

ANTIOXIDANT ASSESSMENT OF SOME THERAPEUTICALLY IMPORTANT PLANTS AS ANTI-AGING AGENTS

UROOJ F^{1*}, IFTIKHAR A², MAZHAR K³, ALTAF R³, GONDAL M⁴, RAFAQAT A⁵, SHABBIR N⁶

^{1*}Ecology and Evolution Laboratory, Department of Botany, University of the Punjab, Lahore, Pakistan

²Akhtar Saeed College of Pharmacy, Rawalpindi, Pakistan

³School of Pharmacy, University of Management and Technology, Lahore, Pakistan

⁴Department of Pharmaceutical Chemistry, Yashfeen College of Pharmacy, Lahore, Pakistan

⁵Yashfeen College of Pharmacy, Lahore, Pakistan

⁶Rashid Latif College of Pharmacy, Lahore, Pakistan

*Correspondence author email address: fareihajaved@gmail.com

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Abstract: Skin, the protective outer covering of the body, acts as an insulator between internal and external environments. Skin diseases are a significant concern today, primarily due to oxidative stress. Although synthetic compounds and drugs are available, they often contain toxic and carcinogenic compounds, leading to cancer. Herbal and natural products, rich in natural antioxidants, are preferred as remedies. **Objective:** To evaluate the antioxidant potential of different solvent extracts at various concentrations from five plants: *Aloe barbadensis*, *Camellia sinensis*, *Cocos nucifera*, *Ficus carica*, and *Rosa indica*, using DPPH, Total Phenolic Content (TPC), and Total Antioxidant Assay. **Methods:** This experimental study was conducted over six months in a laboratory setting. Various solvent extracts (100% v/v) of the five selected plants were prepared, and their antioxidant potentials were evaluated using DPPH radical scavenging assay, TPC assay, and Total Antioxidant Assay. Data were analyzed using ANOVA, and results were expressed as mean \pm standard deviation (SD). **Results:** Among all the plant extracts, the 100% v/v Petroleum ether extract of *Aloe barbadensis* showed the highest radical scavenging value, i.e., 98.6 ± 0.55 . Following the evaluation through DPPH, TPC, and Total Antioxidant Assays, a combination of the five plant extracts was proposed as a potent source of natural antioxidants. **Conclusion:** The study concluded that the selected plant extracts, particularly the Petroleum ether extract of *Aloe barbadensis*, possess significant antioxidant potential. These extracts can be utilized in cosmetics and other industries as natural antioxidant sources, offering a safer alternative to synthetic compounds.

Keywords: Antioxidants, *Camellia sinensis*, *Cocos nucifera*, *Ficus carica*, Oxidative stress, Skin diseases, Total Phenolic Content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Introduction

Skin is the dynamic organ comprising about one-sixth of the body weight (1). It is the body's outermost layer and integument and acts as a barrier between the internal and external environment. The skin protects against serious injuries caused by microbial and chemical agents. It also serves the functions of thermoregulation, absorption, secretion, and sensory (2). Skin diseases are more common nowadays and majorly impact people's quality of life. More than 60% of the general population, including almost all age groups, is now suffering from different skin diseases (3). The most common and general skin problems dermatologists and physicians face nowadays are acne, atopic dermatitis, and psoriasis (4). Some other skin problems like photosensitivity, itching, allergies, eczema, Tinea infections, skin eruptions, pigmentation, folliculitis, pigmentary disorders like vitiligo, and other disorders, including keloid and xerosis, are also in literature. (5). A large number of skin problems are raised due to oxidative stress. The disorders of oxidative stress are psoriasis, polymyositis, atopic dermatitis, eczema, mycosis fungoides, acne, lupus, scleroderma, seborrheic, vasculitis, pemphigoid, and most importantly, photoaging (aging) (2). Uncontrolled release of the reactive oxygen species (ROS)

due to skin exposure to ultraviolet radiation also causes human skin disorders

Including cutaneous neoplasia (6). The most common inflammatory and chronic disease caused by oxidation is psoriasis, which affects about 2% of the population by showing symptoms like itching, thickening, and skin scaling. (7). Generally, synthetic drugs are used to cure skin infections. However, synthetic drugs /creams contain chemical compounds that have hazardous effects. Among these effects, severe allergy is caused due to the presence of chemicals like zinc oxide. At the same time, using these creams may increase the skin's oiliness due to the presence of excessive chemical emulsifiers, so natural drugs are preferred. For example, fairness creams contain harmful ingredients such as parabens. Its exposure estimates the risk assessment to the creams and has a carcinogenic nature, causing breast cancer (8). They also contain the comedogenic ingredient isopropyl myristate, which increases the formation of blackheads.

