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PROXIMATE AND PHYTOCHEMICAL ANALYSIS TO ASSESS THE THERAPEUTIC POTENTIAL OF *OCIMUM SANCTUM*

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ABSTRACT

The potential of medicinal plants to heal chronic illnesses is being investigated more and more. To determine the optimal extraction solvent for phytochemicals and antioxidants from the herb known for its therapeutic properties, *Ocimum sanctum* leaves were extracted in methanol and then fractionated progressively with n-hexane, ethyl acetate, butanol, and aqueous. Phytochemical and proximate analyses were conducted in this study by using standard reference methods. Using AOAC techniques, the moisture content, total ash, crude fiber, crude protein, and nitrogen-free extract of *O. sanctum* leaves were determined. Different extracts of *O. sanctum* leaves were subjected to phytochemical screening for total flavonoid (TFC) and total phenolic content (TPC). Using the DPPH radical scavenging approach, spectrophotometric experimentation was used to assess *O. sanctum's* antioxidant capability. Results of the proximate analysis revealed that moisture, ash, protein, crude fat, crude fiber, and nitrogen-free were 10.61±0.11, 6.66±0.031, 2.68±0.031, 8.86±0.13, 17.92 ±0.13, and 0.280±0.006, respectively. The TPC concentrations in methanol, n-hexane, ethyl acetate, aqueous, and butanol extracts were 92.37±1.81, 4.85±0.02, 20.80±0.15, 72.19±0.59 and 15.08±0.05, respectively. The TFC concentrations in methanol, n-hexane, ethyl acetate, aqueous, and butanol extract were 67.70±1.04, 3.83±0.03, 14.29±0.42, 54.28±0.90, and 10.17±0.11, respectively. Antioxidant activity of all extracts was shown at a concentration as low as 0.1563 mg/ml. The decreasing order of DPPH scavenging activity expressed by the plant extracts was methanol > aqueous > ethyl acetate > butanol > n-hexane. The results of this study showed that all extracts of *O. sanctum* had wielded significant antioxidant and phytochemical potential

Keywords: *Ocimum sanctum*, proximate analysis, phytochemical, TPC, TFC, DPPH.

1. Introduction

Compounds found in medicinal plants have the potential to be therapeutic or serve as building blocks for the development of useful pharmaceuticals [1]. *Ocimum sanctum is* also known as Holy basil widely cultivated in the subcontinent due to its spiritual inviolability, for its miscellaneous curative effects such as anti-inflammatory, hypotensive, antimicrobial, antidiabetic, anxiolytic and antihyperlipidemic is cited in an Indian traditional medicine scripture called Chrkaka Samhita [2]. It's also thought of as an adaptogen that helps with stress adaptation. *O.sanctum* leaf extracts are used in the traditional (ayurvedic and unani) medicinal systems to treat headaches, inflammation, and

common colds. Numerous compounds, including b-caryophyllene, eugenol derivatives, vanillin, rosmarinic acid, ursolic acid, gallic acid, and vanillic acid, are abundant in *O.sanctum* leaves. However, there are still difficulties in identifying and extracting active flavonoids and connecting them to antioxidant qualities. Water and alcohols were found to be superior solvents for flavones, however, acetone was shown to extract more flavones than other organic solvents [3]. In 2017 Agarwal *et al* demonstrated the use of ethyl acetate to improve the extraction of polyphenols from *O.sanctum* [4].

Plants' therapeutic qualities are derived in part from their phytochemicals and secondary metabolites. In addition to acting as reducing agents, metal chelators, free radical absorbers, and neutralizers, polyphenols also effectively guard biological systems against deterioration under conditions of extreme oxidative stress [5]. Plant-based antioxidants, particularly polyphenolic substances, significantly prevent oxidative stress-related disorders from developing as well as rancidity caused by lipid oxidation in food [6]. To enhance the quality of food, nutritional content, and shelf life industrial application of phenolics [7] is enticing scientist interest in the search for natural, safe, and more effective plant-based components. Plants, seeds, and fruits are rich sources of flavonoids that are secondary metabolites and give them their distinctive color, scent, and flavor. Flavonoids have a wide range of roles in plants, including controlling cell division, drawing pollinating insects, and providing defense against biotic and abiotic strains [8]. Plant flavonoids, for example, have many physiological roles in drought, heat, and frost tolerance as well as being able to function as signal molecules, UV filters, and scavengers of reactive oxygen species (ROS) [9, 10].

