towards eco-friendly skincare, the search for natural sources of antioxidants has become more intense [13]. The reason for the shift in demand is related to the known fact that synthetic products impose detrimental effects on the environment and human health [14,15].

Huge quantities of vegetable and fruit waste are produced on a daily basis, often ending up in landfill disposal sites without being fully utilized. This organic waste give rise to various environmental issues related to leachate production, biodegradability, and methane emissions [16]. With proper management, these huge quantities of organic waste offer the potential to serve as a valuable source of phytochemicals and antioxidants, with the advantage of being abundantly available at low costs [17].

Numerous research studies have explored the potential of organic waste from various sources, to serve as a bio resource for extraction of valuable bioactive compounds. These studies have successfully extracted several secondary metabolites, minerals, and vitamins from organic waste using diverse extraction methods [18, 19]. The present study also aims to optimize the extraction of phenolic compounds from seasonal waste of plant materials via Lime leaves (LL), Neem leaves (NL), Radish leaves (RL) and Carrot pulp (CP). Additionally, the goal is to employ an eco-friendly and inexpensive approach to naturally improve anti-aging properties of cosmetic cream samples through the infusion of phytoextracts.

2. Material and Methods

2.1. Plant waste material

Plant waste materials from local sources in Lahore were gathered during the winter season, and the specifics of the collected samples are presented in the table below as Table 1.

2.2. Chemicals and reagents

Folin-Ciocalteu Phenol Reagent (C10H5NaO5S), DPPH 2,2-Diphenyl-1-Picrylhydrazyl (C18H12N 5O6), Sodium Carbonate (Na2CO3), Ethanol (C2H6O), Gallic Acid standard (C7H6O5), Nutrient Agar, Deionized Water. Analytical grade

materials and reagents were utilized throughout.

Table 1. Sampling of plant waste materials.

samples	Collection site
Azadirachta indica leaf	Gardening waste
Citrus aurantifoulia leaf	Gardening waste
Raphanus sativus leaf	Food waste
Daucus carota pulp	Juice waste

2.3. Preparation and extraction of samples

Initially, plant waste materials were collected, thoroughly washed, and subsequently air-dried to eliminate moisture. These dried samples were then finely grounded into a powder. The resulting dry powder was used for extracting phenolic content employing water and ethanol as extraction solvents. The different extraction techniques included: Boiling (2-3 min), Shaking (30 min) and Soaking (30 min and 4-6 hrs.). The ratio of plant material (in grams) to solvent (in milliliters) was consistently maintained at 1:50, for all extractions.

2.4. Evaluation of antioxidant potential in plant extracts

The antioxidant potential of samples was calculated through Folin-ciocalteu's and DPPH assay.

2.4.1. Evaluation of free radical scavenging activity

Method for determination of scavenging activity was followed with slight modifications [20, 21]. To begin, a reaction mixture containing 3mL pure ethanol, 0.5mL plant extract and 0.30mL DPPH (0.5mM) solution was mixed and total volume up to 3.8mL. The control sample contained 0.30mL DPPH (0.5mM) radical solution and 3.5mL pure ethanol. The blank sample contained 0.5mL plant extract and 3.3mL pure ethanol. The reaction mixture was thoroughly mixed and left for incubation for 100min at 25°C room temperature. After the incubation period, the absorbance of the yellow coloration of reaction mixture was measured through UV-Vis spectrophotometer at 517nm wavelength.

2.4.2. Evaluation of total phenolic content

The method for calculation of TPC was adopted with slight modifications [22, 23]. Initially, a reaction mixture containing 0.5mL Folin-Ciocalteu's reagent, 0.5mL plant extract and 5mL deionized water was mixed and allowed to sit for 3-4 min. Later, 1.5mL Na_2CO_3 (20%) solution was mixed in and final volume was adjusted to 10mL. The solution mixture was thoroughly mixed and left for incubation for 2 hours in dark. After the incubation period, the absorbance of the blue coloration was measured through UV-Vis spectrophotometer at 765nm wavelength.