Most of the creams for fairness that are sold in the market contain certain chemicals like steroids and hydroquinone, and long-term use of these creams leads to lethal health issues like permanent pigmentation, skin cancer, and skin burning. To prevent side effects of synthetic drugs plant, plant-based products are the best option to tackle skin

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problems. Plants are the major source of natural antioxidants and thus are helpful in the treatment of skin diseases that are caused by oxidative stress (2). Natural antioxidants are used for the prevention of photo aging and contact dermatitis (2). The extract of *Andrographis paniculata*, *Glycyrrhiza glabra*, *Ocimum sanctum*, and *Azadirachta indica* possess the ability to treat and prevent acne (9). Acne can also be cured with other plants like *Calendula*, *Lavender* and *Citrus*. *Amaranthus* is used to treat various skin problems like acne, psoriasis, and eczema (10). These plants contain active compounds like saponins, flavonoids, phenolics, terpenoids, tannins, linalool, linalyl acetate, polysaccharides, and anthocyanins (10) that contribute to the antioxidant effect and treatment of skin problems due to the oxidative stress. The present study is planned to evaluate the natural antioxidants from plants to cure skin diseases caused by oxidative stress and make a suitable combination of medicinally important plants to avoid synthetic chemicals' harmful aspects.

The present study aims to analyze different combinations of medicinally important plants for their antioxidant ability and to classify the best combination of rose, aloe vera, green tea, fig, and coconut extracts for the medicated creams.

Methodology

This experimental study was designed using a Split-Block Randomized Complete Block Design (RCBD) and conducted in a laboratory setting over six months. Seeds of the experimental plants, including *Aloe barbadensis* (aloe vera), *Camellia sinensis* (green tea), *Cocos nucifera* (coconut), *Ficus carica* (fig), and *Rosa indica* (rose), were obtained from local herbalists and the Punjab Seed Corporation in Lahore.

Four solvents were used for the extraction: petroleum ether, Chloroform, Methanol, and Water. The extraction process was performed using the maceration method, in which plant materials were soaked in the respective solvents at room temperature for a specified period to ensure thorough extraction of the bioactive compounds.

The radical scavenging activity of the plant extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, following the methodology described by Erasto et al. (2004). The DPPH assay involves measuring the reduction in absorbance at 517 nm, indicating the scavenging of the DPPH radical by antioxidants in the plant extracts.

The total antioxidant capacity of the extracts was assessed using the method outlined by Prieto et al. (1999). Antioxidant activity was expressed as the sample absorption at 695 nm. This method quantifies the ability of the extracts to reduce Mo(VI) to Mo(V) and form a green phosphate/Mo(V) complex at acidic pH.

The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu method, as described by Makkar et al. (1993). This assay measures the phenolic compounds' ability to reduce the Folin-Ciocalteu reagent, with results expressed as gallic acid equivalents (GAE).

Data were analyzed using Co-Stat software. Standard deviation (SD) was calculated for each set of measurements. Mean separation was conducted using the Least Significance Difference (LSD) test with a significance level of $p < 0.05$. The analysis was further refined using Duncan's

Multiple Range Test (DMRT) to identify statistically significant differences among the treatments. (Bliss, 1967).

Results

Radical scavenging activity by DPPH Assay

Radical scavenging activity by DPPH assay was accomplished with different concentrations of 30%, 50%, 70%, and 100% v/v of petroleum ether, chloroform, methanol, and distilled water extracts of ethnomedicinally important plants, i.e., *Aloe barbadensis*, *Camellia sinensis*, *Cocos nucifera*, *Ficus carica* and *Rosa indica*; to inspect them quantitatively. The radical scavenging activity of the plant extracts was compared to the available standard antioxidants, i.e., BHT and α -tocopherol.

Various extracts of *A. barbadensis* showed the maximum antioxidant activity. 100% v/v Petroleum ether extract of *A. barbadensis* showed the highest antioxidant value of $98.6 \pm 0.55d$ whereas 70% v/v methanol, 30% v/v distilled water, and 100% v/v distilled water extracts also showed maximum percentage scavenging value, i.e. $98.5 \pm 0.971d$, $98.5 \pm 0.776b$ and $98.3 \pm 0.608a$ respectively. Thus, they can be compared with the standard antioxidants α -tocopherol and BHT and set as standards as their values were much closer to the standard ones. The 70% v/v Chloroform extract showed the lowest value, i.e., $44.4 \pm 1.205b$.