In many physiological processes such as the synthesis of energy in mitochondria, regulation of cell growth, phagocytosis, and intracellular communication and in the human body detoxification of xenobiotics reactive oxygen species (ROS) are primarily formed as the intermediate metabolites. Tobacco smoke, pesticides, chemical pollutants, organic solvents, and UV radiation exposure are a few environmental variables that increase the generation of reactive oxygen species (ROS). Stress brought on by the modern lifestyle increases the overproduction of free radicals and damages tissue, which worsens the situation's health. Many compulsive ailments, including cancer, ischemia, atherosclerosis, arthritis, and damage to several tissues, including the central nervous system, have been linked to high and prolonged levels of stress [11]. The most important issue is human health since it directly impacts productivity and circuitously impacts a person's, a family's, a society's, and a country's economic situation. Antioxidants are being used at an exponentially higher rate to counteract increasing oxidative stress in both diseased and normal states. The use of antioxidants in food technology opens up new prospects for extending the shelf life of edibles. Research into natural antioxidants and their uses is desperately needed, as synthetic antioxidants have been exposed to several negative consequences, like the ability to cause cancer and pose health hazards to humans. Plants produce polyphenols as secondary metabolites in response to environmental stress [12]. Due to their bioactive qualities, these substances have been linked to a wide range of positive health effects in humans, including antiparasitic, immunomodulatory, cardio-protective, anti-inflammatory, neuroprotective, antidiabetic, anti-cancer, antibacterial, anti-aging, and antiviral possessions [13].

According to the studies, the main factor influencing the extraction of phytochemicals and their antioxidant properties is the solvent's polarity. Finding a fraction with the highest yield of polyphenols, flavonoids, and active antioxidant components may be accomplished through sequential fractionation using solvents with varying polarities. However, there are no instances of this herb's leaves being extracted differently. Therefore, this study's objectives were to extract as much polyphenol and flavonoid content as possible from *O. sanctum* leaves through successive fractionation using various solvents and to investigate any relationships between the preparations' contents and antioxidant activity. Thus, this study aimed to get maximum extraction of polyphenols and flavonoids from the leaves of *O. sanctum* by sequential fractionation with different solvents and to explore the correlation between the constituents and the antioxidant activity of the preparations. Through a recent study, an attempt was made to identify major flavonoids and their derivatives in the most effective fractions.

2. Material and Methods

2.1 Plant material

Leaves of *O. sanctum* were collected from a local garden in Faisalabad. The leaves were identified by a taxonomist at the University of Agriculture, Faisalabad. Leaves of *O. sanctum* were washed to remove the dirt particles, dried under the shade to avoid sun damage, and ground into fine particles using the mechanical grinder and sieved to achieve the mesh size of 250 µm.

2.2 Extraction procedure

The extraction of *O. sanctum* in pure methanol proceeded by cold maceration (daily occasional shaking) for seven days with the mass to solvent ratio of 1:10 w/v. The muslin cloth and Whatman filter paper were used to filter the methanolic extract after the completion of maceration. The filtrate was concentrated by evaporating the solvent using rotavapor at reduced pressure under 40 ℃ and saved at -4 ºC by further analysis [14].

2.3 Sequential extraction

The liquid-liquid extraction method was used for fractionation by dissolving dried methanol extract of *O. sanctum* leaves in water. Increasing polarity (from low to high polarity) was considered. Similarly, n-hexane was used three times to extract an aqueous solution, followed by butanol and ethyl acetate. Under the following circumstances, the extract/fraction resulting filtrates were concentrated using a rotary evaporator. While ethyl acetate and n-butanol extracts were dried at 40 ℃, n-hexane was dried at 30 ℃. Under reduced pressure at 50 ℃, the residual aqueous extract was dried. The concentrated extract was then further dried at the appropriate temperature in an oven and kept in a refrigerator between 2 to 8 ℃ [15].

