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CLINICAL RESEARCH

Recovery Optimization of Plant Waste Derived Antioxidants and their Incorporation in Cosmetic Creams to Enhance Antioxidant Potential: A Cost-Effective Approach

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ABSTRACT

SUMMARY

Plants waste is considered a valuable resource for extraction of valuable antioxidants. The prime objective of this study was to optimize extraction of phenolic compounds from four plant waste materials and quantification of their phenolic content to utilize their phytoextracts for loading into cosmetic creams. Dried residue of four plant materials: Neem leaves (NL), Lime plant leaves (LL), Radish leaves (RL) and Carrot pulp (CP) was extracted using water and ethanol solvent under conditions i.e., boiling, soaking and shaking. Phenolic activity was accessed by Folin-ciocalteu and DPPH free radical scavenger method. Neem leaves extract showed the highest TPC of 112.3 mg GAE/g and 92.82% free radical scavenging ability while the other samples also showed significant amounts. Aqueous solvent extracted higher TPC as compared to ethanolic solvent. A positive correlation was observed between TPC and antioxidant activity in aqueous extracts while ethanolic extracts showed no significant correlation. A significant raise in antioxidant activity of creams samples was reported upon loading them with phytoextracts. In one cream sample, the TPC content went from 1.89 to 54.05 mg GAE/g after loading with lime plant extracts. In another sample, the free radical scavenging activity of cream raised from 19.34% to 95.35% by loading cream with 2% lime plant extract. Cosmetic creams containing plant extracts also indicated lesser microbial growth. Slight changes in other parameters e.g., pH, color and odour were also detected in formulated cosmetic products. Conclusively, plant wastes can be used as a valuable resource in cosmetic formulations for skin anti-aging.

KEYWORDS

Sustainability; Waste reduction; Antioxidant; Extraction

INTRODUCTION

Anti-aging treatments for skin nowadays mostly include topical application which contains antioxidants, these anti-aging antioxidants have the capability to slow down the inevitable process of aging by scavenging destructive free radicals [1,2]. It is a known fact that damage from accumulation of free radicals is a leading cause of aging and age related defects [3]. Pro-oxidants like Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are formed in all cells and play an essential role in regulation of several physiological processes in cell, but on availability in excess, they react with structural biomolecules leading to injury of tissue [4].

Skin aging is a complex process that depends on extrinsic or external factors like pollution, ionizing radiation, chronic light exposures, toxins and harmful chemicals etc. and intrinsic or internal factors like cellular metabolism, genetics, hormones and metabolic processes etc. [5,6]. The production of destructive free radicals rises with age while the encountering defense mechanism which internally fights them decreases which creates an imbalance and lead to damage of cellular structures which further leads to aging [7].

The best performing topical treatments involves antioxidants, vitamins, hormone estrogen and minerals which shows the ability to scavenge free radicals from skin cells. Antioxidants have been accepted as anti-aging agents because they can oxidize themselves on interaction with harmful radicals and save the vital molecules from oxidative stress [8], which helps in repairing skin damage, such as wrinkling, loss of elasticity and premature aging. Experiments have revealed that, when antioxidants are applied topically, they can enhance self-repair and protect against long term damage from environmental influences [9-12].

Antioxidants are basically classified in two groups, which include natural and synthetic antioxidants [8]. Natural antioxidants are basically phenolics which may occur in almost every part of plants [13], such as vegetables, nuts fruits, seeds, roots, leaves and barks. The basic core of phenolic compounds is at least made up of one phenol ring with one or more attached hydroxyl groups. In plants, they mostly contain many phenolic rings which make them polyphenols. Polyphenols are widely distributed in nature, and they are considered the second most abundant metabolite in plants. Almost 8000 different structures of phenolic compounds are recognized in plants currently [14]. Recently the search for natural antioxidants from plant-based materials has intensified because of consumers' desire to use natural cosmetic formulations that are eco-friendly [15]. The observed shift in consumers' desire could be because synthetic products have been proved to cause negative effects on environment and health [16,17].

Large amounts of vegetable and fruit waste is produced by many food industries. This waste ends up in landfills and cause problems because of leachate, biodegradability and methane emissions [18]. So globally a huge amount of fruit and vegetable waste gets wasted, this same waste can also be utilized to serve as a potential source of bioactive compounds and phytochemicals. By products obtained from vegetable and fruit industry are getting attention because they are available in large quantities with inexpensive costs [19]. Many kinds of vegetable by-products are considered to prove as a source of certain phytochemicals having antioxidant potential. Many vegetables have been studied on industrial scale as a potentially safe source of natural antioxidants e.g., polyphenols. Now special attention is given to the thought that vegetable and fruit wastes can serve as a rich source of phenolic compounds [20-25].