The content of Total Phenolic Content (TPC) was measured by the following equation:

TPC (g/g) = 2A * 1.957 * (L1/L2 * M)

L1 = the total volume of extract solution (ml)

L2 = the volume of extract solution used for analysis (ml)

M= the mass of extracts in (g)

A= the absorbance of reaction mixture at 540nm.

2.5. Evaluation of antioxidant potential in cosmetic samples

2.5.1. Infusion of cosmetic samples with plan extracts

This phase of research included evaluation of antioxidant activity and cosmetic properties of formulated cream samples after loading phytoextracts into four locally available selected cold cream samples. For confidentiality, the names of the cosmetic creams are not disclosed here. Plant extracts which showed superior antioxidant activities were sundried into solid residue and used further for incorporation in to the cream samples. 2% of extracts were infused in to the cream samples and homogenized with a vortex mixer.

2.5.2. Preparation of cosmetic extracts

Method defined by study in 2017 was followed to make cosmetic extracts from cream samples [24]. Stock solutions were produced by dispersion of 10% cream in a solution of ethanol-water (70:30) solvent. The stock solutions were left to sit for 30 minutes with periodic shaking. Later, the stock solutions were centrifuged for 10min at 5000rpm. The clear supernatant was collected and filtered after centrifugation. The filtrate was used for further analysis.

2.5.3. Evaluation of antioxidant potential in prepared cosmetic extracts

The antioxidant activity of cosmetic extracts prepared from formulated cream samples (Infused with 2% extract) and

control cream samples (Original cream) was further assessed using the DPPH radical and the folin-ciocalteu's assay.

2.5.4. Parameters in creams:

Some of the chemical, physical and biological properties of the formulated creams that are essential for their stability and customer acceptance were also examined.

a) color

Control and formulated samples were visually examined to observe the change in color.

b) Odor

Control and formulated samples were examined for any odor change by smelling them.

c) Appearance and Texture:

Both control and formulated samples underwent visual assessment, additionally the changes in appearance and texture were also detected through topical application.

d) Homogeneity:

The homogeneity of the control and formulated creams was determined through touch and visual appearance.

e) pH measurement:

0.5g cream sample was dispersed in 50mL distilled water. The pH was then measured by dipping the pH meter into the beaker to a depth of 0.5cm.

f) Microbial growth in cream samples:

By using the streak plate method, the formulated and control cream samples were inoculated on the agar media plates. The nutrient plates were kept in the incubator for 24 hours at 37 °C temperature. After growth on media, the microbial colonies were counted with the aid of a colony counter.

2.6 Statistical Analysis

The statistical analyses of the data were analyzed with SPSS computer software version 20.0. Significant differences for the treatments mean data were evaluated by $P<0.05$ through the least significance difference (LSD) method. Microsoft excel (2016) was used to draw figures.

3. Results and Discussion

3.1. Total Phenolic Content

For quantification of TPC content, a calibration curve of Gallic acid was prepared with standard concentrations ranging from 0.05 -0.5mg/ml (R^2= 0.988). TPC concentrations were calculated through the standard curve, TPC values are expressed as milligrams Gallic acid equivalent per gram of sample (mg GAE/g). The solubility of chemical constituents and polarity of solvents play an important role in extraction of TPC [25,26].

A calibration curve of Gallic acid was prepared by making phenol stock solution. And then from stock solution, dilutions of different concentrations (0-0.5 mg/ml) were prepared by adding distill water to make calibration curve. The calibration curve is shown in figure 1.