In *Camellia sinensis* (green tea), 100% v/v Chloroform extract showed the maximum radical scavenging value, i.e. $98.3 \pm 0.754a$ while the 100% v/v Methanol extract has the minimum percentage scavenging value, i.e. $11.9 \pm 0.888e$. All the radical scavenging values of different extracts of *C. sinensis* lie between $11.9 \pm 0.888e$ and $98 \pm 0.754a$. 100% v/v Chloroform, 50% v/v Methanol and 100% v/v Distilled water extracts showed the great radical scavenging values i.e. $98.3 \pm 0.754a$, $96.0 \pm 0.624g$ and $91.0 \pm 1.201a$ respectively that can be compared with the standards. These extracts can be set as a standard for antioxidant potential as their radical scavenging values were much nearer to the standard antioxidants taken.

In *C. nucifera*, 70% v/v Methanol extract showed the highest radical scavenging value, i.e. $94.8 \pm 1.201e$. This value can be compared with the standard antioxidant and set as a standard for others. 100% v/v Methanol extract of *C. nucifera* had the lowest value, i.e., $34.2 \pm 0.709d$. All other various extracts of *C. nucifera* showed values between $34.2 \pm 0.709d$ and $94.8 \pm 1.201e$.

In *F. carica*, 30% v/v Petroleum ether extract showed the highest radical scavenging activity, with a value of $94.5 \pm 0.950d$, whereas 100% v/v Methanol and 50% v/v showed the lowest value of $48.3 \pm 1.050b$ and $48.3 \pm 1.026c$, respectively.

In *R. indica*, 30% v/v Petroleum ether extract had the maximum radical scavenging value, i.e. $98.5 \pm 1.001f$, whereas 100% v/v Chloroform extract had the minimum value, i.e. $36.8 \pm 0.953a$. All other extracts of *R. indica* had values between $36.8 \pm 0.953a$ and $98.5 \pm 1.001f$. 100% v/v Petroleum ether and 100% v/v Distilled water extracts of *R. indica* also showed the values i.e., $98.2 \pm 0.680d$ and $91.9 \pm 0.875a$, respectively, which are close to the highest values and can be compared.

Total Phenolic Content (TPC) Assay

TPC Assay was performed based on results determined from the DPPH Assay with some selected extracts of petroleum ether, chloroform, methanol, and distilled water

with variable concentrations. For this purpose, gallic acid was set as the standard, and from the generated curve, an equation was derived to quantify the phenolic contents in all samples.

This equation calculated phenolic contents as $\mu\text{g}/\text{gram}$ of Gallic acid in all the samples.

The highest TPC value of *A. barbadensis* extracts is shown by the 100% v/v Petroleum ether extract, i.e., $637.7 \pm 6.3d$, and 30% v/v Methanol extract showed the lowest TPC value, i.e., $32 \pm 1e$.

The highest TPC value, $843.3 \pm 4.2e$, among all the extracts of *C. sinensis*, is shown by the 100% v/v Petroleum ether extract, while the TPC value of the 70% v/v Methanol extract was the lowest at $43 \pm 10.8e$. The TPC value of *C. nucifera* extracts is highest in the 70% v/v Methanol extract, $344.6 \pm 6.3d$, and lowest in the 100% v/v Petroleum ether extract, with $15 \pm 5c$.

In *F. carica*, 100% v/v, Distilled water extract showed the highest value of total phenolics, i.e., $436.3 \pm 12.7a$. 70% v/v Chloroform extract of *F. carica* has the lowest phenolic contents, i.e. $25 \pm 1b$. 30% v/v Chloroform extract of *R. indica* showed the greatest total phenolic value, i.e., $578.0 \pm 6.3d$, while 70% v/v Distilled water extract of *R. indica* showed the lowest value, i.e., $19 \pm 2.3b$.

Total Antioxidant Assay

The results obtained from the total phenolic Assay were further screened to analyze them quantitatively for the Total Antioxidant Assay, and consequently, some of the selected samples were run. The results of various plant extracts and

the available standard antioxidants, i.e., α -tocopherol and BHT, have been compared.

