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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, TRACE ELEMENTS AND QUANTITATIVE ESTIMATION OF TOTAL FLAVONOIDS, PHENOLIC CONTENT OF *OCIMUM BASILICUM* LEAVES EXTRACT

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ABSTRACT

The nutritional profile of minerals and antioxidants in Indian spice extracts was evaluated in order to examine their efficacy in treating various diseases, disorders, and allergies in human health. Medicinal plants *Ocimum basilicum*, regularly consumed as spice products in South Asia, have been studied using elemental analysis, antioxidant studies. The basil leaf was extracted by maceration process using methanol and ethylacetate. The methanol and ethylacetate extract were screened of phytochemical content including identification of flavonoid, alkaloid, polyphenols, glycosides, tannin, saponin etc. Estimation of total flavonoids, phenolic content was based on aluminium chloride method in the sample extract by spectrophotometrically. Phytochemical screening test showed that the presence of saponins, proteins, flavonoids, carbohydrates, alkaloids in leaves parts when extracted with methanol and ethylacetate solvents. In this study, *Ocimum basilicum*, has phytochemicals properties in the leaves which are used in curing the ailments and higher flavonoid content indicated the natural antioxidant activity signifying their medicinal importance and potent source in pharma industries. We conclude that *Ocimum basilicum*, is a highly useful medicinal plant. However, there is necessary to explore natural plant sources with their medicinal value used in medical field.

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INTRODUCTION

Medicinal plants in drug discovery are known to us well and the human being used them for various purposes from the beginning of the human history (1). Over the years, medicinal plants have been useful sources of several active compounds of recuperative value and it is used as a substitute medicine for treating numerous diseases (2). Medicinal plants are very important to the health of individuals and communities. These plants have some phytochemical components with their medicinal value that produce a physiological action on the human body. Medicinal plants are a group of species that possesses a wide range of active ingredients that can be utilized to treat various human or animal illnesses. They are the most plentiful source of bio-drugs on earth. Chemical entities and pharmacological intermediaries, modern medications, conventional medical procedures, natural medicines, dietary supplements, and nutraceuticals, as well as counterfeit drugs (3). Alkaloids, carbohydrates, terpenoids, steroids, flavonoids, and tannins are only a few examples of phytochemicals found in medicinal plants that have a certain physiological impact on the human body and are useful for treating and curing human illnesses (4). Figure1. *Ocimum basilicum* L (Lamiaceae) also commonly known as "Holy basil" have possessed different biological effects. The oil of the plant contains eugenol, methyl eugenol, citral, and methyl chavicol (5). *O. Basilicum* leaves are used as antispasmodic, carminative, digestive, stomachic, and tonic (6,7) *O. basilicum*

produces triterpenoids, polyphenols, steroids, and phenylpropanoids some of which, such as baseball, ocimol, basilimoside, rosmarinic acid, hydroxycinnamic acids, oleanolic acid, and betulinic acid which possess various biological properties(8,9) *Ocimum* sp., is shown to exert antibactericidal, antiinflammatory, antioxidative, antiulcer, antidiarrheal, chemopreventive and hypoglycemic properties(10-15) The observed effects of the plants might be due to its antioxidant power which in turn is attributed to the presence of flavonoids and polyphenols (16) Naturally these plants are available in whole worlds. These plants have many properties that are beneficial to human being and complete society in the medication and pharmacological field. Alkaloids, tannins, terpenoids, flavonoids, phenols are active compounds which show the physiological action on the human body (17). Hence, the objective of this study is to determine the phytochemical constituents, trace elements present in leaf extracts of *Ocimum basilicum* and flavonoids component in the plant sources.

MATERIALS AND METHOD

Plant material- Identification and authentication: Matured *O. basilicum* leaves were selectively removed from the plant in and around areas of Pudussery, Palakkad, Kerala and identified by a plant taxonomist. BSI/SRC/5/23/2022/Tech/630.

Preparation of *O.basilicum* leaves extract: 100 g. of *O.basilicum* dried material were exhaustively extracted with methanol and ethyl acetate using maceration for 24 hrs. The extract was evaporated above their boiling points.

Qualitative Phytochemical Screening: The plant extracts obtained by using methanol and ethyl acetate extraction process and it is subjected to different phytochemical tests to identify the plant constituents by using standard following methods (18,19).

Test for protein: The protein content of all the plant extracts was estimated following the method of (20). Values were expressed as μg protein/mg plant extract using the calibration curve of BSA.

Test for Carbohydrates: The presence of carbohydrates was confirmed when 2 ml of extract was treated with 1 ml of Molisch's reagent and a few drops of concentrated sulfuric acid which resulted in the formation of purple or reddish color.

Test for Phenols: 2 ml of distilled water, followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

Test for Tannins: To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Flavonoids: To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for Saponins: 2 ml of extract, 2 ml of distilled water were added and shaken in a graduated cylinder for 15 min lengthwise. It resulted in the formation of 1 cm layer of foam that indicated the presence of saponins.