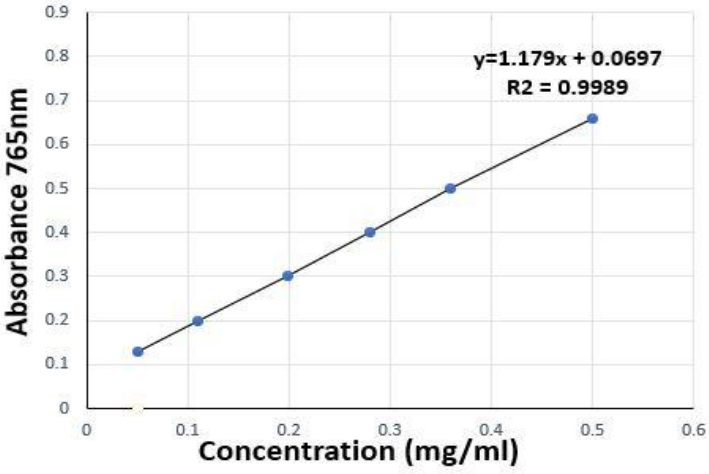
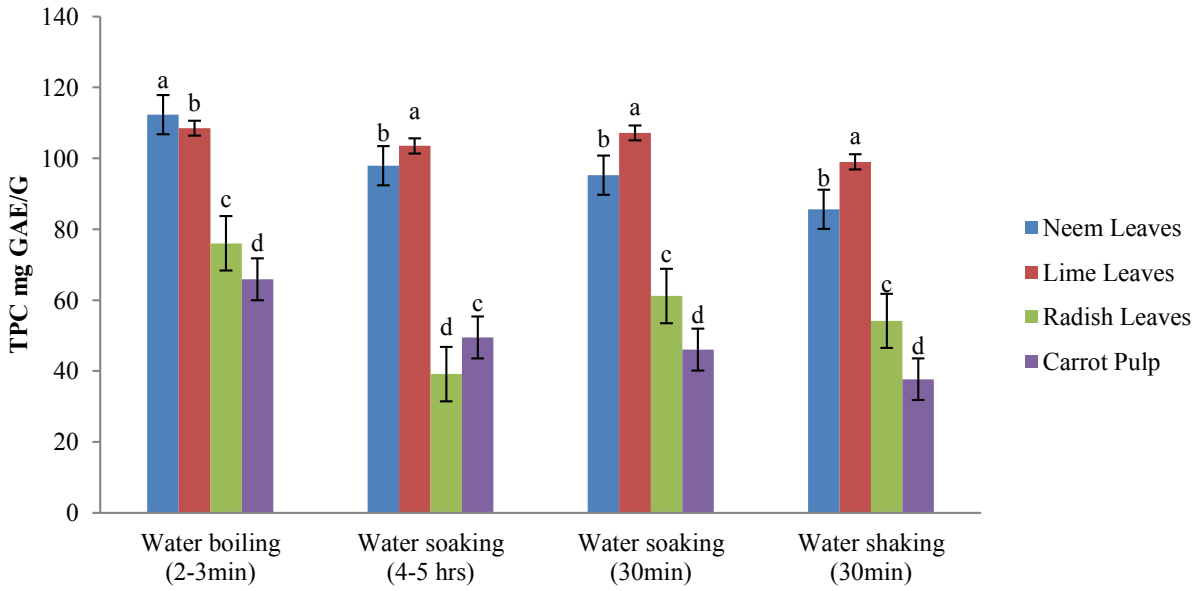


Figure 1. Calibration curve of gallic acid.

In order to optimize extraction of TPC in this work, two solvents of extraction and four extraction conditions were employed. The findings showed that aqueous extracts outperformed ethanolic extracts in their ability to extract TPC from plant waste (See figure 2 & 3). It is probable due to the stronger polarity of water and high aqueous solubility of phenolic compounds present in plant materials [27, 28]. Phenolic content in all extracts prepared with an aqueous solvent was reported in the range of 37.68-112.3mg GAE/g (see Figure 3). In contract, extracts prepared with ethanol as the solvent showed the TPC values in range of 5.45-101.97mg GAE/g. Water demonstrated better potential as an extraction solvent than ethanol (Figure 2 & 3). Water's polarity makes it an effective, quick, inexpensive, facile, and cleaner option for extraction of phenolic contents from plant materials [29]. The influence of various extraction conditions on achievable yields

of phenolic content was studied by application of four different extraction conditions: Shaking 30min, shaking 4-6hrs, boiling 2-3 min and soaking 30min. The results revealed that the highest TPC for all plant materials was produced on boiling them in water for two to three minutes (Figure 2). Studies suggested that extraction at high temperatures tend to have a positive influence on to the extraction of polyphenols. In general, Neem and lime extracts consistently exhibited significantly higher TPC values compared to carrot and radish extracts prepared in water at different extraction conditions. In contract, highest phenolic content in ethanol solvent was extracted by soaking the plant material for a long period of 4-6 hours (Figure 3).