Current study aims at optimizing experimental conditions for extraction of bioactive compounds from plant waste viz Neem leaves (NL), lime plant leaves (LL), radish leaves (RL) and carrot pulp (CP). And to utilize an eco-friendly and cost-effective approach to enhance the anti-aging activity of locally available cosmetic creams by loading natural extracts.

MATERIALS AND METHODS

Plant Materials

All the samples were collected locally in the month of January 2019. The details of plant material collection are mentioned in table 1.

Table 1: Collection of plant materials.

Sample	Botanical Name	Part Used	Collection Site
Neem	*Azadirachta indica*	Leaf	Gardening waste
Lime	*Citrus aurantifoulia*	Leaf	Gardening waste
Radish	*Raphanus sativus*	Leaf	Food waste from market
Carrot	*Daucus carota*	Pulp	Juice waste

Chemicals

Reagents used in this experiment include DPPH 2,2-Diphenyl-1-Picrylhydrazyl (C18H12N5O6), Folin-Ciocalteu Phenol Reagent ($C_{10}H_5NaO_5S$), Ethanol (C_2H_6O), Sodium Carbonate (Na_2CO_3), Gallic Acid ($C_7H_6O_5$), Distilled Water, Nutrient Agar. All the chemicals and reagents used were of analytical grade.

Preparation of Samples and Extraction

All the samples were collected and washed and then dried under direct sunlight to remove their water content and grinded properly into powdered form. Then the dry powder was used to extract in water and ethanol solvents by using soaking (30 minutes & 4 hours - 6 hours), shaking (30 minutes) and boiling (2 minutes - 3 minutes) as different conditions. The ratios of plant material (g) and solvent (ml) was 1:50 respectively, for all extracts.

Determination of Antioxidant Potential in Plant Materials

In this research study, antioxidant potential of plant materials was determined by performing DPPH assay and folin-ciocalteu's assay.

Determination of free radical scavenging activity by DPPH assay

Briefly, a 3.8 ml of reaction mixture consisting of 0.5 ml of extract, 3 ml of absolute ethanol and 0.3 ml of 0.5 mM DPPH radical solution was prepared. The control set contained 3.5 ml absolute ethanol and 0.3 ml of 0.5 mM DPPH radical solution. The blank solution was prepared by adding 3.3 ml absolute ethanol and 0.5 ml extract. The tubes containing the reaction mixture were mixed thoroughly and incubated for 100 minutes at room temperature. The changes in color (violet to light yellow) were read at 517 nm using UV-Vis spectrophotometer [26,27].

Determination of TPC by Folin-ciocalteu's method

Firstly, a reaction mixture of 0.5 ml sample, 0.5 ml Folin-Ciocalteu's reagent and 5 ml distilled water was prepared and left for 2 minutes - 3 minutes. Then, 1.5ml 20% sodium carbonate solution was added and volume up to 10

ml. The reaction mixture was incubated for 2 hours in dark and then absorbance of the blue coloration was measured at 765nm in UV-Vis spectrophotometer [28-30].

Determination of Antioxidant Potential in Cosmetic Creams before and after Loading Extracts

Preparation of formulated creams

This phase of the study was conducted to evaluate the difference in antioxidant potential and various properties of cream after loading natural plant extracts in locally available creams. Two plant materials that showed superior antioxidant potential were dried to produce solid residue which was used further used for addition in four selected creams of locally available brands. The solid residue was finely grounded into fine powdered form and then 2% solid residue was added in collected cream samples and mixed thoroughly by using vortex mixer.

Preparation of cosmetic extracts

Stock solutions of creams were prepared by making 10% solution in a solvent mixture of water-ethanol (30:70). The suspensions of each cream in the solvents were incubated with periodical shaking for 30 minutes. After the incubation period ended, the solutions were centrifuged at 5000 rpm at room temperature for 10 minutes in a centrifuge. After centrifugation, the supernatant was filtered and clear solvent extract was collected and used for further evaluation [31].

Evaluation of antioxidant potential in cosmetic extracts

Cosmetic extracts obtained from formulated samples (creams with extracts) and control samples (creams without extracts) were used further to determine antioxidant potential by using DPPH radical scavenging assay and folin-ciocalteu's assay.

Evaluation of other parameters in formulated creams

Formulated samples were also analyzed for some of their physical, chemical and biological properties that are important for their acceptance by consumers and for their stability.

Color

Visual changed in color of control samples and formulated cream samples were observed.

Odor

Odor of both control and formulated cream samples were smelled and change in odor after adding extract was detected.

Appearance and texture

The change in appearance and textural feel of both control and formulated cream samples was detected visually.

Homogeneity

The formulations and control samples were tested for the homogeneity by visual appearance and by touch.

pH measurement

0.5 g of cream was dissolved in 50 ml of distilled water. Then pH meter was dipped to a depth of 0.5 cm in beaker to measure pH [32].

