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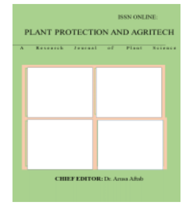

Research article

Optimization of Solvent Extraction for Maximizing Antioxidant and Phenolic Yield from Functionally Important Medicinal Plants

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Abstract

Oxidative stress is a major reason of many skin diseases. Natural antioxidants are more effective and there are no side effects of them. A number of skin diseases are caused due to antioxidant deficiency. These drugs although cure the problem but with a lot of side effects. Therefore, present investigation was done to explore the antioxidant potential of five medicinal species (*Camellia sinensis* L., *Rosa indica* L., *Cucumis sativus* L., *Olea europaea* L. and *Foeniculum vulgare* Mill.*)*. All the plants were extracted by maceration method in both polar and non-polar solvents in a series of 30 %, 50%, 70% and 100% v/v concentrations. Antioxidant evaluation was done through DPPH Assay, Total Phenolic Assay (TPC) and Total Antioxidant Assay. Among all 30% v/v methanol extract of *Rosa indica* showed maximum radical scavenging activity. Methanol extract (100% v/v) of *Camellia sinensis* showed maximum phenolic content and maximum antioxidant potential. The extracts showing best results are recommended as best anti-aging plants for pharmaceutical industry.

Keywords: Antioxidants, Maceration, Oxidative stress, Phytochemicals. Pharmacological, networking

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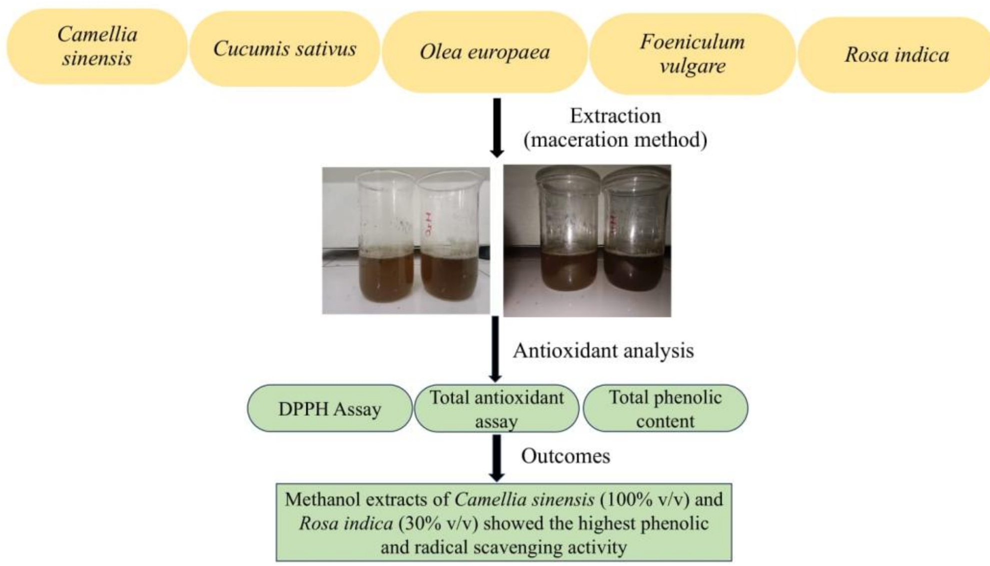
Graphical Abstract