Figure 2. Effect of extraction conditions on TPC content evaluated by Folin-Ciocalteu assay (Aqueous extracts). Vertical bars represent ± S.D. Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$).



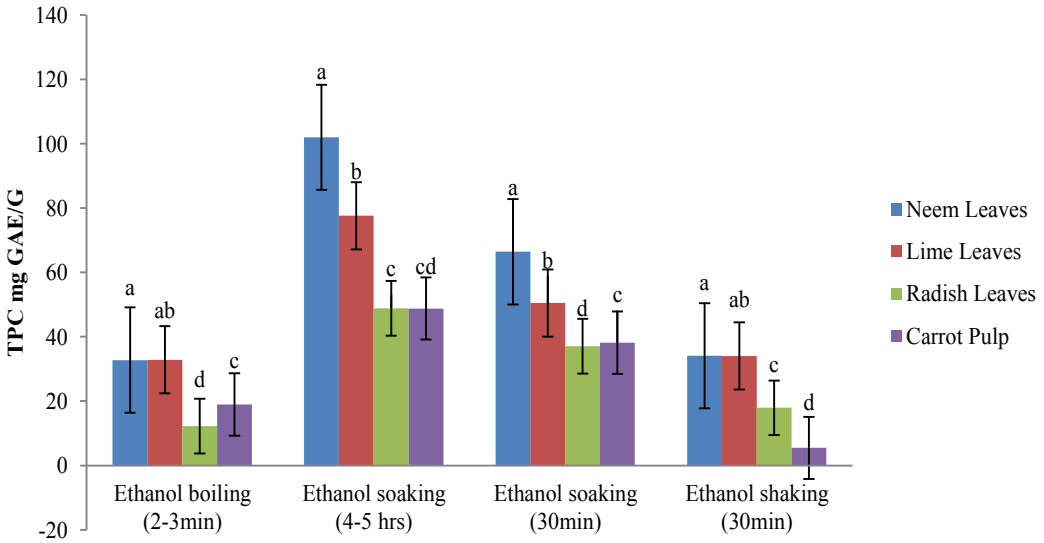


Figure 3. Effect of extraction conditions on TPC content evaluated by Folin-Ciocalteu assay (Ethanolic extracts). Vertical bars represent ± S.D. Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$

Once again, Neem and lime leaves followed the same trend as it was in aqueous solvent and produced higher TPC than other plant materials in ethanolic solvent as well. Neem leaf extracts exhibited the greatest phenolic content overall, measuring 112.3mg GAE/g in aqueous solvent and 101.97mg GAE/g in alcoholic solvent, declaring Neem leaves as the waste material with best antioxidant potential in this study.

3.2. Free radical scavenging activity

DPPH assay is a widely adopted method used to evaluate the free radical scavenging activity of natural antioxidants in plants extracts [30]. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical which undergoes a color change to deep purple upon gaining H+ from antioxidants, hence providing a quick and facile method to measure antioxidant activity through spectrophotometry [31]. The prepared extracts were assessed for their antioxidant capacity using the DPPH assay and were reported as a percentage (AA %).

Measurements taken by DPPH assay revealed significant levels of antioxidant activity in all plant materials.