Test for microbial growth in formulated creams

The formulated creams and the control cream samples were inoculated separately on the plates of agar media by streak plate method. The plates were incubated for 24 hours in the incubator at 37°C. After the incubation period, plates were taken out and microbial colonies were counted and compared between both control and formulated samples.

RESULTS AND DISCUSSION

Total Phenolic Content

A calibration curve prepared with Gallic acid concentrations (0.05 mg/ml - 0.5 mg/ml) as standard was used to determine the total phenolic content in various extracts. TPC values calculated were expressed as milligrams Gallic acid equivalent per gram of sample (mg GAE/g). Extraction of phenolic content may be influenced by the polarity of the extracting solvent and the solubility of chemical components in the extracting solvent [33,34]. Therefore, two extraction solvents along with four different extraction conditions were implemented for the extraction of TPC in this study. The outcomes revealed that aqueous extracts proved to be more potent than ethanolic extracts in extraction of TPC from plant residues (Figure 1 & Figure 2). This is most likely because water has stronger polarity and improved solubility for phenolic contents found in plant residues [35,36]. The phenolic contents among all plant materials prepared in aqueous extracts were in range of 112.3 mg - 37.68 mg GAE/g (Figure 1). On the other hand, the phenolic contents in ethanolic extracts were in range of 101.97 mg - 5.45 mg GAE/g, which was lower than aqueous extracts (Figure 2). Because of water polarity, water is a fast, economic, simple and non-toxic option to efficiently extract phenolic components from plant materials [37,38].

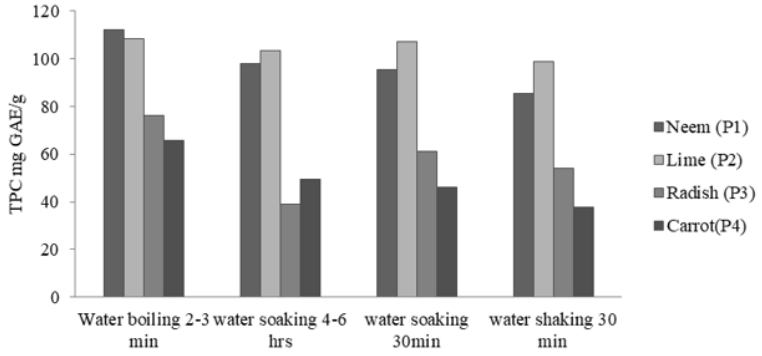

Figure 1: Comparative analysis on TPC of four plant materials evaluated by folin-ciocalteu method in aqueous extracts.

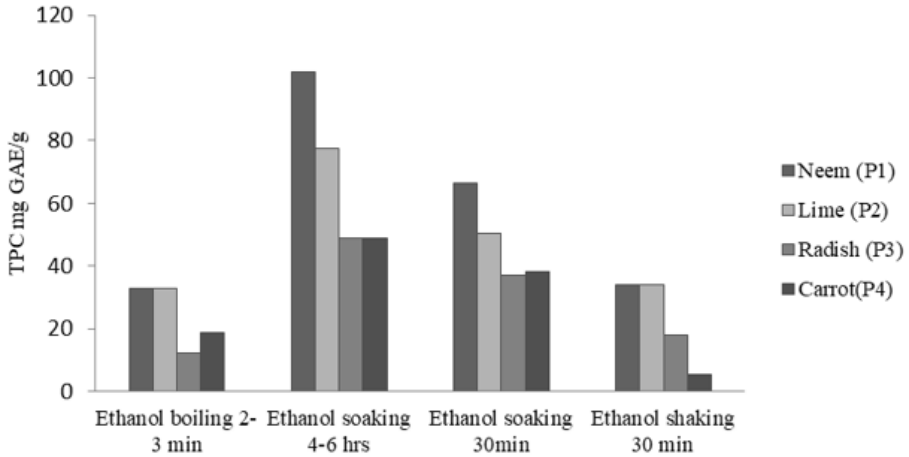

Figure 2: Comparative analysis on TPC of four plant materials evaluated by folin-ciocalteu method in ethanolic extracts.

Optimization of extraction condition to achieve maximum yields was also performed in this study. The effect of a range of extraction conditions including boiling 2 minutes - 3 minutes, soaking 30 minutes, soaking 4 hours – 6 hours, shaking 30 minutes was investigated to determine the yields of phenolic content from four plant materials. It is a safe conclusion to make that boiling of extracts for 2 minutes - 3minutes yielded the highest quantities of TPC from all four plant materials in aqueous extracts (Figure 1). A previous study done on effect of various solvent ratios and temperatures on extractability of phenolic compounds, also suggested that higher extraction temperatures cause a positive effect on the extractability of phenolic compounds [39,40]. Overall, Neem leaves and lime leaves showed exceptionally high yields of TPC than radish leaves and carrot pulp extracted under all conditions in aqueous solvent.