2.4 Extraction yield

The yield of the extract in percentage was calculated after determining the mass of the extract by using the following formula [16].

$$Extraction\ yield\ (\%) = \frac{Dry\ weight\ of\ extract}{Dry\ weight\ of\ plant\ material} \times 100$$

2.5 Proximate analysis

The proximate analysis of *O.sanctum* leaf extracts, such as moisture content, crude protein, crude fat, crude fiber, total ash, and Nitrogen Free Extract (NFE) was quantified according to the respective standard methods of AOAC (2012) and the results were expressed on a dry-weight basis

2.6 Phytochemical analysis

2.6.1 Determination of total phenolic content (TPC)

With minor modification, the FC assay was used to evaluate the TPC of crude extracts and fractions [17]. After making a methanolic solution of each extract (1 mg/mL), 9 ml of distilled water was added. Add 1 mL of FC reagent to it, give it a good shake, and let it incubate for 5 minutes. Ten milliliters of 7% w/v sodium bicarbonate solution were added to the prior mixture. Distilled water was added to the volume to make it to 25 mL. whereas the methanol-containing blank solution. By making gallic acid standard solutions with concentrations of 10, 20, 40, 80, and 120 mg/mL, a calibration curve for gallic acid was also created. For 1.5 hours at 37 ºC, every solution was incubated. The wavelength at which absorbance was measured was 725 nm, and TPC was expressed as gallic acid equivalent (mg of gallic acid/g of extract).

2.6.3 Total flavonoid content (TFC)

By following the previous methodology, these contents of plant extracts were determined using the Aluminum chloride method, with catechin serving as a reference [18]. Catechin solutions of 10, 20, 40, 80, and 120 mg/mL were produced, along with a methanolic solution of each extract (1 mg/mL). In test tubes, 200 mL of each of the sample and standard solutions were combined with 100 mL of

aluminum chloride and 100 mL of 1M potassium acetate. The total volume was then adjusted to 5 mL using distilled water. The blank solution had methanol in it. Every solution was maintained for 0.75 hours at 37 °C. At 520 nm in wavelength, absorbance was calculated. TFC was expressed as the catechin equivalent (mg of CE/g of extract) and a calibration curve for catechin was created.

2.7 Estimation of in vitro antioxidant activity

2.7.1 DPPH radical scavenging assay

In this test, 1 mL of the plant sample solution and 1 mL of methanol were combined with 2 mL of the DPPH solution (0.04 g/100 ml methanol). Using the twofold dilution procedure, sample solutions of plant extracts were made in methanol. The absorbance was measured at 517 nm after a 30-minute incubation period. Methanol was the blank. Standard control was provided by ascorbic acid solution [19]. The following formula was used to calculate percentage inhibition.

$$\%\, inhibition = \frac{Abs.Control - Abs.Sample}{Abs.Control} \times 100$$

2.8 Statistical Analysis.

One-way ANOVA and Tukey's comparison were used to statistically analyze the data at the significance level (P 0.05) using Graph Pad Prism 6.01.

3. Results and discussion

Each extract's yield (%) was calculated after the extraction and fractionation process. *Ocimum sanctum* extracts with various solvents yielded 14.7% in methanol, 1.2% in n-hexane, 3.4% in ethyl acetate, and 6.7% in butanol and water.