1. Introduction

The skin serves as the primary interface between the human body and the external environment, functioning as a waterproof barrier that protects underlying tissues from physical, chemical, and biological insults (McKnight et al., 2022). Environmental changes and lifestyle factors significantly contribute to the rising prevalence of skin-related disorders. Skin diseases represent the fourth most common non-fatal health condition worldwide, affecting approximately one-third of the global population and imposing a substantial socioeconomic and medical burden (Han et al., 2025). Such common dermatological problems as alopecia, psoriasis, atopic dermatitis, vitiligo, hypertrophic scarring, melanoma, acne, and other infectious skin diseases can also be given. Although these conditions are very prevalent, most of them do not have a therapeutic solution which is reliable and lasting and as such, it is important to develop better treatment methods. According to etiology, skin diseases are divided into eczemas, infections and pyodermas, viral and fungal infections, infestations, acne, pigmentary disorders, urticaria, pruritus, cystic lesions, psoriasis, nevi and other dermatological disorders (Shrestha et al., 2014). Psoriasis and rosacea are inflammatory skin diseases that are typified by immune-mediated chronic inflammation on the skin (Zhao et al., 2024). Also, the bacteria, viruses, and fungi are likely to cause diseases like swelling, impaired wound healing, dandruff, scabies, tinea infections, impetigo, leucoderma, leprosy, and rashes (Sivarajan and Ramakrishnan, 2012). It is becoming more and more evident that oxidative stress is at the center of pathogenesis of such disorders, as it causes inflammation, breaks down the skin barrier, and increases allergen penetration, which results in the appearance of clinical symptoms, including pruritus, xerosis, lichenification, and eczema-like erythematous lesions (Kvedariene et al., 2025). Therefore, oxidative stress is an important pathophysiological mechanism which increases the severity and development of disease. Synthetic drugs are traditionally the topical creams that are widely used to treat the skin diseases. These formulations may be effective but with a long-term use, it may be coupled with an adverse effect like photosensitivity, premature aging of the skin, pigmentation disorders, and carcinogenic risk. The increasing issues surrounding the safety of synthetic compounds have motivated the quest towards safer and more sustainable substances as an alternative to dermatological therapy. Over the past several years, a trend has been towards natural and plant-based methods of the treatment of skin diseases. It has been demonstrated that plant-derived phenolic compounds such as ascorbic acid, ellagitannins, and carotenoids can prevent oxidative stress and inflammation and regulate cellular survival signaling pathways (Porwal et al., 2024). The relation between a number of medicinal plants and dermatology has been traditional; Datura metel leaves are applied in therapy of skin swellings (Sivararajan and Ramakrishnan, 2012), Glycyrrhiza glabra stems are used in treating leucoderma and inflammatory skin (Razia and Sivaramakrishnan, 2014), and Mimosa pudica leaves and roots are applied in treating itchiness and wound healing. By the same analogy, the Eclipta alba leaves have been used as a treatment of swellings and as a skin aging inhibitor; mainly because of its phenolic components (Sivarajan and Ramakrishnan, 2012). Leaves of Lawsonia inermis have been found to be useful in the treatment of psoriasis, flavonols and phenolic acids were also found to be some of the important bioactive constituents (Zohourian et al., 2011). Many plants and phytochemicals have also proven to have an effect in the treatment of psoriasis (Gupta et al., 2025). Plant secondary metabolites including alkaloids, flavonoid, terpenoids, and steroids have a considerable therapeutic activity and are promising alternatives to synthetic compounds (Jose et al., 2014; Sahoo et al., 2024). Common bioactive compounds in the dermatological formulations are usually presented by niacinamide (peanuts and sunflower seeds), sodium ascorbyl phosphate (potatoes and citrus fruits), tocopherol (olives), pyridoxine (sunflower seeds), allantoin (comfrey), and derivatives of fatty acids contained in coconut oil. Therefore, the present study aims to investigate different combinations of plant-derived phytochemicals with antioxidant potential, targeting oxidative stress as a key underlying mechanism in the development and progression of skin diseases.

2.Materials and methods

The proposed study was conducted in Molecular taxonomy Lab, Lahore College for Women University Lahore College for Women University, Lahore.

2.1. Plant material

The current research work deals with the study of antioxidant activity of some functionally important plant that are *Rosa indica* (rose), *Olea europaea* (olive), *Cucumis sativus* (cucumber), *Camellia sinensis* (black tea), *Foeniculum vulgare* (fennel seed).

2.2. Experimental design

Experimental design for the present study was split Block RCBD. Whole experiment comprised of four solvents (P. ether, Chloroform, Methanol and Water). Extraction was done by Maceration Method.

2.3. Solvent Extraction by Maceration Method

250g of ground plant material was extracted in sequence with 500ml of polar and non-polar solvents. The extraction was carried out by soaking the powder in each of the solvent for the period of 8 days. e.g. P. ether, Chloroform, Methanol and Water. The residue was filtered and the filtrate was preserved in the labeled amber colored glass jars, whereas the residue was further soaked in the next solvent in series (Kebede *et al.*, 2025).

2.4 Antioxidant activity

For antioxidant evaluation of the plant extracts, DPPH assay and Total antioxidant assay were performed. The details of the methods used for antioxidant activity are as follows:

2.4.1.Radical Scavenging Activity by DPPH Assay

2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) assay was performed by following the methodology of Kebede *et al.*, (2025). The preparation of extract solutions was done by dissolving 0.5mg/ml of each extract in the respective extraction solvent that is Dimethyl Sulphoxide (DMSO). 1ml of 0.5mg/ml DPPH in DMSO was mixed with the 1ml of test sample and kept in dark for half an hour. The scavenging activity was measured by observing the absorbance at 517 nm and the whole procedure was done in triplicate manner. The antioxidant activity was expressed as % age of scavenging activity (SC %) on DPPH radical as

SC% = [1-(absorbance of sample) / (absorbance of control) ×100.