In case of ethanol as extraction solvent (Figure 2), it is clearly depicted that soaking of plant material for 4 hours - 6 hours in ethanol resulted in highest yields of TPC in comparison to other extraction conditions. But again, as mentioned above for aqueous extracts, the Neem leaves and lime leaves also gave the highest quantities of TPC than radish leaves and carrot pulp extracts prepared under all conditions in ethanol as solvent. Overall, the highest phenolic content of 112.3 mg GAE/g in water and 101.97 mg GAE/g in ethanol was found in Neem leaves extracts which makes them the plant material with exceptional antioxidant properties.

Free Radical Scavenging Activity

For the study of natural antioxidants, DPPH assay is a very popular tool for evaluation of the scavenging activity of various plant materials [41]. DPPH is a free radical which is also stable. On gaining H^+ from contributor, its solution starts gaining deep violet coloration which helps in providing an easy and quick method to evaluate antioxidants by spectrophotometry [42]. Antioxidant activity of the prepared extracts was determined by DPPH assay and expressed in terms of percentage.

Results obtained in this study showed a significant level of antioxidant activity in all extracts prepared from neem leaves, lime leaves, radish leaves and carrot pulp. Antioxidant activity achieved among various extracts prepared in water was in range of 92%-59% (Figure 3). On the other hand, the antioxidant activity of ethanolic extracts was achieved in range of 68%-30% (Figure 4). The maximum scavenging activity of 92% was obtained in Neem leaves extracts prepared from boiling 2 minutes - 3 minutes in water which was in correlation with the highest TPC content obtained. So, all the extracts showed greater variability among different conditions and predominant changes were detected in antioxidant activity of different plant material prepared under different extraction conditions.

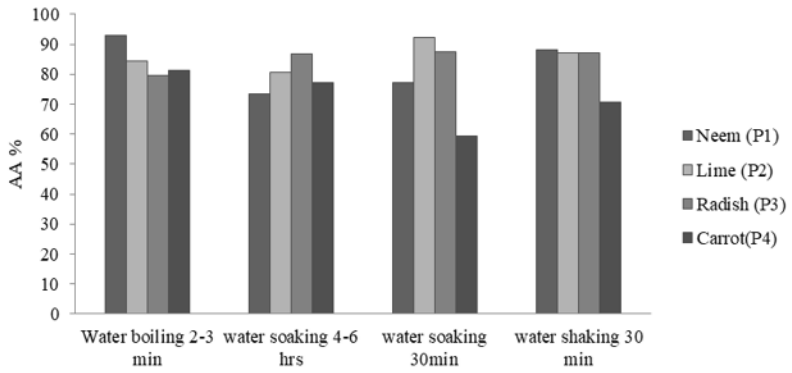

Figure 3: Comparative analysis on antioxidant activities of aqueous extracts evaluated by DPPH assay.

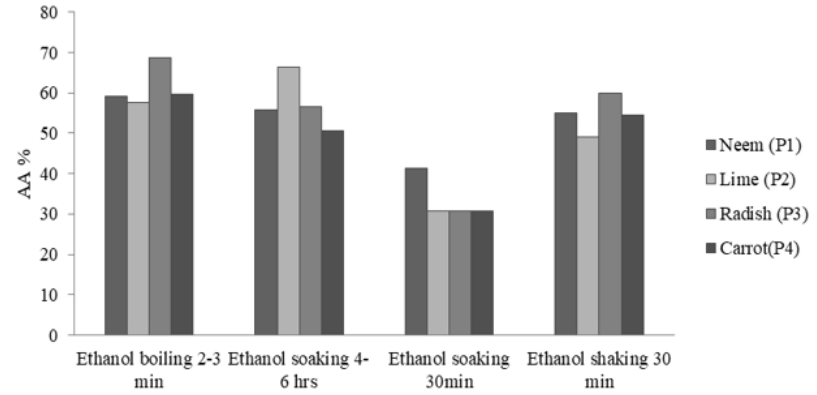

Figure 4: Comparative analysis on antioxidant activities of ethanolic extracts evaluated by DPPH assay.

According to the literature available, there's no general conclusion concerning the correlation between total phenolic contents and antioxidant capacity of plant material. Some authors found great correlation between total phenolic content and antioxidant capacity [43-47] while others could not find any correlation between these two [44,48,49]. The possible reason for this may be because of difference in extract preparation methods, interpretation of results, type of method used for analysis, effect of interfering substances (saccharides, carotenoids and ascorbic acid), etc. [50].

Results in current study showed no significant correlation between polyphenolic content and antioxidant activity of plant materials prepared in alcoholic extracts, which is in agreement with results reported by Ulewicz-Magulska and Wesolowski [51], where alcoholic extracts showed no significant correlation. While in aqueous extracts a significant correlation between TPC and antioxidant activity was observed. Results of aqueous extracts were consistent with previous studies, which reflected that antioxidative activity enhances with increasing total phenol content [52-54]. It could be established that, extraction solvents of high polarity show higher yields of phytochemicals as compared to low polarity solvents [54,55]. The alterations in yield may be attributed to the fact that high polarity solvents facilitate the breakdown of hydrogen bonds in phenol structure and enhances solubility [56].