In *A. barbadensis*, 30% v/v and 50% v/v of distilled water extracts showed the maximum antioxidant values, i.e., $0.240 \pm 0.0005b$ and $0.220 \pm 0.001g$, respectively. These values are very close to the standard ones. Hence, these extracts of *A. barbadensis* can be used as standard antioxidants by others. In *C. sinensis* (green tea), 30% v/v Petroleum ether extract showed the highest antioxidant potential, having a value of $0.801 \pm 0.001g$. Numerous extracts showed great antioxidant values, e.g., 50% v/v Methanol, 50% v/v Petroleum ether, 30% v/v Methanol, and 70% v/v Petroleum ether extracts showed the values $0.796 \pm 0.002f$, $0.740 \pm 0.01g$, $0.729 \pm 0.002f$ and $0.628 \pm 0.002f$ respectively. These antioxidant values are much closer to the standard antioxidants, i.e., α -tocopherol and BHT. Thus, these extracts can be set as standard antioxidants.

70% v/v Petroleum ether extract of *C. nucifera* has the highest antioxidant value, i.e., $0.211 \pm 0.002e$ among all the other extracts of *C. nucifera*.

In *F. carica*, 100% v/v Methanol extract showed the highest antioxidant value $0.520 \pm 0.01e$. This value is very close to the standard antioxidant. The values of 50% v/v and 70% v/v methanol extract are $0.465 \pm 0.002g$ and $0.304 \pm 0.002g$, respectively. These extracts can be set as standard. The highest antioxidant value among the various extracts of *R. indica* is $0.332 \pm 0.0015f$ of 70% v/v Methanol extract

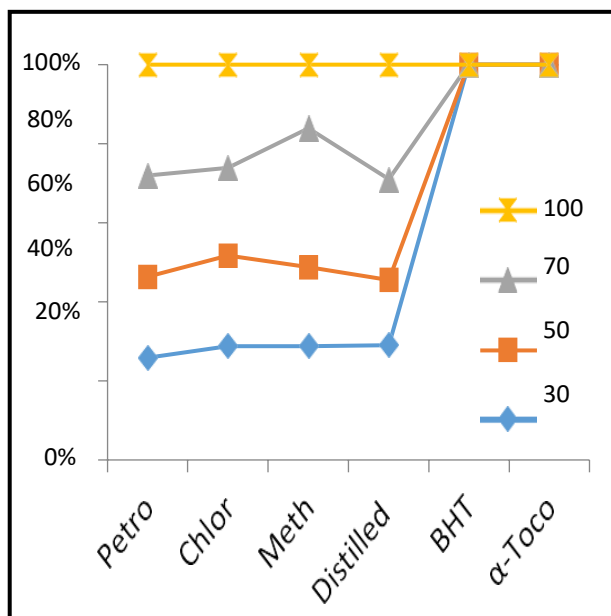


Fig. 1: Antioxidant Evaluation of Various Extracts at Different Conc. of *Aloe barbadensis* (Aloe vera) through DPPH Assay

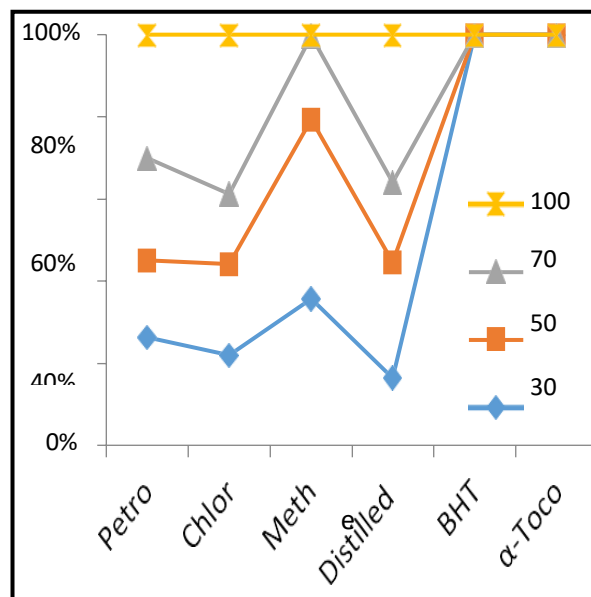


Fig. 2: Antioxidant Evaluation of Various Extracts at Different Conc. of *Camellia sinensis* (Green Tea) through DPPH Assay