Test for Glycosides: To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Steroids: To 1 ml of fruit extract equal volume of chloroform is added and a few drops of concentrated sulphuric acid added appearance of brown ring indicates the presence of steroids and appearance of the bluish brown ring indicates the presence of phytosteroids.

Test for Terpenoids: 0.5 ml of the extract was treated with 2 ml of chloroform and conc. sulphuric acid. Formation of red brown color at the interface indicates the presence of terpenoids.

Test for Alkaloids: To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

Quantitative Determination of Secondary Metabolites

Estimation of Flavonoids: The total flavonoid content in the sample was estimated by the method of Chang. A volume of 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 μl of 5% sodium nitrite was added and six minutes 0.1 5 ml of aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read at 510 nm along with standard quercetin at concentration. The results are expressed as mg of flavonoids as quercetin equivalent / gm of dried sample.

Total Phenolic Content (TPC): Total phenolic content of the extract was determined according to the Folin-Ciocalteu method of Slinkard and Singleton with some modifications. Briefly, 0.1 ml of extract, 1.9 ml distilled water and 1 ml of Folin-Ciocalteu's reagent were seeded in a tube, and then 1 ml of sodium carbonate was added.

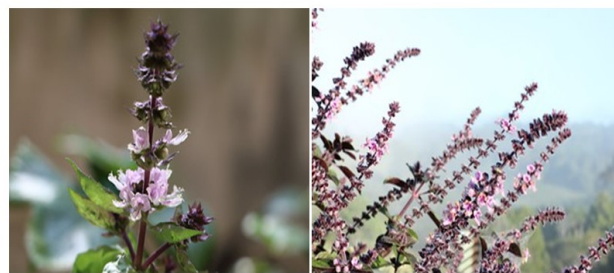


Figure 1. *Ocimum basilicum* wholeplant

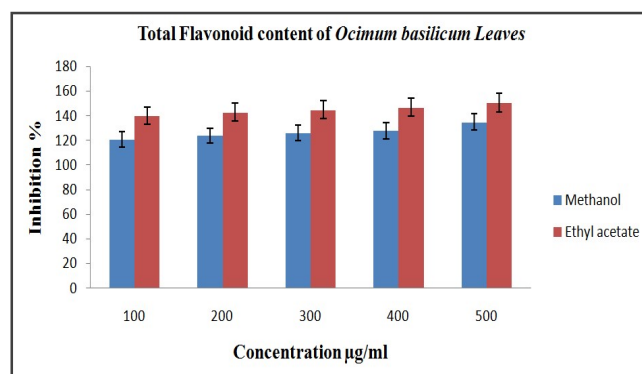


Figure 2. Shows the total flavonoid content *O. basilicum* leaves extract

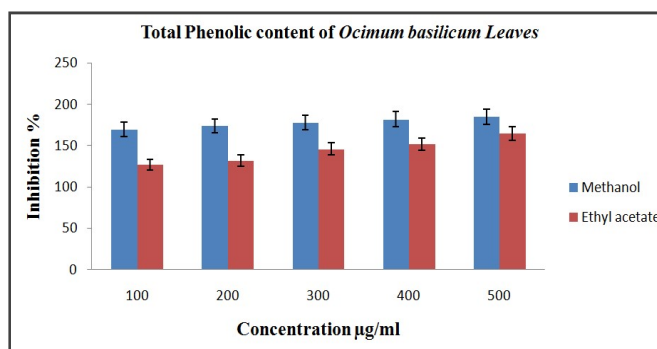


Figure 3. Shows the total phenolic content *O. basilicum* leaves extract

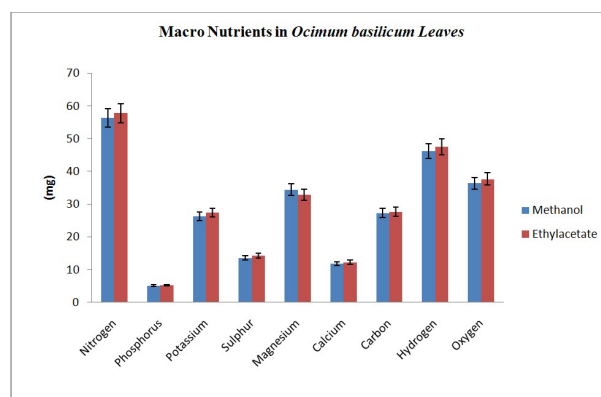


Figure 4. Shows the Macro nutrients *ocimum basilicum* leaves extract

The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained.