Antioxidant Potential of Formulated and Control Cream Samples

The total phenolic content in formulated and control cream samples was evaluated by folin-ciocalteu's assay. It is evident from figure 5 that, the formulated cream samples (with 2% extract) showed higher antioxidant potential as compared to control samples (without extract). Formulated creams with Lime leaves extracts (42.51 mg GAE/g - 100.4 mg GAE/g) showed higher TPC than the creams formulated with Neem leaves extracts (32.59 mg GAE/g - 64.99 mg GAE/g).

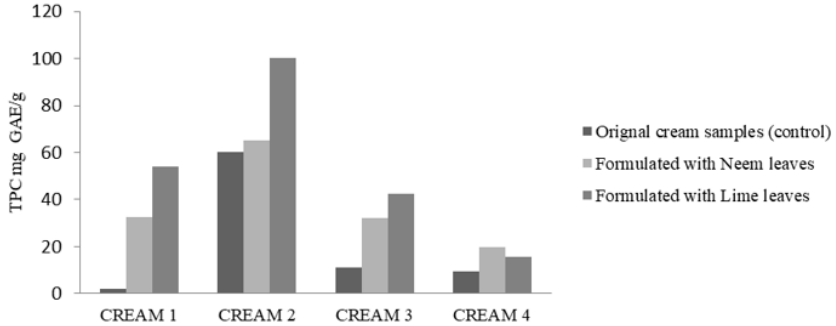

Figure 5: Evaluation of antioxidant potential in formulated creams by folin-ciocalteu method.

Analysis of the cosmetic extracts for free radical scavenging activity demonstrated a significant increase in antioxidant potential of cosmetic creams upon addition of natural extracts. Figure 6 shows DPPH scavenging activity of formulated cream samples containing extracts, where cream samples with Lime leaves extracts (55.91% - 95.35%) showed better antioxidant capacity followed by cream samples with Neem leaves extracts (37.74% - 90.14%). So, the results clearly demonstrated that addition of natural extracts derived from waste plant material provides an eco-friendly option to enhance the antioxidant activity and total phenolic content in cosmetic creams.

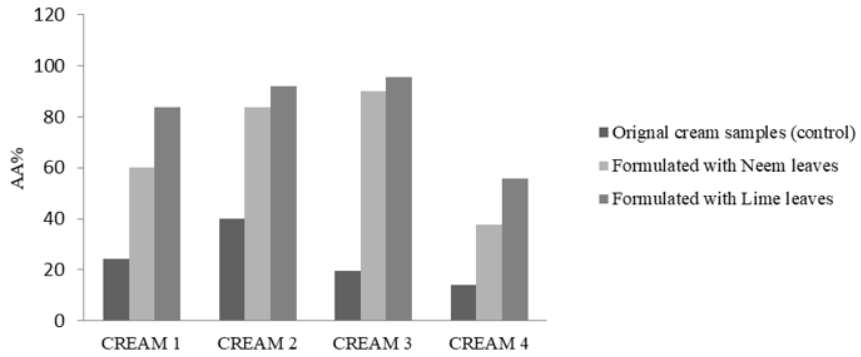

Figure 6: Evaluation of antioxidant potential in formulated creams by DPPH assay.

Effect on various parameters in formulated creams

The parameters evaluated were color, odor, homogeneity, appearance and texture, pH and microbial activity.

Color

Samples of creams were detected for any color change visually. Change in color was detected in original and formulated cream samples based on visual observations. It was clearly observed that, the addition of 2% Neem extracts imparted brownish color to the cream samples. On the other hand, addition of 2% Lime extracts imparted a yellowish hue to the cream samples.

Odor

All cream samples were smelled for their odor change. All the original cream samples had pleasant odor. On the other hand, a slight change in odor of formulated cream samples was detected after the addition of 2% plant extract. It was not on fragrance side anymore.

Homogeneity

The cream formulations were tested for the homogeneity by touch and visual appearance. On visual appearance, the creams showed homogenous distribution of extracts in creams.

pH measurement

The pH analysis of creams is a significant parameter when considering their efficacy and stability. The acceptable and normal range of pH for skin is 4.5-6, so the formulations with their purpose of application on skin should have pH near this range [57]. pH analyses were performed on formulated creams and original creams and change in pH values was compared. The pH of the cream samples after adding 2% extract clearly seems to be lower than the pH of originally available creams. Although the formulations analyzed in this study, found to have pH values nearby normal range (Figure 7).