3.1 Proximate Analysis

Proximate analysis is vital in assessing the quality attributes of the raw material, tulsi (*Ocimum sanctum*) leaves. Its powder was examined for several parameters to determine the overall composition of *O. sanctum* leaves. Results of proximate analysis (table#1) showed that the moisture content of *O. sanctum* leaves was (10.61±0.11), crude protein was 2.68±0.031, ash content was 6.66±0.031, crude fiber 17.92±0.13, crude fat 8.86±0.13 and nitrogen free extract was 0.280±0.006. Although these values were obtained in triplicate, there was no difference due to the negligible standard deviation. The findings of the proximate analysis showed non-significant variation. The proximate composition of *O. sanctum* leaves has been shown in numerous investigations. The current study's findings were developed with Idris *et al*. [20]. Their study examined the stem and leaves of *Ocimum Gratissimum* for mineral and proximate composition. Results illustrated that leaf and stem moisture content was 82.60 ± 0.11% and 82.60 ± 0.01% respectively. Protein contents of leaves and stem were 1.65±0.02% and 3.33 ± 0.07 % respectively. Ash contents of leaves and stem were 13.67±0.02% and 13.67 ± 0.13% respectively. The crude fiber of leaves and stems were 19.65 ± 0.03% and 9.52±0.01%, respectively, while crude fat of leaves and stem3.00±0.15% and 8.50 ± 0.04%, respectively. High concentration of carbohydrates in the stems (62.03 ± 0.04%) and leaves (64.98± 0.01%) gave a consistent proliferation in energy that is 278.42 ±0.11 kcal/100g for stem and 343.08 ± 0.01 kcal/100g for leaves respectively. In a study conducted by Sexena *et al*. (2015), they made value-added products of Emblica Officinalis Gaertn (gooseberry) and *Ocimum basilium* (basil: a form of tulsi) and examined the proximate composition of both plants. The proximate analysis results of *Emblica Officinalis Gaertn and Ocimum basilium* were compared with standards. Results showed that the moisture contents of gooseberry were 71.65%, crude fat content was 0.1%, crude fiber was 3%, and carbohydrate and protein contents were 13.2%, respectively. While *O. sanctum* proximate analysis results indicated that moisture, ash, protein, fat, fiber, and carbohydrate were 80.48%, 96.51%, 4.71%, 0.7%, 2.62%, and 2.37% respectively. 50 kcal energy content was found in gooseberry.

Table 1: Proximate analysis of *Ocimum sanctum* leaves

Treatments	Moisture (%)	Crude fiber (%)	Ash (%)	Fat (%)	Crude protein (%)	NFE (%)
T_1	10.61±0.11[a]	17.92±0.13[a]	6.66±0.14[a]	8.86±0.13[a]	2.68±0.031[a]	0.280±0.006[a]
T_2	10.34±0.03[a]	17.92±0.13[a]	6.66±0.14[a]	8.85±0.13[a]	2.68±0.031[a]	0.270±0.000[a]
T_3	10.62±0.08[a]	17.92±0.16[a]	6.66±0.14[a]	8.54±0.11[a]	2.65±0.012[a]	0.267±0.003[a]

3.2 Estimate of TPC and TFC

According to the data, the maximum TPC concentrations were found in the methanolic and aqueous extracts (92.37±1.81 mg GAE/g and 72.19 ± 0.59 mg GAE/g, respectively). TFC concentrations in the methanolic and aqueous extracts were 67.70 ± 1.04 mg CE/g and 54.28 ± 0.90 mg CE/g, respectively. Estimate of TPC and TFC is given in the table #3. *Ocimum Canum Sim* leaves were studied for chemical and phytochemical composition. The findings of proximate analysis showed that these leaves had a high carbohydrate content which is 639.6±30.9 g/kg, crude protein was 0.40 ± 0.10 g/kg, crude fat was 70.0 ± 14.1 g/kg, ash content were 120.0 ± 28.3 g/kg and 170.0 ± 14.2 g/kg crude fiber. According to a phytochemical study, they have modest alkaloids and phenolics but are high in flavonoids, tannins, and saponin [21].

Results of another study carried out and revealed that total high ash contents of *O. sanctum* leaves depicted that there a high level of minerals in it and higher content of acid insoluble ash, indicating that *O.sanctum* has higher digestibility properties when it consumed as a whole.

Experiments showed that 19.27% of the leaves' dry weight could be extracted as a crude extract from an aqueous methanol solution. Using n-hexane, ethyl acetate, and n-butanol for differential fractionation, we were able to obtain 1.22–13.66% of the complete extracted soluble constituents from the methanol extract, which corresponds to 0.24–2.63% of the dry mass of the leaves. Regarding extraction efficiency, n-butanol produced a higher yield of soluble components (13.66%) than ethyl acetate (4.46%). Essential oils with shorter carbon chains that may contain phenolic compounds or other hydrophilic moieties coupled with hydrophobic lipids made up the bulk of the extractable soluble components, but only a minor fraction (1.22%) were extractable with n-hexane. Prior research by Chaudhary *et al*. [22] highly influences the academic community.