The control contained all reagents except the plant extract. The DPPH radical scavenging activity of BHT (Butyl Hydroxytoluine) and Alpha Tocopherol were used as standard. All tests were performed in triplicate.

2.4.2. Total Antioxidant Capacity Determination

The total antioxidant capacity of all the extracts was determined by following the methodolgy of (Imtiaz *et al*., 2025). 0.1ml of each solution (0.5mg/ml) was mixed with 1.9ml of reagent solution (0.6M sulphuric acid, 4Mm ammonium molybdate and 28Mm sodium phosphate). The incubation of reaction mixture was done at 95C° for 60 minutes and then allowed to cool at room temperature. The antioxidant activity was expressed as the sample absorption at 695nm. BHT was used as standard for comparison.

2.4.3. Total Phenolic Content Determination

The total phenolics of all the plant samples were determined by following the methodology of Kebede *et al.*, (2025). 0.1 ml of each of the plant extract (0.5mg/ml) was mixed with 2.8ml of 10% Na_2CO_3 and 0.1ml of 2N Folin- Ciocalteu reagent was also added to it. The absorbance of the reaction mixture was measured at 725nm by UV visible spectrophotometer. The results were expressed as milligrams of Gallic acid equivalents (GAE) per gram of the sample extract.

2.5. Statistical Analysis

For statistical analysis Co-stat software was used. It was evaluated by calculating Standard deviation (S.D) was calculated. The analysis was done using Duncan’s Multiple Range test (DMRT).

3. Results

3.1 DPPH radical scavenging activity

Radical scavenging activity with DPPH Assay was performed with petroleum ether, chloroform, methanol and distilled water plant extracts to analyze them quantitatively. The results of the plant extracts were being compared with the standards antioxidant available (BHT and α-Tocopherol). Extracts showed values much higher than the standards. The 30% v/v chloroform extract of *Camellia sinensis* showed the highest antioxidant values i.e. 98.6±0.3[d] and the 50% v/v methanol extract of *Camellia sinensis* showed the lowest antioxidant values i.e. 24.57±0.45[h]. The antioxidant values of extracts of *C. sinensis* was shown (Figure 1a). The 30% v/v methanol extract of *Rosa indica* petals showed the maximum antioxidant values i.e. 99.17±0.38[g] and the 30% v/v distilled water extract of *Rosa Indica* petals showed the minimum antioxidant value i.e. 48.4±0.35[d]. Figure 1b shows the antioxidant values of extracts of *R. indica.*

The 30% v/v chloroform extract of *Cucumis sativus* seeds showed the highest antioxidant values i.e. 98.8±0.58[d]and the 70% v/v petroleum ether extract of *Cucumis sativus* seeds extract showed the lowest antioxidant value i.e. 39.27±0.35[f]. Figure 1 shows the antioxidant values of extracts of *C.sativus.* The 100% v/v chloroform extract of *Olea europaea* fruits showed the maximum antioxidant values i.e. 97.79±0.79[a]. Fig 3.1d shows the antioxidant values of extracts of *O. europaea*The 100% v/v distilled water extract of *Foeniculum vulgare* seeds showed the highest antioxidant values i.e. 99.07±0.40[a]. Figure1e shows the antioxidant values of extracts of *F. vulgare.*