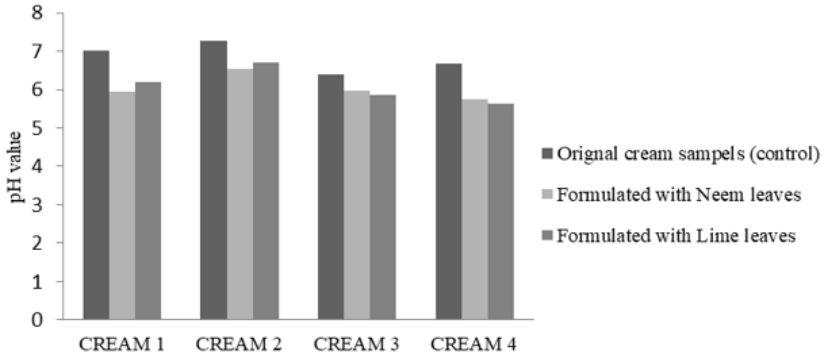

Figure 7: Change in pH value of formulated and control cream samples.

Appearance and texture

All the creams samples showed smooth texture. Appearance of creams formulated with Neem extracts was in form of light brown semisolid cream and for the Lime extract formulated creams it was as yellowish semisolid cream. No significant change in texture of cream samples was detected before and after adding extracts. But appearance of cream samples slightly changed on adding extracts.

Microbial activity test

Cosmetic products with high antioxidant potential also have an inhibition effect on growth of microbes present over skin [58]. Microbial activity in cream samples before and after adding extracts is shown in Figure 8.

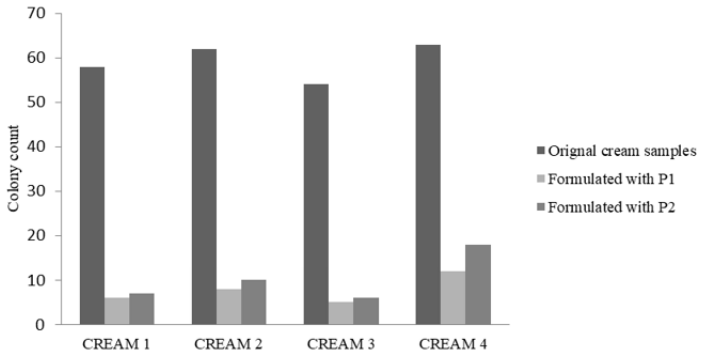

Figure 8: Microbial growth in formulated and control cream samples.

It is clearly evident from the results that, the formulated cream samples containing extracts showed lesser microbial growth than the original cream samples without extract. These results can be justified by the fact that, antioxidants can have antimicrobial effect when they were added in samples. Cream samples with Neem extracts showed more antimicrobial effect than the cream samples containing Lime extracts. The results of this study suggested that natural extracts obtained from plant materials contain significant amounts of phytochemicals and have a good potential for eco-friendly cosmetic products formulation and development.

CONCLUSION

From the current study, it may be concluded that optimum conditions which allowed fast and maximum extractions of TPC from four samples (Neem leaves, Lime leaves, Radish leaves and Carrot pulp), were obtained through boiling for 2 minutes - 3 minutes. Water was found to be the best solvent for extraction of polyphenols. The extracts of *Azadirachta indica* (neem) leaves demonstrated superior antioxidant and free radical scavenging activities over other extracts. Also, addition of extracts into cosmetic creams significantly improved their

antioxidant potential. From the results, it can be concluded that plant waste materials can serve as a good source of phenolic compounds and can be useful further as a cheap, eco-friendly and natural source of topical antioxidants. Further isolation and purification of polyphenols from extracts and study of their mechanism of actions, their stability when added as an active ingredient in cosmetics, are needed to be known for estimation of their cosmeceutical value.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Mukherjee PK, Maity N, Nema NK et al. (2011) Bioactive compounds from natural resources against skin aging. Phytomedicine 19(1): 64-73.
2. Hoang HT, Moon JY, Lee YC (2021) Natural antioxidants from plant extracts in skincare cosmetics: Recent applications, challenges and perspectives. Cosmetics 8(4): 106.
3. Hussain F and Kayani HUR (2020) Aging-Oxidative stress, antioxidants and computational modeling. Heliyon 6(5): e04107.
4. Griendling KK, Touyz RM, Zweier JL et al. (2016) Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: A scientific statement from the American Heart Association. Circulation Research 119(5): e39-e75.
5. Parrado C, Mercado-Saenz S, Perez-Davo A et al. (2019) Environmental stressors on skin aging. Mechanistic insights. Frontiers in Pharmacology 10: 759.
6. Gu Y, Han J, Jiang C et al. (2020) Biomarkers, oxidative stress and autophagy in skin aging. Ageing Research Reviews 59: 101036.
7. Allemann IB and Baumann L (2008) Antioxidants used in skin care formulations. Skin Therapy Letter 13(7): 5-9.
8. Babbush KM, Babbush RA, Khachemoune A (2020) The therapeutic use of antioxidants for melasma. Journal of Drugs in Dermatology 19(8): 788-792.
9. Comino-Sanz IM, López-Franco MD, Castro B et al. (2021) The role of antioxidants on wound healing: A review of the current evidence. Journal of Clinical Medicine 10(16): 3558.
10. Abdelsattar AS, Makky S, Nofal R et al. (2022) Enhancement of wound healing via topical application of natural products: *In vitro* and *in vivo* evaluations. Arabian Journal of Chemistry 15(6): 103869.
11. Neves JR, Grether-Beck S, Krutmann J et al. (2022) Efficacy of a topical serum containing L-ascorbic acid, neohesperidin, pycnogenol, tocopherol, and hyaluronic acid in relation to skin aging signs. Journal of Cosmetic Dermatology 21(10): 4462-4469.
12. Kyi MT (2020) A split-face double-blind randomized placebo-controlled trial of the efficacy of kakadu plum extract for reduction of periorbital wrinkles. In Rangsit Graduate Research Conference 15(2563): 2837-2858.