The fatty acid composition of the methanol extract of *O. sanctum* leaf was reported to be 1.93% by Ali and Ali ([23]). Harichandan et al. ([24]) analyzed the holy basil leaf methanol extract and found it includes alkaloids, terpenes, phenols, tannins, and steroids. Several variables can affect polyphenol and flavonoid recovery, such as the sample's chemical makeup, the solvent's properties, and the extraction process's settings. Studies by Iloki-Assanga *et al*. ([3]) and Kchaaou *et al*. [25] suggest that methanol has better extraction capacities for polar polyphenols and flavonoids than water.

Table 3: Total phenolic and flavonoid contents in different extracts of *Ocimum sanctum*

Treatments	Total phenolic (mg GAE/g)	Total flavonoid (mg CE/g)
Methanol	92.37±1.81[a]	67.70±1.04[a]
n-hexane	4.85±0.02[e]	3.83±0.03[e]
Ethylacetate	20.80±0.15[c]	14.29±0.42[c]
Aqueous	72.19±0.59[b]	54.28±0.90[b]
Butanol	15.08±0.05[d]	10.17±0.11[d]

3.3 DPPH scavenging activity

The antioxidant activity of *Ocimum sanctum* plant extracts was evaluated by DPPH scavenging assay. Results indicate that the antioxidant activity of all extracts was shown at a concentration as low as 0.1563 mg/ml. Table 4.3 showed that aqueous (84.56 ± 0.35%) and methanolic (87.50 ± 0.61%) extracts were compared to ascorbic acid (82.55 ± 0.35%). The decreasing order of DPPH scavenging activity expressed by the plant extracts was methanol > aqueous > ethyl acetate > butanol > n-hexane. Root extracts from *O. sanctum* and *O. kilimandscharicum* were fractionated in various solvents in a 2017 study by Agarwal *et al*. The ethyl acetate fraction was analyzed, and flavonoids

and their compounds were found. Plants contain polyphenolic chemicals in various complex forms, including esters, glycosides, glucuronides, and free monomers. Similar results have been observed in previous investigations by Ali and Ali ([23]) and Harichandan *et al*. ([24]). The amounts and characteristics of other biomolecules in the compound can distress the retrieval and movement of polyphenols in various solvents. Antioxidant activities have been linked to the phenolic content of extracts, even if our understanding of the structure-activities connections of phenolic composites in herbs is still sketchy [4, 26]. The antioxidant capability was measured by testing their ability to quench different types of free radicals and neutralize metal ions. Another study revealed that *O.sanctum* leaves contained 28.38 mg/g flavonoids. The presence of flavonoids confirms that the plants have high antioxidant value and justifies their antimicrobial, anti-inflammatory, anti-mutagenic, antiviral, and anti-allergic actions [27].

Recent advances in biomedicine showed the pathological involvement of free radicals and oxidative stress in various inflammatory and immune diseases like arthritis, diabetes, neurodegeneration, and cancer [28]. The methanolic and aqueous extracts of *O. sanctum* exhibited the maximum DPPH scavenging activity, possibly due to higher TPC and TFC than other extracts. Another study revealed that *Moringa olifera* and *P.braunii* aqueous and methanolic extract showed the maximum DPPH and hydrogen peroxide scavenging activities and reducing power, possibly due to the presence of higher total phenolic and flavonoid contents than other extracts [29]. Other species of *Moringa* have also shown significant *in vitro* and *in vivo* antioxidant potential (Verma *et al*., 2009).

The current study revealed that methanolic extract of *O. sanctum* had higher DPPH scavenging activity than ascorbic acid at the higher tested concentration. It has previously been shown that plant extracts might have higher antioxidant activity than ascorbic acid due to the presence of phenolics and flavonoid compounds [30].