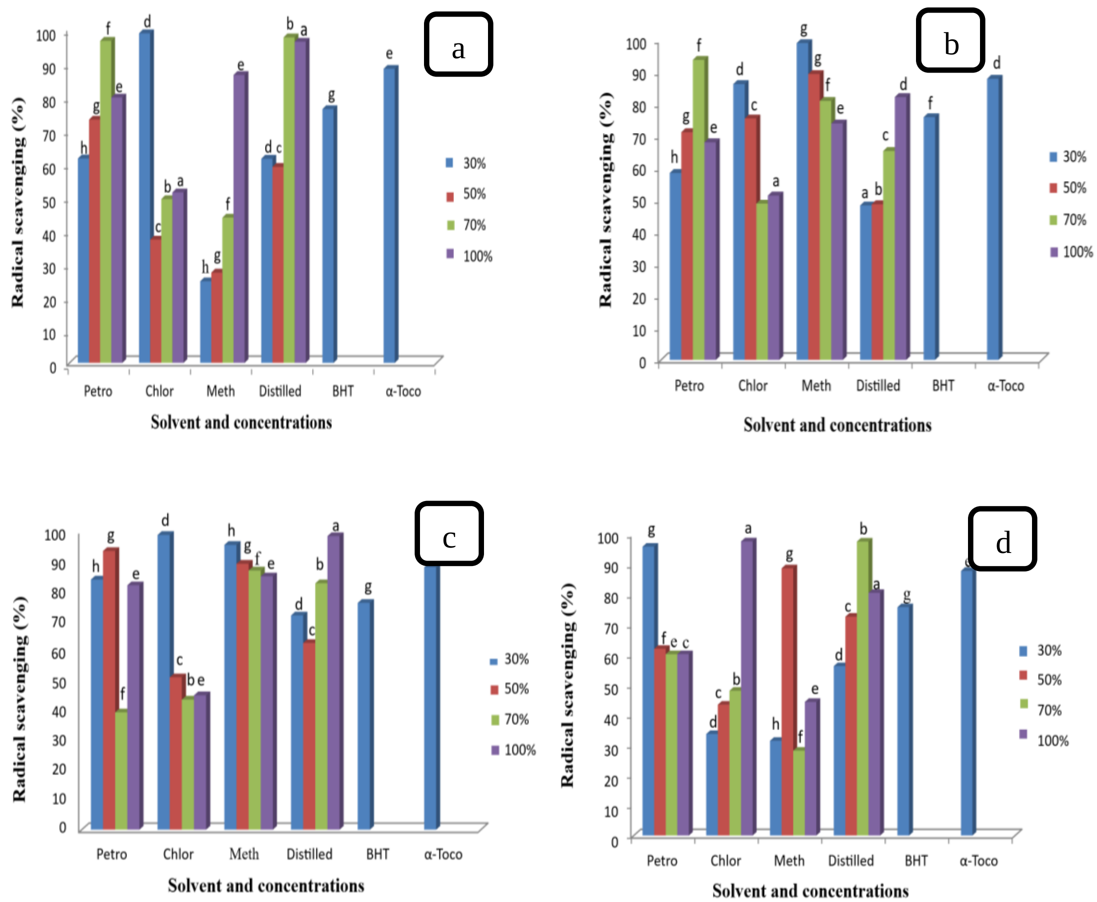
3.2. Total Phenolic Content (TPC) Assay

The results for Total phenolic Assay were noted down by comparing them with the Gallic acid equivalent and the resultant values were recorded in the form of mg/g of Gallic acid.

The 100% v/v methanol extract of *Camellia sinensis* showed the highest phenolic content i.e. 939.1±4.15[b] and the 100%v/v chloroform extract of *Camellia sinensis* showed the lowest phenolic content i.e. 8.34±1.49[a]. The 50% v/v petroleum ether extract of *Rosa indica* petals showed the maximum phenolic content i.e. 822.5±4.15[f] and the 50% v/v distilled water extract of *Rosa indica* petals showed the minimum phenolic content i.e. 56.9±0.43[c]. The 100% v/v petroleum ether extract of *Cucumis sativus* seeds showed the highest phenolic content i.e. 615.56±6.37[c] and the 100% v/v chloroform extract of *Cucumis sativus* seeds showed the lowest phenolic content i.e. 41.95±0.9[a]. The 50% v/v methanol extract of *Olea europaea* fruit showed the maximum phenolic content i.e. 686.37±63.64[d] and the 100% v/v methanol extract of *Olea europaea* fruit showed the minimum phenolic content 127.17±3.6[d]. That 30% v/v chloroform extract of *Foeniculum vulgare* seeds showed the highest phenolic content i.e. 537.39±6.67[d] and the 50% v/v methanol extracts of *Foeniculum vulgare* seeds showed the lowest phenolic content i.e. 29.14±0.02[f]. Figure 2a – 2e shows the phenolic content of different plants extracts.