13. Asif M (2015) Chemistry and antioxidant activity of plants containing some phenolic compounds. Chemistry International 1(1): 35-52.
14. Dai J and Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15(10): 7313-7352.
15. Jesumani V, Du H, Aslam M et al. (2019) Potential use of seaweed bioactive compounds in skincare - A review. Marine Drugs 17(12): 688.
16. Khan AD and Alam MN (2019) Cosmetics and their associated adverse effects: A review. Journal of Applied Pharmaceutical Sciences and Research: 1-6.
17. Bijauliya RK, Alok S, Kumar M et al. (2017) A comprehensive review on herbal cosmetics. International Journal of Pharmaceutical Sciences and Research 8(12): 4930-4949.
18. Oktiawan W, Wardhana IW, Sutrisno E et al. (2019) Municipal solid waste management using bioreactor landfill in the treatment of organic waste from Jatibarang Landfill, Semarang-Indonesia. In E3S Web of Conferences 125: 07002.
19. Figuerola F, Hurtado ML, Estévez AM et al. (2005) Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chemistry 91(3): 395-401.
20. Celano R, Docimo T, Piccinelli AL et al. (2021) Onion peel: Turning a food waste into a resource. Antioxidants 10(2): 304.
21. Castrica M, Rebucci R, Giromini C et al. (2019) Total phenolic content and antioxidant capacity of agri-food waste and by-products. Italian Journal of Animal Science 18(1): 336-341.
22. Sindhu R, Gnansounou E, Rebello S et al. (2019) Conversion of food and kitchen waste to value-added products. Journal of Environmental Management 241: 619-630.
23. Montenegro-Landívar MF, Tapia-Quirós P, Vecino X et al. (2021) Fruit and vegetable processing wastes as natural sources of antioxidant-rich extracts: Evaluation of advanced extraction technologies by surface response methodology. Journal of Environmental Chemical Engineering 9(4): 105330.
24. Sharma P, Gaur VK, Sirohi R et al. (2021) Sustainable processing of food waste for production of bio-based products for circular bioeconomy. Bioresource Technology 325: 124684.
25. Pavlović N, Jokić S, Jakovljević M et al. (2020) Green extraction methods for active compounds from food waste-cocoa bean shell. Foods 9(2): 140.
26. Hegazy AE and Ibrahium MI (2012) Antioxidant activities of orange peel extracts. World Applied Sciences Journal 18(5): 684-688.
27. Brand-Williams W, Cuvelier ME, Berset CLWT (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology 28(1): 25-30.
28. Singleton VL and Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16(3): 144-158.
29. Zheng W and Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food chemistry 49(11): 5165-5170.
30. Juranović Cindrić I, Kunštić M, Zeiner M et al. (2011) Sample preparation methods for the determination of the antioxidative capacity of apple juices. Croatica Chemica Acta 84(3): 435-438.
31. Singh M, Seth P, Poddar S (2017) Comparative analysis of four facial foundation lotions with reference to its antioxidant richness and bio-safety. Cosmetics 4(2): 12.

