

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT PROFILE OF *OCCIMUM SANCTUM LINN*

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ABSTRACT

The medicinal plants have become important in the global context today as it offer solutions to the major concerns of human mankind. *Ocimum sanctum Linn.* which have been normally used in traditional puja at every Indian Hindu home. This medicinal plant has numerous properties such as Phytochemical and Antioxidants filled with good aroma. The soxhlet hot extraction method was used for extracting *Occimum sanctum linn* using various solvents like chloroform, aqueous, ethanol, methanol, acetone and hexane. The extract was tested under standard qualitative phytochemical screening method and the test results revealed the presence of phytochemical constituents such as carbohydrates, proteins, steroids, alkaloids, flavanoids, phenols and tannins. The quantitative analysis illustrated that the alkaloids, flavonoids, tannin, saponin and phenol. Free radical scavenging activity were analyzed by Ferric reducing antioxidant power (FRAP) assay and ABTS assay.

Keywords: ABTS, FRAP, Free radical scavenging activity, *Occimum sanctum linn*, Phytochemicals.

I. INTRODUCTION

Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function [1]. Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species (ROS) in the cells. Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases [2]. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage and oxidative stress is the main cause of several diseases: cancer, cataracts, age related diseases and Parkinson's disease. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases.

This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation [3]. Medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry [4]. The earliest mention of medicinal use of plants in Hindu culture is found in

“Rigveda “which is said to have written between 4500-1600 BC. It is Ayurveda, the foundation of medicinal science of Hindu culture in its eight division deals with specific properties of drugs and various aspects of science of life and art of healing. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grown in different parts of the country [5]. *Ocimum sanctum* L. (family Lamiaceae) is an aromatic perennial herb widely grown in India. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [6].

In the present study the phytochemical screening and antioxidant activities of *Ocimum sanctum* (Tulsi) belongs to the family *Lamiaceae* and found mostly in countries including: Libya, India, North and South America, Mexico and Brazil where it is popularly known as alfavaca-cravo, alfavacao, alfavaca [7]. It's traditionally used to relief pains and used in the treatment of rheumatism, diarrhea, high fever, convulsions, diabetes, eczema, piles and as a repellent [8][9]. The decoction of the stem is inhaled for the treatment of catarrh and bronchitis [10]. *Ocimum sanctum* is popularly used in folk medicine for the treatment of upper respiratory tract infection, diarrhea, cough, fever, gonorrhoea, worm infection, stomach aches, headaches, pile, pneumonia and surface wound. It is also implicated in blood coagulation, anti-inflammatory, cardiovascular and renal function properties have been observed [11]. The plant is used as food spice and for the treatment of ailments such as; malaria, diabetes, respiratory and urinary tract infections, cough, fever, diarrhea, abdominal pains, pneumonia, conjunctivitis, oral wounds and tooth infection [12][13].

II. MATERIALS AND METHODS

1.1 Collection of Plant Materials

Fresh leaves and stem of *Ocimum sanctum*, was purchased from local market Vellore district. It was dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve and stored in a labeled air tight container for further studies. The plants material powder was subjected to soxhlet extraction by using various solvents like double distilled water, methanol, ethanol, acetone, hexane and chloroform for about 24h. Each solvent extract was evaporated to dryness.

1.2 Preliminary Phytochemical Screening

The various solvents extracts of *Ocimum sanctum* linn plants was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures [14, 15, 16].

1.2.1 Test for Tannins:

1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black coloration.

1.2.2 Test for Phlobatannins: When crude extract of each plant sample was boiled with 2 % aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

1.2.3 Test for Saponins: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

1.2.4 Test for Flavonoids: 5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

1.2.5 Test for Steroids: 2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in samples indicates the presence of steroids.

1.2.6 Test for Alkaloids: Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.

1.2.7 Test for Carbohydrates: Few drops of extract are heated with Fehling's A and B solution. Appearance of orange red precipitate indicates the presence of carbohydrates.