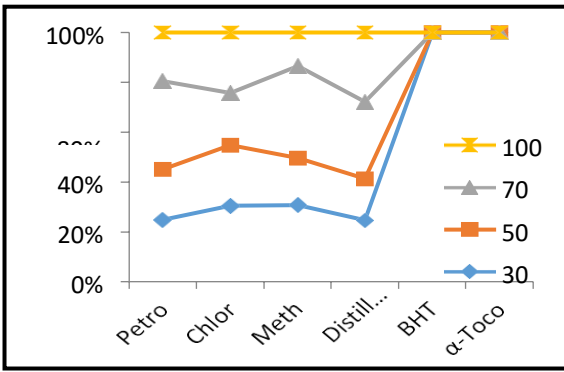


Fig. 3: Antioxidant Evaluation of Various Extracts at Different Conc. of *Cocos nucifera* (Coconut) through DPPH Assay

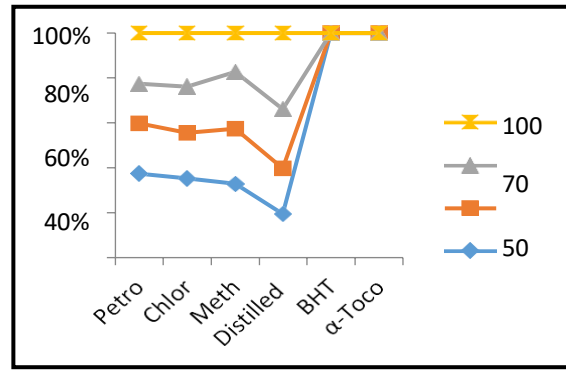


Fig. 4: Antioxidant Evaluation of Various Extracts at Different Conc. of *Ficus caria* (Fig) through DPPH Assay

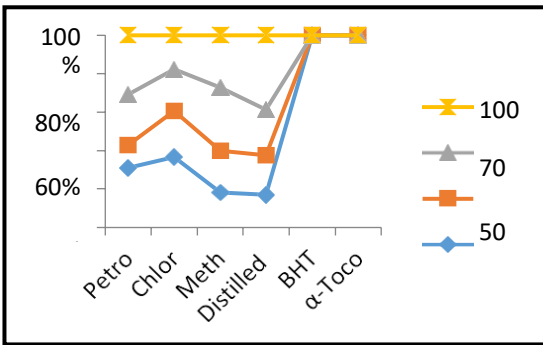


Fig. 5: Antioxidant Evaluation of Various Extracts at Different Conc. of *Rosa indica* (Rose) through DPPH Assay

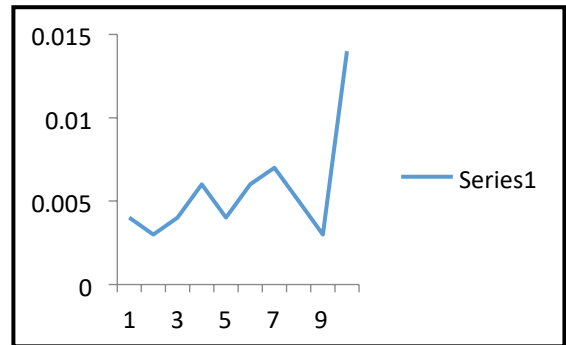


Fig. 6: Graph Showing the Curve of Gallic Acid

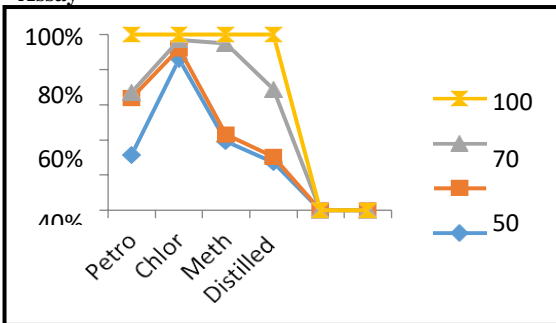


Fig. 7: Total Phenolic Contents Estimation of Various Extracts at Difference Conc. of *Aloe barbadensis* (Aloe vera) by TPC Assay

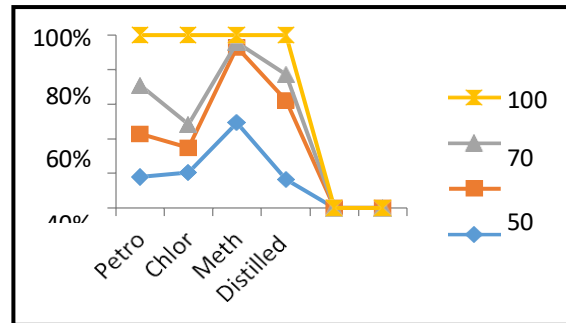


Fig. 8: Total Phenolic Contents Estimation of Various Extracts at Difference Conc. of *Camellia sinensis* (Green Tea) by TPC Assay