32. Muthukumarasamy R, Ilyana A, Fithriyaani NA et al. (2016) Formulation and evaluation of natural antioxidant cream comprising methanolic peel extract of Dimocarpus longan. International Journal of Pharmaceutical and Clinical Research 8(9): 1305-1309.
33. Rotta EM, Haminiuk CWI, Maldaner L et al. (2017) Determination of antioxidant activity and phenolic compounds of *Muntingia calabura Linn.* peel by HPLC-DAD and UPLC-ESI-MS/MS. International Journal of Food Science & Technology 52(4): 954-963.
34. Kumar N and Goel N (2019) Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports 24: e00370.
35. Zhao M, Yang B, Wang J et al. (2006) Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. Food Chemistry 98(3): 539-544.
36. Ng ZX, Samsuri SN, Yong PH (2020) The antioxidant index and chemometric analysis of tannin, flavonoid, and total phenolic extracted from medicinal plant foods with the solvents of different polarities. Journal of Food Processing and Preservation 44(9): e14680.
37. Takao LK, Imatomi M, Gualtieri SCJ (2015) Antioxidant activity and phenolic content of leaf infusions of Myrtaceae species from Cerrado (Brazilian Savanna). Brazilian Journal of Biology 75: 948-952.
38. Waszkowiak K and Gliszczyńska-Świgło A (2016) Binary ethanol–water solvents affect phenolic profile and antioxidant capacity of flaxseed extracts. European Food Research and Technology 242: 777-786.
39. Bucic-Kojic A, Planinic M, Tomas S et al. (2011) Effect of extraction conditions on the extractability of phenolic compounds from lyophilised fig fruits (Ficus carica L.). Polish Journal of Food and Nutrition Sciences 61(3): 195-199.
40. Che Sulaiman IS, Basri M, Fard Masoumi HR et al. (2017) Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacanthus nutans Lindau leaves by response surface methodology. Chemistry Central Journal 11(1): 1-11.
41. Villaño D, Fernández-Pachón MS, Moyá ML et al. (2007) Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta 71(1): 230-235.
42. Akar Z, Küçük M, Doğan H (2017) A new colorimetric DPPH• scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. Journal of Enzyme Inhibition and Medicinal Chemistry 32(1): 640-647.
43. Aryal S, Baniya MK, Danekhu K et al. (2019) Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants 8(4): 96.
44. Feduraev P, Skrypnik L, Nebreeva S et al. (2022) Variability of phenolic compound accumulation and antioxidant activity in wild plants of some Rumex species (*Polygonaceae*). Antioxidants 11(2): 311.
45. Li Z, Lee HW, Liang X et al. (2018) Profiling of phenolic compounds and antioxidant activity of 12 cruciferous vegetables. Molecules 23(5): 1139.
46. Derakhshan Z, Ferrante M, Tadi M et al. (2018) Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. Food and Chemical Toxicology 114: 108-111.
47. Pang Y, Ahmed S, Xu Y et al. (2018) Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. Food Chemistry 240: 212-221.
48. Ahmed AF, Attia FA, Liu Z et al. (2019) Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (Ocimum basilicum L.) plants. Food Science and Human Wellness 8(3): 299-305.

49. Dong X, Hu Y, Li Y et al. (2019) The maturity degree, phenolic compounds and antioxidant activity of Eureka lemon [*Citrus limon (L.)* Burm. f.]: A negative correlation between total phenolic content, antioxidant capacity and soluble solid content. Scientia Horticulturae 243: 281-289.
50. Stratil P, Klejdus B, Kubáň V (2007) Determination of phenolic compounds and their antioxidant activity in fruits and cereals. Talanta 71(4): 1741-1751.
51. Ulewicz-Magulska B and Wesolowski M (2019) Total phenolic contents and antioxidant potential of herbs used for medical and culinary purposes. Plant Foods for Human Nutrition 74: 61-67.
52. Sopee MSM, Azlan A, Khoo HE (2019) Comparison of antioxidants content and activity of Nephelium mutabile rind extracted using ethanol and water. Journal of Food Measurement and Characterization 13: 1958-1963.
53. Stanger MC, Steffens CA, Soethe C et al. (2017) Phenolic content and antioxidant activity during the development of 'Brookfield'and 'Mishima'apples. Journal of Agricultural and Food Chemistry 65(17): 3453-3459.
54. Andres AI, Petron MJ, Lopez AM et al. (2020) Optimization of extraction conditions to improve phenolic content and in vitro antioxidant activity in craft brewers' spent grain using response surface methodology (RSM). Foods 9(10): 1398.
55. Sepahpour S, Selamat J, Abdul Manap MY et al. (2018) Comparative analysis of chemical composition, antioxidant activity and quantitative characterization of some phenolic compounds in selected herbs and spices in different solvent extraction systems. Molecules 23(2): 402.
56. Khan MK, Paniwnyk L, Hassan S (2019) Polyphenols as natural antioxidants: sources, extraction and applications in food, cosmetics and drugs. Plant Based "Green Chemistry 2.0" Moving from Evolutionary to Revolutionary: 197-235.
57. Matousek JL, Campbell KL, Kakoma I et al. (2003) Evaluation of the effect of pH on in vitro growth of Malassezia pachydermatis. Canadian Journal of Veterinary Research 67(1): 56-59.
58. de Lima Cherubim DJ, Buzanello Martins CV, Oliveira Fariña L et al. (2020) Polyphenols as natural antioxidants in cosmetics applications. Journal of Cosmetic Dermatology 19(1): 33-37.