1.2.8. Test for Proteins: Add 2 ml of Biuret reagent to 2 ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins.

1.2.9 Test for Terpenoids (Salkowski test): 5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H₂SO₄ was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

1.2.10 Test for Cardiac glycosides (Keller-Kiliani test): 5 ml of extract was treated with 2 ml of glacial acetic acid containing one ~ 24 ~ Journal of Pharmacognosy and Phytochemistry drop of ferric chloride solution. This was underlaid with 1 ml of concentrated H₂SO₄. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

II. 2.1 Quantitative Determination of Phytochemical Constituents Determination of TPC

Total phenolic content of the methanolic extract of all selected plants was determined by standard method [17] with little modifications, using tannic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of tannic acid/ml. 250 µl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin - Ciocalteu reagent. The samples were mixed well and then allowed to for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Then, 2.5 ml of 7 % sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the samples for 90 min. All the experiment was conducted in three replicates.

2.2 Determination of Alkaloids

5 g of the dried powder of each sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reaches to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated [16].

2.3 Determination of Saponins

20 g of each sample was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and saponin content was calculated as percentage [18].

2.4 Determination of Flavonoids

10 g of each plant sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 41. The filtrate was allowed to be evaporated into dryness over a water bath and weighed to a constant weight [19].

III. INVITRO ANTIOXIDANT ACTIVITY

3.1. Determination of FRAP radical scavenging activity

A modified method of Benzie and Strain [20] was adopted for the ferric reducing antioxidant power (FRAP) assay. It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue colour which can be read at 593nm. 1.5 mL of freshly prepared FRAP solution, containing 25µmL of 300 mM acetate buffer pH 3.6, 2.5µmL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40µmMµHCl, and 2.5µmL of 20 mM ferric chloride {FeCl₃·6H₂O} solution, was mixed with 1µmL of the extracts, and the absorbance was read at 593 nm. The standard curve was linear between 100 and 500µM FeSO₄·7H₂O. Results are expressed in µM Fe (II)/g dry plant material and compared with that of ascorbic acid.

3.2. ABTS radical cation decolorization assay

ABTS stock solution was prepared by mixing 7mM ABTS [21] was modified stock solution with 2.45 mM potassium persulfate (1/1, v/v) and allowing the mixture to stand in the dark at room temperature for 12–16 h until the reaction was complete and the absorbance was stable. The ABTS solution was diluted with ethanol (about 1:89 v/v) to an absorbance of 0.700±0.05 at 734nm. The photometric assay was conducted by adding 0.9ml of ABTS solution to different concentration of samples and incubated for 15 min, measurement were taken immediately at 734 nm. The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$E = ((A_c - A_t) / A_c) * 100$$

Where,

At and Ac are the respective absorbance of tested samples and ABTS was expressed as µmol.

III.RESULTS AND DISCUSSION

Plant are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolics, lignins, tannins, flavonoids, quinines, alkaloids, and other metabolites, which are rich in antioxidant activity [22]. Studies have shown that many of the phytochemicals possess anti-inflammatory, anti-diabetic and antimicrobial activities [23]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [24]. Plant derived substances have recently become a great interest owing to the versatile applications. Medicinal plants and herbs are the richest bio-resource of drugs of traditional systems of medicine, modern medicine, pharmaceutical intermediates and chemical entities for synthetic drugs. Phytochemical screening of *Occimum sanctum* using various extracts like aqueous, methanol, ethanol, acetone, hexane and chloroform. Phytochemical qualitative analysis of *Occimum sanctum* presented in the Table 1. The screening analysis was performed in order to identify various secondary metabolites which is present in *Occimum sanctum* using a wide range of solvents namely aqueous, methanol, ethanol, acetone, hexane and chloroform. The screening analysis of *Occimum sanctum* using various solvents revealed the presence of carbohydrate, protein, alkaloids, and tannins, phenols, in the methanolic and aqueous extracts. While the presence of saponins was noted in chloroform extract. The qualitative phytochemical analysis results explored the presence of a wide range of phytochemical constituents which signifies the *Occimum sanctum* as a valuable therapeutic natural source which will serve as an effective herbal option to combat dreadful infectious diseases.