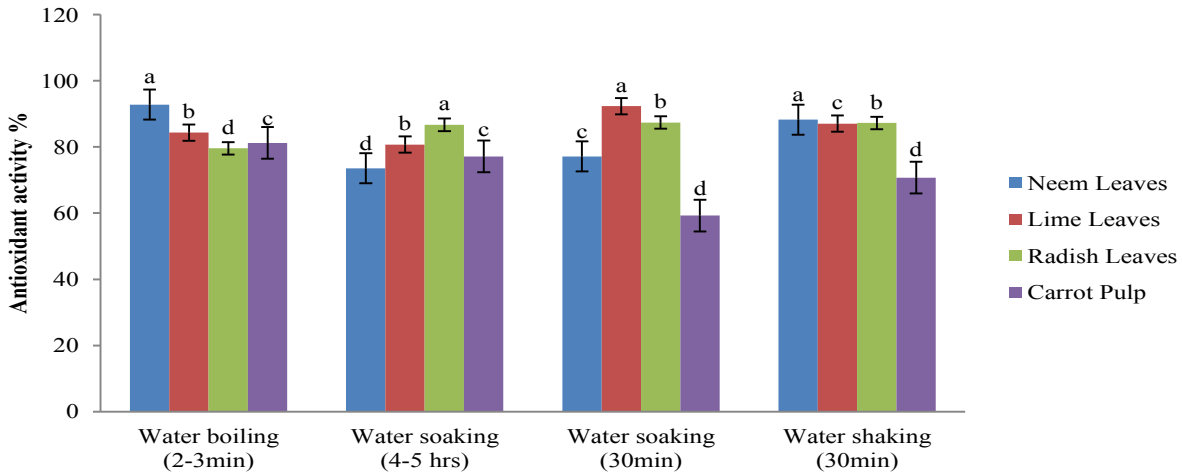


Figure 4. Antioxidant activity in aqueous extracts of four plant materials evaluated by DPPH assay. Vertical bars represent ± S.D. Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$).

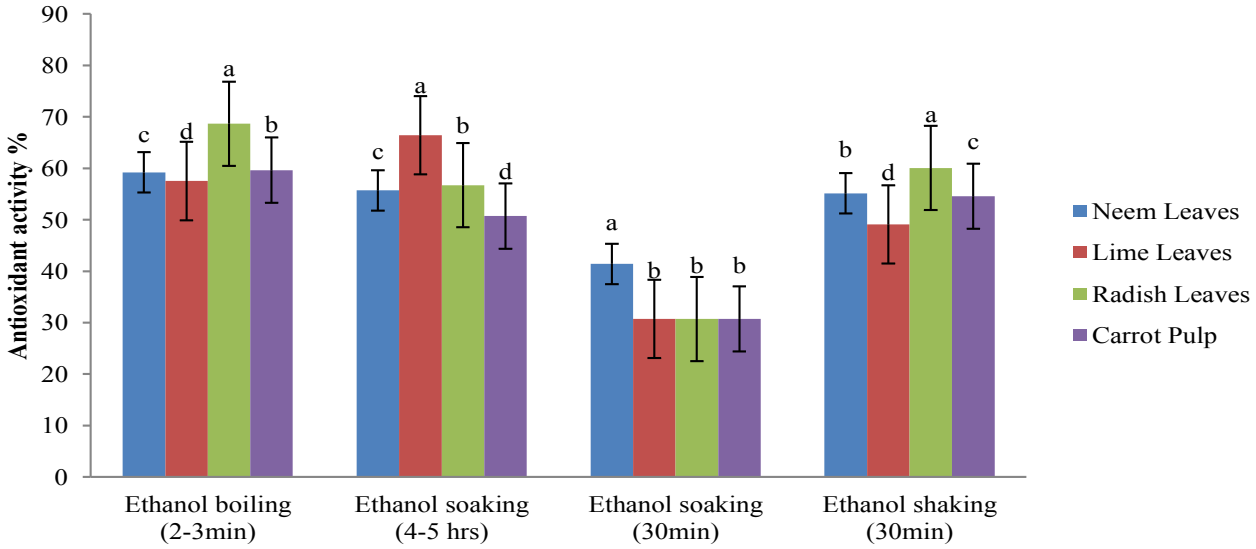


Figure 5. Antioxidant activity in ethanolic extracts of four plant materials evaluated by DPPH assay.Vertical bars represent ± S.D. Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$)

All extracts produced in aqueous solvent under various conditions showed the range of antioxidant activity from 59-92% (Figure 4). In contrast, ethanolic extracts showed a range from 30-68% antioxidant activity (Figure 5).The highest free radical scavenging activity of 92% was again observed in the aqueous Neem extracts prepared through 2-3 min of boiling, which also correlated with the maximum TPC values obtained under same conditions for Neem. Based on the available literature, no general understanding of correlation between total phenolic content and antioxidant activity can be made. As some researchers discovered a great correlation between two [32], however other researchers could not identify any correlation between phenolic content and antioxidant activities [33,34]. This can be due to the variations in extract preparation techniques, how findings are interpreted, the kind of analytical method used, the influence of interfering compounds (carotenoids, saccharides and ascorbic acid), etc. [35].