3.1 Total Antioxidant Assay

The results of plant extracts were being compared with the standard antioxidants available i.e. α-Tocopherol, and BHT. Some of the extracts showed values very much closer to standards used, 0.513and 0.476 respectively. Hence, they can be used as standards in future after refining. The 100% v/v methanol extract of *Camellia sinensis* showed highest antioxidant values i.e. 1.65±0.002[a] and the 70% v/v chloroform extract of *Camellia sinensis* showed the lowest antioxidant values i.e. 0.03±0.001[b] as shown in Table 1a. The 100% v/v methanol extract of *Rosa indica* petals showed the maximum antioxidant value i.e. 0.512±0.001[e] which is as same as value of standard α-Tocopherol. The 100% v/v methanol extract of *Cucumis sativus* seed showed the highest antioxidant value i.e. 0.42±0.001[e]. The 70% v/v chloroform extract of *Cucumis sativus* seed showed the lowest antioxidant value i.e. 0.049±0.002[b] as shown in Table 1c. The 70% v/v petroleum ether extract of *Olea europaea* fruits showed the maximum antioxidant value i.e. 0.66±0.002[f] and 70% v/v methanol, 70%v/v, 50% v/v, 30%v/v chloroform extract of *Olea europaea* fruits showed the minimum antioxidant value i.e. 0.02±0.002[d], 0.02±0.001[b], 0.02±0.001[c] and 0.02±0.001[d], correspondingly as shown in Table 1d. The 70% v/v petroleum ether extract of *Foeniculum vulgare* seeds showed the highest antioxidant value i.e. 0.26±0.002[d] as shown in Table 1e.



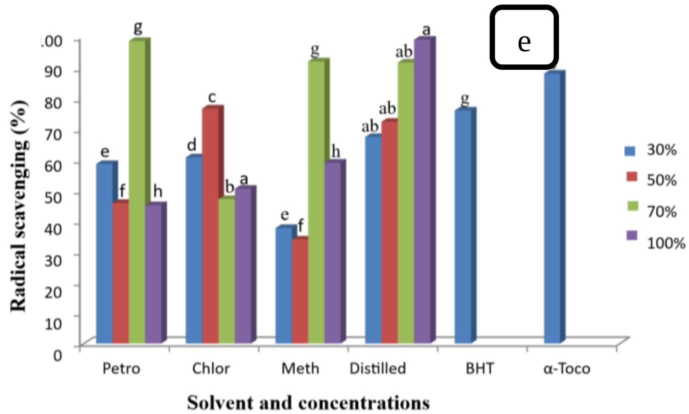
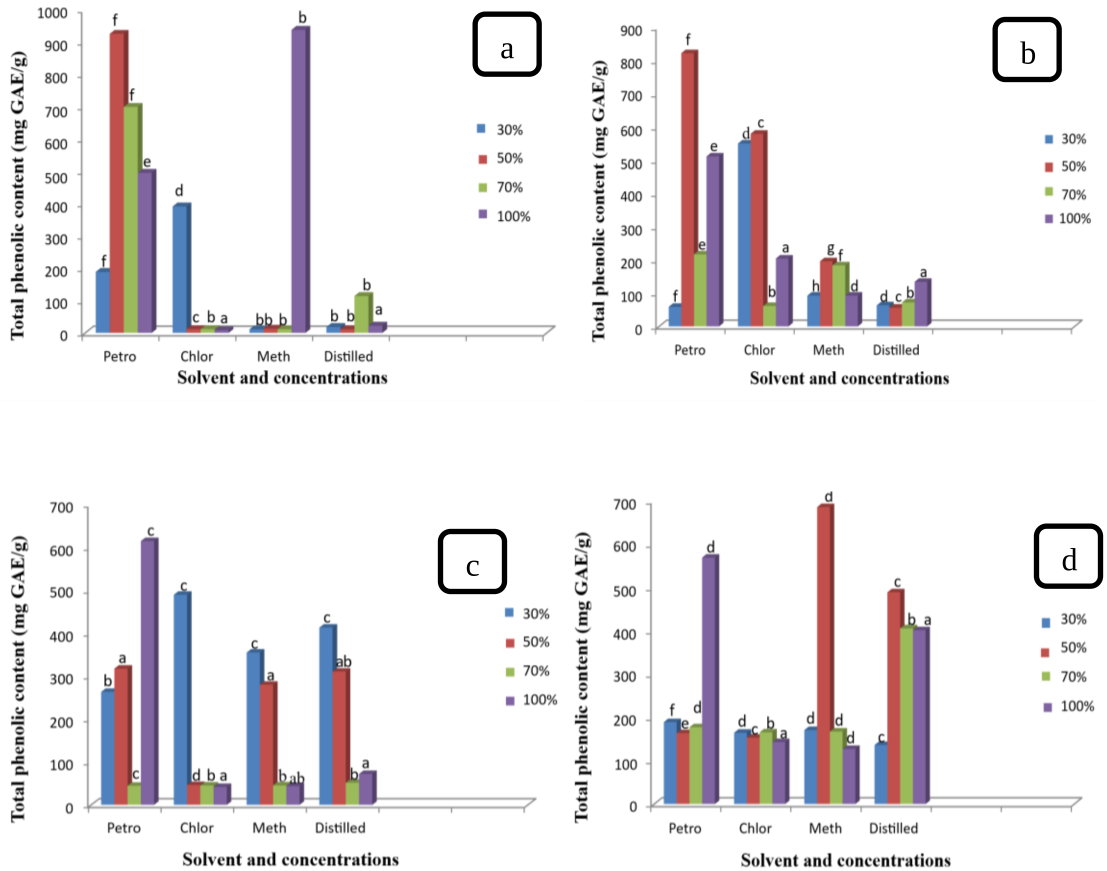