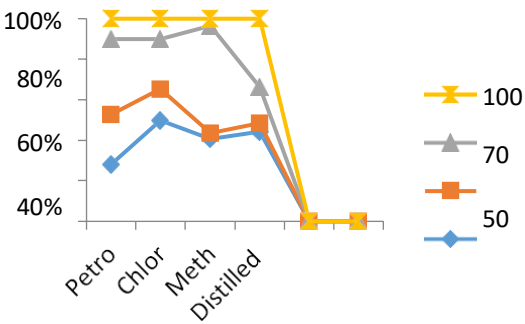


Fig. 9: Total Phenolic Contents Estimation of Various Extracts at Difference Conc. of *Cocos nucifera* (Coconut) by TPC Assay

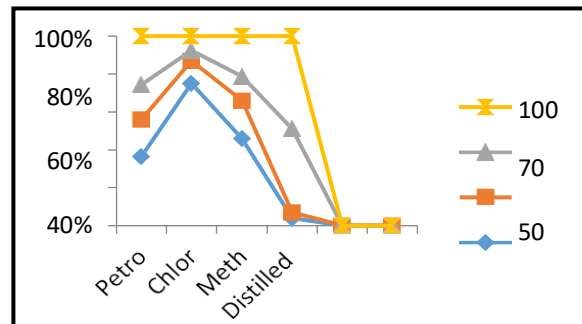


Fig. 10: Total Phenolic Contents Estimation of Various Extracts at Difference Conc. of *Ficus caria* (Fig) by TPC Assay

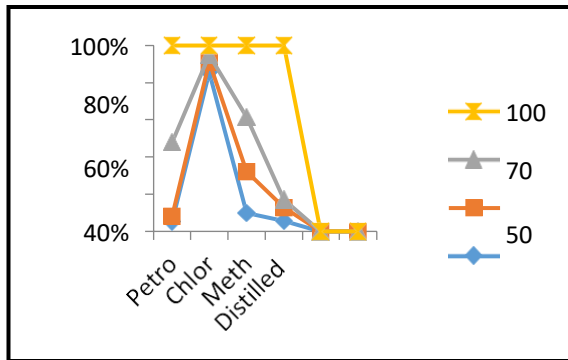


Fig. 11: Total Phenolic Contents Estimation of Various Extracts at Difference Conc. of *Rosaindica* (Rose) by TPC Assay

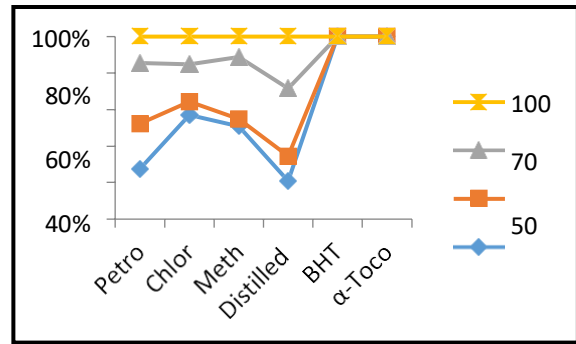


Fig. 12: Antioxidant Evaluation of Various Extracts at Different Conc. of *Aloe barbadensis* (Aloe vera) by Total Antioxidant Assay

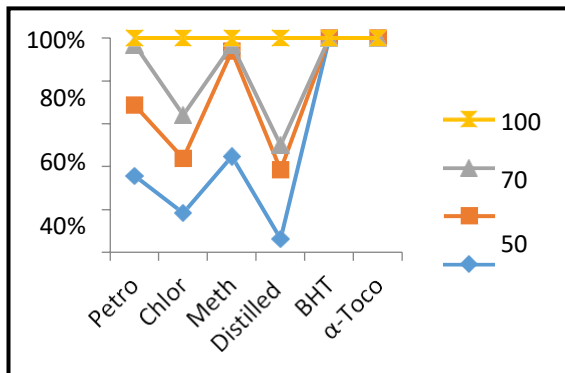


Fig. 13: Antioxidant Evaluation of Various Extracts at Different Conc. of *Camellia sinensis* (Green Tea) by Total Antioxidant Assay