The findings of the current study were in agreement with previous study who found no correlation between the phenolic content and antioxidant potential in alcoholic extracts of plant materials [36]. But, TPC and antioxidant activity were shown to be significantly correlated in aqueous extracts. The findings of the aqueous extracts were in line with other research studies, which showed that the amount of total phenol increases with an increase in antioxidant activity [37,38].

Briefly, it can be determined that high polarity extraction solvents result in extraction of high TPC quantities when compared with low polarity solvents [37,39]. The variations in extraction yields may result from the ability of high-polarity solvents to break down hydrogen bonds within the phenol structure, thereby improving their solubility.

Total phenolic content (TPC) values extracted from other plant materials by employing various extraction techniques and extraction solvents are mentioned in Table 2.

3.3. Antioxidant Potential of Formulated and Control Cream Samples

Cosmetic extracts of all cream samples were prepared to determine the total phenolic content of creams through Folin-Ciocalteu's assay. Figure 6 clearly depicts that the antioxidant potential of the formulated creams (infused with 2% plant extract) significantly increased when compared to the control samples (without extract). On comparison to creams infused with Neem extract (32.5 - 64.9mg GAE/g); creams infused with Lime extract (42.5 - 100.3mg GAE/g) showed greater TPC values.

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Table 2. Comparison of TPC values found in other organic waste materials.

Plant material	Extraction solvent	TPC value (mg GAE/g)	Extraction Technique	References
Mango peel	Methanol/water (20:80)	525	Centrifugation	[40]
Apple peel	Methanol/water (20:80)	245	Centrifugation	[40]
Grapefruit waste	Ethanol	71.0	Ultrasonication	[41]
Artichoke waste	Ethanol/ butanone (50:50)	514.2	Stirring and aging	[42]
Golden rod	Ethanol/ butanone (50:50)	251.4	Stirring and aging	[42]
Neem leaves	Aqueous	112.3	Boiling	Present study
Lime leaves	Aqueous	108.5	Boiling	Present study
Radish leaves	Aqueous	76.03	Boiling	Present study
Carrot pulp	Aqueous	65.84	Boiling	Present study

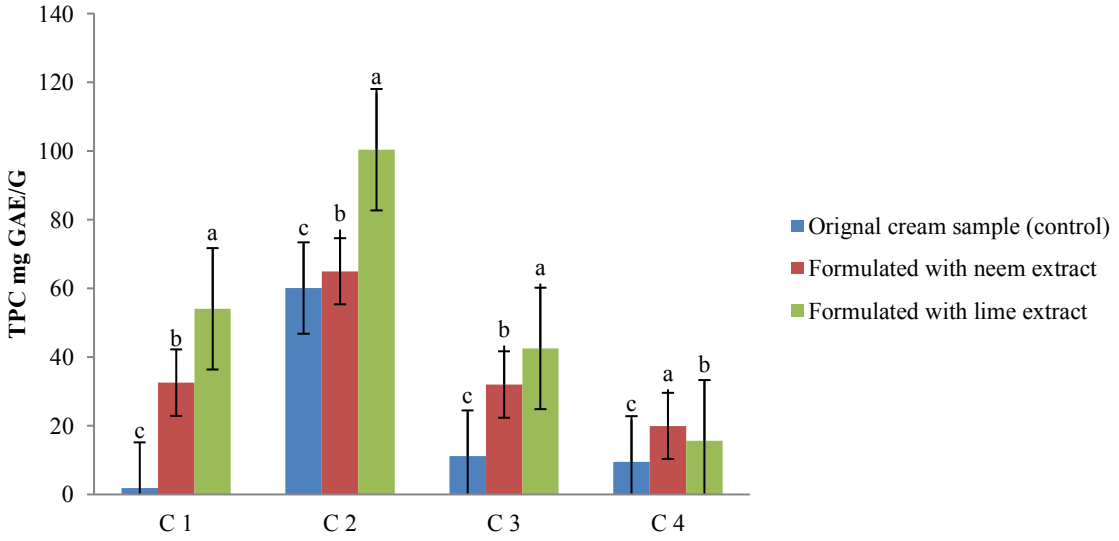

Figure 6. Effect of 2% phytoextracts infusion on TPC values of four locally available creams (C1, C2, C3, C4). Vertical bars represent ± S.D. Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$)