Figure. 1: Antioxidant activity of different plant extracts. (a) *Camellia sinensis,* (b) *Rosa indica,* (c) *Cucumis sativus,* (d) *Olea europae,* (e) *Foeniculum vulgare*



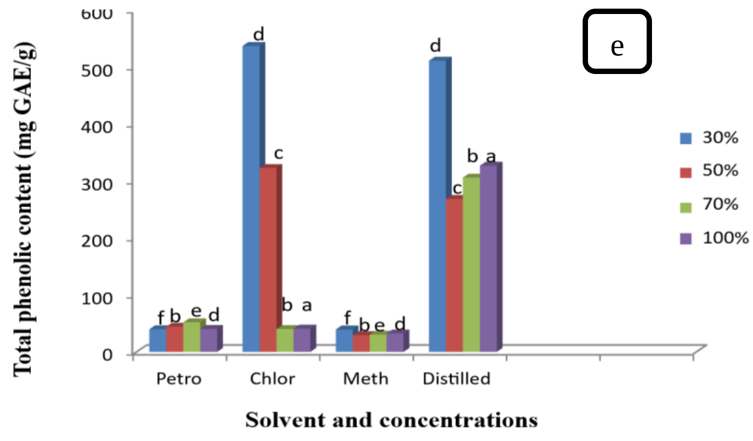


Figure 2: Total phenolic content of different plant extracts. (a) *Camellia sinensis,* (b) *Rosa indica* (c) *Cucumis sativus,* (d) *Olea europae,* (e) *Foeniculum vulgare*

Table 1a: Antioxidant Evaluation of Various Extracts at Different Conc. of *Camellia sinensis by*

No of obs.	Absorption of Various Conc. at 695 (nm)															
	Distilled water conc. (v/v)				Methanol conc. (v/v)				Chloroform conc. (v/v)				Petroleum ether conc. (v/v)			
	100	70	50	30	100	70	50	30	100	70	50	30	100	70	50	30
Mean ± st. dev	0.41± 0.001[a]	0.35± 0.001[b]	0.04± 0.001[c]	0.17± 0.001[d]	1.65± 0.002[a]	0.04± 0.001[a]	0.04± 0.001[a]	0.04± 0.001[a]	0.035± 0.001[a]	0.03± 0.001[b]	0.04± 0.001[c]	0.13± 0.002[d]	0.04± 0.001[e]	0.143± 0.002[f]	0.09± 0.001[fg]	0.52± 0.001[g]

Table 1b: Antioxidant Evaluation of Various Extracts at Different Conc. of *Rosa indica.*by DPPH Assay

No of obs.	Absorption of Various Conc. at 695 (nm)															
	Distilled water conc. (v/v)				Methanol conc. (v/v)				Chloroform conc. (v/v)				Petroleum ether conc. (v/v)			
	100	70	50	30	100	70	50	30	100	70	50	30	100	70	50	30
Mean ± st. de	0.17± 0.002[a]	0.13± 0.001[b]	0.04± 0.001[c]	0.04± 0.003[d]	0.512± 0.001[e]	0.29± 0.001[f]	0.40± 0.002[g]	0.26± 0.001[g]	0.039± 0.001[a]	0.04± 0.002[a]	0.06± 0.001[b]	0.05± 0.001[b]	0.06± 0.001[bc]	0.11± 0.001[c]	0.10± 0.001[c]	0.04± 0.002[c]

Table 1c: Antioxidant Evaluation of Various Extracts at Different Conc. of *Cucumis sativus* DPPH Assay

No of obs.	Absorption of Various Conc. at 695 (nm)															
	Distilled water conc. (v/v)				Methanol conc. (v/v)				Chloroform conc. (v/v)				Petroleum ether conc. (v/v)			
	100	70	50	30	100	70	50	30	100	70	50	30	100	70	50	30
Mean ± st. dev	0.06 ±0.002[a]	0.09±0.001[b]	0.08±0.002[c]	0.11±0.001[d]	0.42±0.001[e]	0.05±0.001[f]	0.23±0.001[g]	0.21±0.002[h]	0.06±0.001[a]	0.049±0.002[b]	0.06±0.002[bc]	0.09±0.002[cd]	0.09±0.002[de]	0.06±0.002[de]	0.075±0.001[de]	0.22±0.002[e]