Medicinal plants constitute the group of plants mainly used for health care. Use of them as traditional medicine is known since time immemorial. Chemicals present universally in all the plants can be classified as primary and secondary metabolites. Primary metabolites include proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, chlorophylls etc., while secondary phytochemicals as alkaloids to terpenoids and acetogenins to different phenols. These are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [25]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [26,27,28]. Quantitative analysis of *Occimum sanctum* presented in the Table 2. In our present study revealed that Total phenolic content (41.55) shows higher amount compared to other phytochemicals Alkaloids (6.52), Saponins (0.62) and Flavonoids (17.11). ABTS radical cation scavenging activity is relatively recent one, which is oftenly used for screening of complex antioxidant mixtures such as plant extract beverages and biological fluids. ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and chain-breaking antioxidants [29]. FRAP assay had been used to determine antioxidant activity as it is simple and quick [30]. Besides that, the reaction is reproducible and linearly related to molar concentration of the antioxidants. However, some disadvantage was found in this method, as FRAP assay does not react fast with some antioxidants such as glutathione [31]. This investigation revealed given concentration, the extract of *Occimum sanctum* showed higher antioxidant potential by FRAP method in Table 4. FRAP assay still can be used for assessment of antioxidant activity in plant materials as humans only absorb limited amount of glutathione [32].

ABTS is an excellent substrate for peroxidases and is frequently used to study the antioxidant properties of natural compounds [33]. ABTS scavenging radical scavenging activity examined at different concentrations of

ethanol extracts of *P. aquilinum* (1.0, 2.0, 3.0, 4.0 and 5.0mg) was depicted. In the present study of methanolic extract of *Occimum sanctum* in the concentration range from (200,400,600,800& 1000) showed higher antioxidant potential by ABTS radical scavenging method in Table 5.

IV. TABLES

Table: 1. Qualitative phytochemical analysis of *Occimum sanctum* extracts

Test	Aqueous	Ethanol	Acetone	Chloroform	Methnol	Hexane
Phlobatannins	-	-	-	-	-	-
Saponins	+	+	-	+	+	-
Flavonoids	+	+	-	+	+	+
Steroids	+	+	-	+	+	-
Alkaloids	+	+	-	-	+	-
Carbohydrates	+	+	-	+	+	+
Proteins	+	+	-	+	+	+
Terpenoids	+	+	-	+	+	-
Glycosides	-	-	-	-	-	-

+:Presence; - :Absence

Table :2. *Occimum sanctum* Methanolic extracts of Total Phenolics, Alkaloids, Saponins and Flavonoids

S.no	Phytochemicals	Results (w/w)
1.	Total Phenolics	41.55
2.	Alkaloids	6.52
3.	Saponins	0.62
4.	Flavonoids	17.11

Table 3: FRAP radical scavenging activity of methanolic extract from *Occimum sanctum*

S.NO	Sample Conc.(µg/ml)	FRAP (mM)	
		Standard	<i>Occimum sanctum</i>
1	200	210	374
2	400	406	444
3	600	599	548
4	800	795	780
5	1000	990	1067

Table 4: ABTS radical scavenging activity of methanolic extract from *Occimum sanctum*

S.NO	Sample Conc.(µg/ml)	ABTS (µmol)	
		Standard	<i>Occimum sanctum</i>
1	200	44	64
2	400	51	76
3	600	54	88
4	800	56	93
5	1000	70	95

V. CONCLUSION

Phytochemical screening of aqueous, methanolic and ethanol extract had revealed the presence Alkaloids, flavonoids, saponin and protein by positive reaction with the respective reagent. It was observed that the leaf and stem extract of *Occimum sanctum* contained high level of phenolic content that might have accounted for the strong activity observed against free radical scavenging antioxidant activity. The finding of this study suggests that this plant leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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