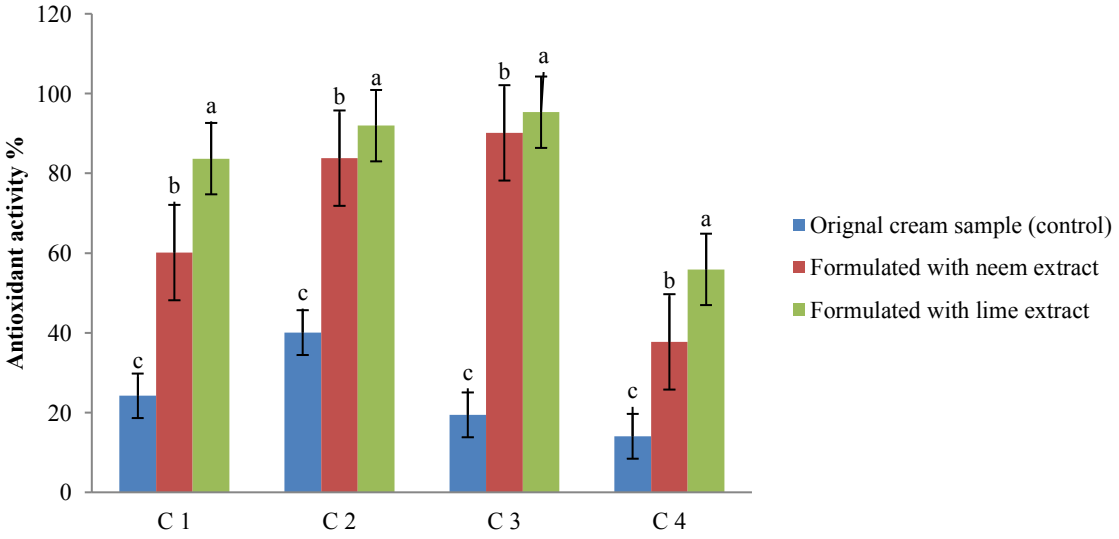

Figure 7. Effect of 2% phytoextracts infusion on free radical scavenging activity of four locally available creams (C1, C2, C3, C4). Vertical bars represent ± S.D. Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$).

Free radicals scavenging activity in cosmetic extracts displayed a significant boost after infusion of creams with plant extracts. Figure 7 illustrates the DPPH scavenging activity of the cosmetic cream samples. Scavenging activity of creams infused with lime extracts (ranging from 55.91% to 95.35%) exhibited superior potential than creams infused with neem extracts (ranging from 37.74% to 90.14%). Thus, the findings of this study undoubtedly validate the utilization of this environmental-friendly approach to naturally enhance antioxidant potential in cosmetic creams by incorporating plant waste extracts

3.3.1. Effect of phytoextracts infusion on various parameters in cosmetic creams.

The evaluated parameters included color, odor, homogeneity, appearance and texture, pH and antimicrobial activity of creams.

a) ***Color:*** Cream samples were visually checked for any color changes. Based on visual observations, a notable change in

color of cream samples was found after infusion. It was evident that, the 2% Neem plant extracts gave the cream samples a brownish color after addition. However, adding 2% lime plant extracts gave the cream samples a yellowish tint.

b) ***Odor:*** Each cream sample was smelled to detect for any odor changes. The aroma of the original creams was pleasant due to the fragrances added by manufacturers. However, after infusion of 2% plant extracts, a detectable change in the scent of samples

was noticed. It was no longer on the fragrance side.

c) ***Homogeneity:*** By touch and visual inspection, the homogeneity of the cream formulations was evaluated. Visually, the cream samples showed the uniform distribution of extracts.

d) ***pH measurement:*** When evaluating the efficacy and stability of cosmetics creams for skin, pH analysis is a crucial factor. The acceptable pH range for skin lies between 4.5 and 6, so formulations designed for skin application should fall within this range [43]. The pH of the original and formulated creams was analyzed, and the pH change was compared. Following the addition of 2% extract, the pH values of the cream samples clearly appear to be lower than the pH values of the control cream sample (see figure 8). Although the formulations examined for this study had pH values within the acceptable range for skin pH.