Table 4: DPPH scavenging activity of (%) of *Ocimum sanctum* different extracts

Methanol	**n-hexane**	**Ethyl acetate**	**Butanol**	**Aqueous**	**Ascorbic acid**
87.50 ± 0.61	64.49 ± 0.36	76.20 ± 0.19	74.31 ± 0.24	83.46 ± 0.34	81.55 ± 0.35
81.68 ± 0.5	56.43 ± 0.28	65.36 ± 0.28	66.64 ± 0.40	75.23 ± 0.12	74.26 ± 0.23
78.19 ± 0.14	46.39 ± 0.34	60.08 ± 0.51	58.53 ± 0.43	66.56 ± 0.21	60.54 ± 0.35
67.35 ± 0.19	35.42 ± 0.14	49.65 ± 0.28	45.56 ± 0.43	51.5 ± 0.21	53.41 ± 0.11
50.16 ± 0.24	29.48 ± 0.23	35.68 ± 0.13	32.55 ± 0.41	46.61 ± 0.35	46.49 ± 0.33
38.73 ± 0.49	17.55 ± 0.47	30.86 ± 0.54	26. 64 ± 0.41	35.94 ± 0.81	42.35 ± 0.35

Conclusion

Research on *O. sanctum* leaf extract and fractions showed that great antioxidant capability and a high phenolic and flavonoid content could be extracted using methanol and aqueous solvents. The flavonoid-enriched methanol and aqueous extract capacity as antioxidants supports their potential utility as a defense against oxidative stress-related damage. These extracts may be used therapeutically or neutraceutically to include the antioxidants found in herbs, with potential consequences for human health, nutrition, and food.

Author's Contribution: the study was designed by Hafiza Sadia Younas and Madiha Ilyas. The experimental trials were carried out and the samples were examined by Hafiza Sadia Younas and Faqir Muhammad. The results were analyzed by Madiha Ilyas, Saima Tehseen, and Ayesha Riaz. Hafiza Sadia Younas wrote the original document. After a thorough assessment, each contributor gave their final approval to the work.