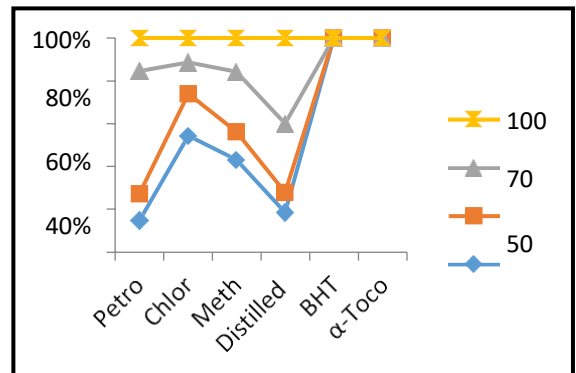


Fig. 14: Antioxidant Evaluation of Various Extracts at Different Conc. of *Cocos nucifera* (Coconut) by Total Antioxidant Assay

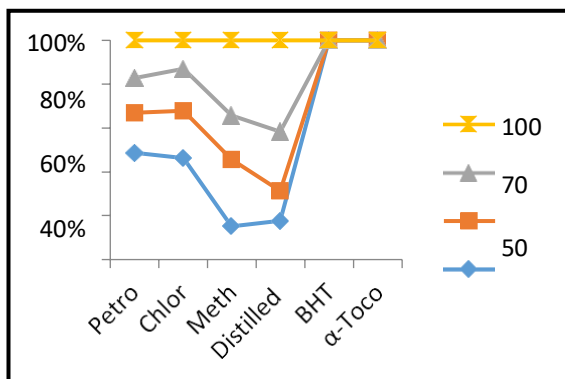


Fig. 15: Antioxidant Evaluation of Various Extracts at Different Conc. of *Ficus caria* (Fig) by Total Antioxidant Assay

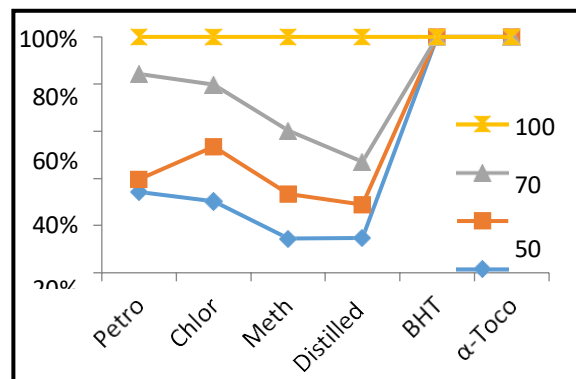


Fig. 16: Antioxidant Evaluation of Various Extracts at Different Conc. of *Rosa indica* (Rose) by Total Antioxidant Assay

Discussion

Antioxidants are helpful as they protect living organisms from the damage caused by reactive oxygen species. Many skin, inflammatory, and chronic diseases also arise due to oxidative stress. Antioxidants are also helpful in increasing the shelf life of many food and cosmetic products (11). Antioxidants also play an essential role in aging (12). This need for antioxidants can be fulfilled by artificial synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary

butylhydroquinone (TBHQ) (13). However, these synthetic chemicals used by different industries contain toxic compounds and are carcinogenic. Thus, natural antioxidants are preferred in the food and cosmetic industry as they do not have any side effects and are not toxic.

The DPPH Assay, along with Ferric Thio Cyanate (FTC) and linoleic acid system method, was performed on the ethanol extracts of *Aloe barbadensis*. (13, 22, 23) Ethanolic extracts of *Aloe barbadensis* showed significant antioxidant

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activity, and the results were compared with BHT and α -tocopherol, which are standard antioxidants. The wide domain of antioxidants will be analyzed if solvents with a polarity range have been used as both standards taken and ethanol extracts are non-polar. In the present study, the antioxidant activity of *A. barbadensis* in different solvents with a range of polarity is evaluated and compared with the standard antioxidants. 100% v/v petroleum ether, 70% v/v methanol, 30% and 100% v/v distilled water showed the results of $98.6 \pm 0.55d$, $98.5 \pm 0.971d$, $98.5 \pm 0.77b$ and $98.3 \pm 0.608a$ respectively by DPPH Assay. Thus, on the basis of present results and the results of (13, 22), it is observed that *A. barbadensis* extracts provided higher or equivalent antioxidant activity as compared to BHT and α -tocopherol. Thus, *A. barbadensis* can be used as a natural antioxidant in food, medicine, and cosmetics.

The antioxidant capacity of methanolic and aqueous extracts of *Camellia sinensis* by DPPH, FRAP, FIC, and Total Phenolic Assay was evaluated (14, 24, 25). Methanolic extracts have eminent antioxidant activity. The results include only the polar fraction of the solvent.