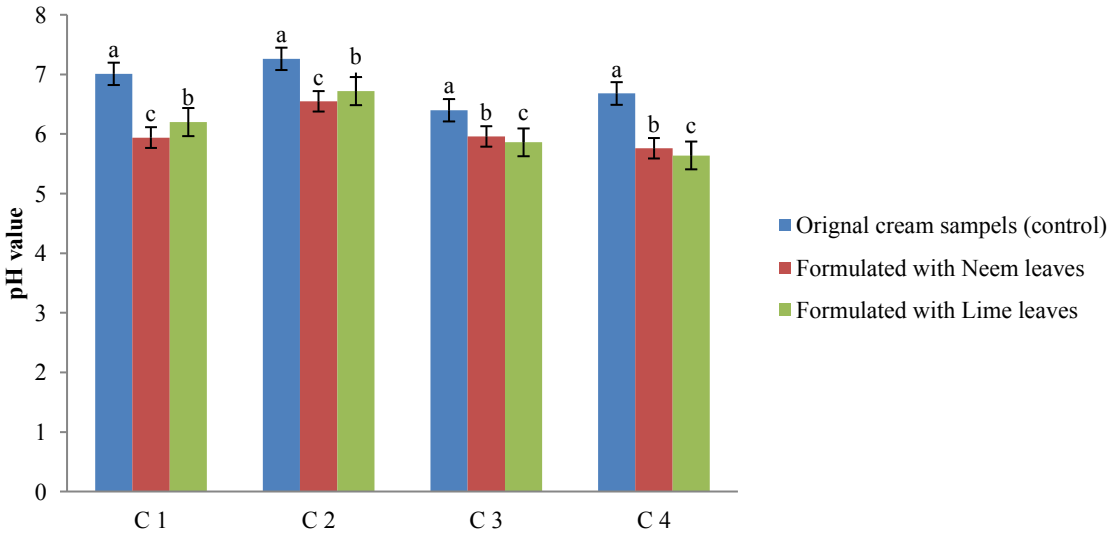

Figure 8. Effect of plant extracts on pH of four locally available creams (C1, C2, C3, C4). Vertical bars represent ± S.D. Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$).

e) ***Appearance and Texture:*** All examined samples were found to have a smooth texture. The creams infused with Neem extracts showed a semisolid light brown appearance, while those with Lime extract had a yellowish semi-solid appearance.

4. Conclusion

From this research study, it can be deduced that boiling for 2-3 minutes is the best extraction condition which allowed the quick and maximal TPC extractions from four materials (Neem leaves, Lime leaves, Radish leaves, and Carrot pulp). The optimum solvent for extracting polyphenols was discovered to be water. Azadirachta indica leaf extracts outperformed other extracts in terms of antioxidant and free radical scavenging activity. Additionally, infusion of plant extracts significantly increased the antioxidant activity of cosmetic creams. The findings suggest that plants waste is a bio resource providing cheap, readily available, environmentally friendly, and natural source of anti-aging chemicals. It is necessary to further isolate and purify polyphenols from extracts, examine their mechanisms of action, and determine their stability when used as a bioactive component in skincare, in order to estimate their true

cosmeceutical worth.

Conflicts of Interest

There are no conflicts of interest reported by the authors.

Authors contribution

Wajeeha Rafiq (W.R), Maira Kaleem (M.K). and Sofia Nosheen (S.N).; Conceptualization, W.R. and M.K.; formal analysis, W.R. and M.K.; methodology, W.R.; software, W.R. and M.K.; writing—original draft preparation, S.N.; supervision, W.R.; writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability statement

All the data which is presented in this article can be given on request from the first author.

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