References

1. Olaleye, O., et al., *Chemical composition, antioxidative and antimicrobial activities of different extracts of the leaves of parquetina nigrescens (asclepiadaceae).* Progress in Chemical and Biochemical Research, 2021. **4**: p. 359-371.
2. Satyamitra, M., et al., *The antioxidant flavonoids, orientin and vicenin enhance repair of radiation-induced damage.* SAJ Pharmacy and Pharmacology, 2014. **1**(1): p. 1.
3. Iloki-Assanga, S.B., et al., *Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of Bucida buceras L. and Phoradendron californicum.* BMC research notes, 2015. **8**: p. 1-14.
4. Agarwal, K., et al., *Antioxidative potential of two chemically characterized Ocimum (Tulsi) species extracts.* Biomedical Research and Therapy, 2017. **4**(9): p. 1574-1590.
5. Lobo, V., et al., *Free radicals, antioxidants and functional foods: Impact on human health.* Pharmacognosy reviews, 2010. **4**(8): p. 118.
6. Sarija, K., et al., *In vitro antioxidant studies and phytochemical screening on the seeds of corchorus trilocularis.* Der Pharmacia Sinica, 2014. **5**(5): p. 82-85.
7. Naczk, M. and F. Shahidi, *Extraction and analysis of phenolics in food.* Journal of chromatography A, 2004. **1054**(1-2): p. 95-111.
8. Rodríguez De Luna, S.L., R. Ramírez-Garza, and S.O. Serna Saldívar, *Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties.* The Scientific World Journal, 2020. **2020**(1): p. 6792069.
9. Di Ferdinando, M., et al., *Flavonoids as antioxidants in plants under abiotic stresses.* Abiotic stress responses in plants: metabolism, productivity and sustainability, 2012: p. 159-179.
10. Dias, M.C., et al., *The antioxidant system in Olea europaea to enhanced UV-B radiation also depends on flavonoids and secoiridoids.* Phytochemistry, 2020. **170**: p. 112199.
11. Mazandarani, M., et al., *Effects of solvent type on phenolics and flavonoids content and antioxidant activities in Onosma dichroanthum Boiss.* J Med Plants Res, 2012. **6**(28): p. 4481-8.
12. Nunes, P., et al., *Biological oxidations and antioxidant activity of natural products.* Phytochemicals as nutraceuticals-Global Approaches to Their Role in Nutrition and Health, 2012. **278**.
13. Fraga, C.G., et al., *The effects of polyphenols and other bioactives on human health.* Food & function, 2019. **10**(2): p. 514-528.
14. Akhtar, M.F., et al., *Association of textile industry effluent with mutagenicity and its toxic health implications upon acute and sub-chronic exposure*. Environmental monitoring and assessment, 2018. **190**: p. 1-13.
15. Alamgeer, A.M. Uttra, and U.H. Hasan, *Anti-arthritic activity of aqueous-methanolic extract and various fractions of Berberis orthobotrys Bien ex Aitch.* BMC complementary and alternative medicine, 2017. **17**: p. 1-16.
16. Naseem, Z., et al., *Green extraction of ethnomedicinal compounds from Cymbopogon citratus Stapf using hydrogen-bonded supramolecular network.* Separation Science and Technology, 2021. **56**(9): p. 1520-1533.
17. Naseem, Z., et al., *Use of hydrogen-bonded supramolecular eutectic solvents for eco-friendly extraction of bioactive molecules from Cymbopogon citratus using Box–Behnken design.* Journal of Food Measurement and Characterization, 2021. **15**: p. 1487-1498.
18. Ahmed, S., et al., *Antioxidant activity of Pistacia Khinjuk supported by phytochemical investigation.* 2017.
19. Naseem, Z., et al., *Ultrasound-assisted deep eutectic solvent–based extraction of phytochemicals from Mentha arvensis: optimization using Box-Behnken design.* Biomass Conversion and Biorefinery, 2021: p. 1-11.
20. Idris, S., et al., *Nutritional composition of the leaves and stems of Ocimum gratissimum.* Journal of Emerging Trends in Engineering and Applied Sciences, 2011. **2**(5): p. 801-805.
21. Aluko, B., O. Oloyede, and A. Afolayan, *Phytochemical and nutrient compositions of the leaves of Ocimum canum Sims.* African Journal of Biotechnology, 2012. **11**(63): p. 12697-12701.

22. Chaudhary, A., et al., *Phytochemical and antioxidant profiling of Ocimum sanctum.* Journal of Food Science and technology, 2020. **57**(10): p. 3852-3863.
23. Ali, A. and M. Ali, *New fatty acid derivatives from Ocimum sanctum L. leaves.* Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, 2012.
24. Harichandan, S.P., et al., *Phytochemical screening and antioxidant activity of methanolic extract of Ocimum sanctum Linn. Leaves.* GSC Biological and Pharmaceutical Sciences, 2019. **8**(2): p. 022-033.
25. Kchaou, W., et al., *Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (Phoenix dactylifera L.).* Industrial crops and products, 2013. **45**: p. 262-269.
26. Saeed, N., M.R. Khan, and M. Shabbir, *Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L.* BMC complementary and alternative medicine, 2012. **12**: p. 1-12.
27. El-Awady, M.A., et al., *Comparison of the antimicrobial activities of the leaves-crude extracts of Moringa peregrina and Moringa oleifera in Saudi Arabia.* Int. J. Curr. Microbiol. App. Sci, 2015. **4**(12): p. 1-9.
28. Khojah, H.M., et al., *Reactive oxygen and nitrogen species in patients with rheumatoid arthritis as potential biomarkers for disease activity and the role of antioxidants.* Free Radical Biology and Medicine, 2016. **97**: p. 285-291.
29. Saleem, A., et al., *Exploration of acute and chronic anti-inflammatory potential of Quercus leucotrichophora A. Camus extracts in Wistar rats: A mechanistic insight.* Frontiers in Pharmacology, 2023. **14**: p. 1002999.
30. Soare, L.C., et al., *Antioxidant activity, polyphenols content and antimicrobial activity of several native pteridophytes of Romania.* Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 2012. **40**(1): p. 53-57.