Table 1d: Antioxidant Evaluation of Various Extracts at Different conc. of *Olea europaea* by DPPH Assay

No of obs.	Absorption of Various Conc. at 695 (nm)															
	Distilled water conc. (v/v)				Methanol conc. (v/v)				Chloroform conc. (v/v)				Petroleum ether conc. (v/v)			
	100	70	50	30	100	70	50	30	100	70	50	30	100	70	50	30
Mean ± st. de	0.07± 0.002[a]	0.063± 0.001[b]	0.06± 0.001[c]	0.20± 0.002[c]	0.02± 0.002[d]	0.02± 0.002[d]	0.06± 0.001[d]	0.02± 0.001[e]	0.04± 0.001[a]	0.02± 0.001[b]	0.02± 0.001[c]	0.02± 0.001[d]	0.03± 0.001[e]	0.66± 0.002[f]	0.02± 0.002[f]	0.29± 0.002[f]

Table 1e: Antioxidant Evaluation of Various Extracts at Different Conc. of *Foeniculum vulgare* by DPPH Assay

No of obs.	Radical Scavenging Activity. at 517 (nm)															
	Distilled water conc. (v/v)				Methanol conc. (v/v)				Chloroform conc. (v/v)				Petroleum ether conc. (v/v)			
	100	70	50	30	100	70	50	30	100	70	50	30	100	70	50	30
Mean ± st. dev	99.07±0.40[a]	91.6±0.4[ab]	72.3±0.4[ab]	67.33±0.61[ab]	58.95±0.35[ab]	92±0.17[ab]	33.9±0.3[b]	37.65±0.34[b]	50.43±0.12[a]	47.08±0.54[b]	76.61±0.42[c]	60.7±0.4[d]	45.07±0.40[e]	98.6±0.4[f]	45.83±0.35[g]	58.53±0.45[h]