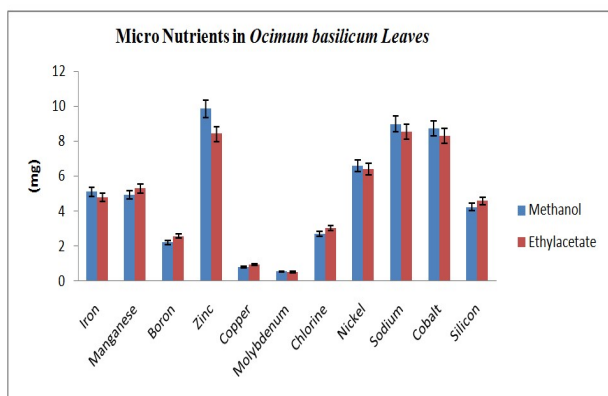


Figure 5. Shows the Micro nutrients *ocimum basilicum* leaves extract

Table. 1 Shows the Phytochemical screening of *Passiflora foetida* flowers extract

SNo	Qualitative test	<i>Passiflora foetida</i> flowers extract	
		Hexane	Petroleum ether
1	Proteins	-	+
2	Carbohydrates	+	-
3	Phenols	-	+
4	Tannins	+	+
5	Flavonoids	+	+
6	Sapoin	-	+
7	Glycosides	+	+
8	Steroids	+	+
9	Terpenoids	-	+
10	Alkaloids	+	-

The results were compared with the cortical calibration curve and the total phenolic content of the sample was expressed as mg of catechol equivalents per gram of extract.

Mineral concentration: Trace minerals, namely Cu, Co, Fe, and Zn were estimated in concentrate methanol and ethylacetate extract of *Ocimum basilicum* leaf and resultant two fractions by using an atomic absorption spectrophotometer (AAS 4141, ECIL-Elements, India) and macro-minerals like Na and K were measured by using flame photometer (Model no. 1381, ESPIO, Japan). The accurately weighed amount of samples were digested by 10 ml of Tri acid solution (Nitric acid: sulphuric acid: perchloric acid- 9:2:1) and then volume was made up to 25 ml by adding triple distilled water to the digested solution. Ca was estimated by Arsenazo method (Kit supplied by Reckon Diagnostics Pvt. Ltd, India (Chempak)), inorganic phosphorus (Pi) by phosphomolybdate complex method (kit supplied by Chemelex, S.A., India). Magnesium in the hot water extract and its fractions was estimated by kit supplied by Lab-Care Diagnostics (India) Pvt. Ltd. (Accucare™ Magnesium Xylidyl Blue). For estimation of Mg in leaf powder, leaf powder was digested in tri-acid solution and was estimated using an atomic absorption spectrophotometer (ECIL-Elements AAS 4141). All the results were expressed as $\mu\text{g mg}^{-1}$ of extract.

Statistical analysis: All the assays were carried out in triplicate. Experimental results are expressed as mean \pm standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

RESULTS AND DISCUSSION

The phytochemical screening and quantitative estimation of chemical components of the plants studied showed that the leaf and stem were rich in flavonoids, saponins, amino acids, proteins, diterpenes. These phytochemicals has medicinal and physiological activity (20). Phytochemicals are the chemical constituents present in plants which

show physiological action on the human body (21). Table 1 shows the Alkaloids, flavonoids, phenols, diterpenes, carbohydrates, proteins, glycosides, and essential oils are some of the important bioactive phytochemicals (22). A number of reports are available that represent the phytochemical presence in plants such as flavonoids, glycosides, tannins, alkaloids phenols, proteins in medicinal plants (23, 24). Major phytochemicals are reported in *Ocimum basilicum*. are flavonoids, glycosides, Alkaloids, proteins, tannins and phenols (25). It has been determined that antioxidant activity present in flavonoid component and its effect on human nutrition and their health. The mechanism of action of flavanoids is through scavenging or chelating process (26). Flavonoids are the common antioxidants present in various medicinal plants (27, 28). Figure 2 showed the flavonoid component present in the leaves of *Ocimum basilicum*. Various reports also available that indicates as a good source of flavonoids (29). Since, phenolic and flavonoid components present in high amount in *Ocimum* species. Thus, widely used in traditional medicine systems (30). Flavonoids have one hydroxyl group that is substituted aromatic ring. Phenolic combine with metal ions and form a chelate complex and can easily oxidize and donating electrons to scavenge free radicals (31). It has been reported that good amount of total flavonoid contents present in the methanol extract of *Ocimum basilicum* (Figure 3). Trace elements are a group of naturally occurring elements that are required for various biochemical and physiological functions. The inadequate supply of these micro-nutrients results in a variety of deficiency diseases or syndromes. Figure 4,5 shows the presence of trace elements showed that the extract might usefully influence various body functions. These elements are used extensively in chemotherapy and are essential in human and animal health (32,33,34). The presence of high concentration of some elements seen in this analysis of *O. basilicum* may be due to the topography, soil-water-plant exchange complex and evapotranspiration of the environment (35). Some classes of chemical compounds and elements found in the extract have been known to exert pharmacological effects while others are capable of protecting the active ingredients in the herb from decomposing either chemically or physiologically (36).

CONCLUSION

The present study showed that methanol and ethyl acetate extract of *O. basilicum* in basic nutrients. Qualitative phytochemical screening showed that it is abundant in phytochemicals such as alkaloids, carbohydrates, saponins, reducing sugars, flavonoids, phenols, proteins, tannins, terpenoids and glycosides especially it was found in higher amounts in the methanol extract than other extracts. Quantitative analysis showed that methanolic extract contains higher amounts than ethyl acetate extract). From the findings of the study, it may be concluded that the methanolic extract of *O. basilicum* acts as the potential source of phytochemical which may be used traditional medicinally for prevention of several diseases.

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