Examination of the scavenging ability of distilled water and 70% ethanol extracts with chloroform, ethyl acetate, and n-butanol of *Camellia sinensis* has been done by DPPH Assay (15). Ethanol extracts have shown higher phenol content. The results are more genuine as solvents having a range of polarity are used to investigate the antioxidant potential

In the current study, a range of solvents, i.e., petroleum ether, chloroform, methanol, and distilled water at various concentrations, are used to evaluate the antioxidant capacity of *Camellia sinensis* by DPPH, TPC, and Total Antioxidant Assay. 100% v/v Chloroform, 50% v/v Methanol, and 100% Distilled water extracts showed higher radical scavenging values, i.e., $98.3 \pm 0.754a$, $96.0 \pm 0.624g$, and $91.0 \pm 1.201a$ respectively. Maximum phenolic contents lie in the 100% Petroleum ether extract, having a value of $843.3 \pm 4.2e$. These values resemble, to a great extent, the standard antioxidants taken. Thus, *C. sinensis* can be used to control the diseases caused by oxidative stress. These polyphenols present in the *C. sinensis* are the anti-aging agents (16)

Free radical scavenging properties of aqueous extracts of *Cocos nucifera* were revealed by the in vitro experiments using the DPPH Assay and the HPLC technique (17, 26). This technique is beneficial to identify different phytochemicals. In the present study, 70% v/v Methanol extract has the highest antioxidant capacity among polar and non-polar extracts of *C. nucifera* by DPPH Assay, having a value of 94.8 (27, 28)

$\pm 1.201e$. This radical scavenging value of *C. nucifera* is very near to the BHT and α -tocopherol, taken as standard.

An investigation of the in vitro antioxidant activity of the screened methanolic extracts of *Ficus carica* was conducted using a DPPH Assay (18). Purification of these extracts requires the thin layer chromatography. This technique provides a better approach to the studies as components present in the extract that are responsible for the antioxidant activity are isolated. In the present study, various solvents with a wide range of polarity are used for extraction to explore the range of bio-constituents accountable for the antioxidant activity. Among all the extracts of *F. carica* in different solvents, 30% v/v Petroleum ether extract has the highest radical scavenging activity, having a value of $94.5 \pm 0.950d$. A comparison of the antioxidant potential of 30% v/v Petroleum ether extract with the standard

antioxidants BHT and α -tocopherol shows that this extract can replace synthetic artificial

antioxidants. *F. carica* can be used in cosmetics, food, and medicine as a natural antioxidant and is helpful in protecting the skin from inflammatory diseases.

The antioxidant activity of 80% ethanol extracts of *Azadirachta indica* has been evaluated through DPPH and Total Antioxidant Assay. (19, 20, 21) The root bark extract of *A. indica* possesses strong antioxidant potential. The Phospho-molybdenum Assay further affirmed the results. In the current study, polarity solvents are used to investigate the bioavailability responsible for the antioxidant capacity of *Rosa indica* (29, 30). 30% v/v Petroleum ether extract of *R. indica* showed the maximum radical scavenging activity of $98.5 \pm 1.001f$. The antioxidant values by DPPH Assay of 100% v/v Petroleum ether and 100% v/v Distilled water, i.e., $98.2 \pm 0.680d$ and $91.9 \pm 0.875a$, respectively, are closely comparable to the standard antioxidants. 70% v/v Methanol extract contained maximum antioxidant capacity, i.e., $0.332 \pm 0.0015f$ calculated by the Total Antioxidant Assay. The results showed that both polar and non-polar extracts of *R. indica* have the maximum antioxidant potential.

Conclusion

The present study infers that these plant extracts mentioned above have shown remarkable antioxidant activity and were comparable to the standard antioxidants selected. Hence, they can easily fulfill the need for natural antioxidants in cosmetics and other industries in the near future.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate.

Approved by the department concerned.

Consent for publication

Approved

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Not applicable

Conflict of interest

The authors declared an absence of conflict of interest.

Authors Contribution

FAREIHA UROOJ & ASRA IFTIKHAR (Assistant Professor

Data Analysis)

KANWAL MAZHAR (Lecturer) & RABIA ALTAF (Associate Professor)

Revisiting Critically

MAHPARA GONDAL (Assistant Professor)

Final Approval of version

AQSA RAFAQAT (Lecturer)

Concept & Design of Study

NABA SHABBIR (Lecturer)

Drafting

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