4. Discussion

Oxidative stress is widely recognized as a central factor in the development and progression of numerous skin-related disorders, including premature aging, inflammatory dermatoses, pigmentation abnormalities, and impaired wound healing. The overproduction of reactive oxygen species (ROS) impairs the redox balance of cells, destroys lipids, proteins, and nucleic acids, and triggers the activation of inflammatory pathways, including NF- 0 B and MAPKs, which ultimately undermine the integrity of the skin barrier and cellular homeostasis (Porwal et al., 2024; Kvedariene et al., 2025). Under this regard, the current paper will give an overall assessment of the solvent extraction efficiency of five medicinally significant plants: Camellia sinensis, Rosa indica, Cucumis sativus, Olea europaea and Foeniculum vulgare; especially in the dermatological and cosmeceutical uses. These findings are clear that solvent polarity and solvent concentration are decisive factors of establishing the content of total phenols and antioxidant capacity. High phenolic concentrations and antioxidant activities were always observed with polar solvents, especially methanol and aqueous mixtures of methanol, in most plant species. This finding is consistent with the recent findings that indicate that hydroalcoholic solvents enhance the solubility and extraction efficacy of hydroxyl-rich phenolic compounds, flavonoids, and tannins by increasing the hydrogen bonding and diffusion kinetics (Imtiaz et al., 2025; Kebede et al., 2025: Tariq and Reyaz,2013). Conversely, non-polar solvents (petroleum ether and chloroform) that are less effective in recovering total phenolic were also able to show strong DPPH radical scavenging activity in a number of cases, suggesting that the lipophilic antioxidants tocopherols, sterols and essential oil constituents were extracted. Of the plants that were studied, Camellia sinensis had the highest total phenolic content especially in 100% methanolic extracts as a well-documented source of catechins, epigallocatechin gallate (EGCG), and other flavan-3-ols. These polyphenolic compounds have been identified to possess a great electron-donating capacity, metal-binding capacities, and have the capacity to inhibit lipid peroxidation hence effectively neutralizing ROS (Hossain et al., 2013; Han et al., 2025). The very high DPPH scavenging activity of lower concentration chloroform extracts further indicates that it contains moderately polar antioxidant compounds which work in synergy with phenolics. New studies in the dermatology field confirm the application of green tea polyphenols in anti-aging and anti-inflammatory preparations because these are capable of stimulating collagen production and preventing the oxidative damage caused by UV radiation (Porwal et al., 2024; Zhao et al., 2024: Dekdouk *et al.*, 2015). Rosa indica petals exhibited extremely good antioxidant activity, especially in 30 v/v methanol extracts, and considerable amounts of phenols in petroleum ether extracts. This trend implies the co-occurrence of the presence of both hydrophilic classes of phenolics, including gallic acid, quercetin derivatives and anthocyanins, and lipophilic antioxidant compounds. According to the previous and recent research, rose petals have a complex phytochemical matrix that is able to rapidly transfer the hydrogen atom, and this is the reason why the entire amount of DPPH radicals was inhibited in the current study (Zahid et al., 2018). These properties demonstrate that R. indica has a great potential in the formulation of cosmetics to address oxidative stress effects on skin aging and inflammation. Cucumis sativus seeds showed antioxidant activity that was pronounced especially in chloroform and methanol extracts and their scavenging values nearly matched those of common antioxidants. The findings are in line with the previous reports that have found the cucurbitacins, flavonoids, and phenolic acids among the key contributors to antioxidant capacity of cucumber seeds (Shah et al., 2013). Recent uses in the dermatology field highlight the application of cucumber extracts in the process of soothing and cooling, and hydrating the skin and the capacity to scavenge ROS and decrease skin irritation, which means they should be incorporated in the formulation to treat sensitive or inflammatory skin conditions (Porwal et al., 2024). The fruit extracts of Olea europaea had good antioxidant activity especially in chloroform and petroleum ether, which demonstrate the presence of lipophilic antioxidants like tocopherols, squalene, and oleuropein derivatives (Hossain *et al.*, 2013; Zahid *et al.* 2018; Khan *et al*., 2025). The compounds are reputedly known to block lipid peroxidation and regulate the inflammatory reaction in skin tissues (Dekdouk et al., 2015; Yangui et al., 2015). The moderate to high level of phenolic compounds in methanolic extracts also substantiates the polyfunctional antioxidant effects of olive-derived products that are increasingly being used in cosmeceutical preparations as photo protectants and elasticity retardants on the skin. The antioxidant activity of Foeniculum vulgare seeds was found to be very high in aqueous extracts and petroleum ether extracts and this could be explained by the volatile phenylpropanoids like trans-anethole, fenchone and estragole. These bioactive components have been widely reported to possess antioxidant, anti-inflammatory and antimicrobial effects (Khan et al., 2025). Recent research emphasizes the usefulness of the fennel extracts in the treatment of oxidative stress-induced inflammatory diseases and microbial skin infection, which underlines its conventional application in dermatological preparations (Sahoo et al., 2024). Despite overall positive association between total phenolic content and antioxidant activity, some extracts had high radical scavenging capacity, yet had relatively low phenolic content. This shows the non-phenolic antioxidant agents and synergistic effects of various phytochemicals also plays a large role in the overall antioxidant activity. These results are in line with the recent literature that notes that the antioxidant activity cannot be reduced to the concentration of phenolics but can be due to the joint activity of various bioactive compounds obtained at particular solvent conditions (Akbari et al., 2022; Imtiaz et al., 2025). In general, the findings of this paper have provided evidence on the significance of maximizing the solvent polarity and concentration to maximize the antioxidant recovery of medicinal plants. Some of the plant extracts were found to be as antioxidant as or better than the synthetic antioxidants like the BHT and -tocopherol, thus the potential of the plant extracts as safer alternatives to pharmaceutical and cosmetic use. The success of low solvent levels in the attainment of high antioxidant efficacies also underline the possibility of low cost-efforts and ecologically viable extraction methods that can be used in large-scale industrial application.

Conclusion

The antioxidant potential of all the studied plants was high as revealed in the DPPH, total antioxidant capacity, and total phenolic content tests in the presence of diverse solvents and different concentrations. Maximum antioxidant activity was noted in selected chloroform, methanol, and aqueous extracts and the maximum phenolic content was attributed mainly to methanolic and petroleum ether extracts. Altogether, all the plants showed high efficacy as antioxidants individually, yet their synergistic effect can be achieved when they are used simultaneously, which is why their use is promising as a pharmaceutical and cosmeceutical agent.

Author Contributions:

Conceptualization, MS.; methodology, MS.; software, AS.; validation, SI. formal analysis, SI.; investigation, SI.; data curation, AS and SI.; writing—original draft preparation, MS.; writing—review and editing, AS and SI.; visualization, AS.; supervision, MS.; All authors have read and agreed to the published version of the manuscript

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Conflicts of Interest: The authors declare no conflicts of interest

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