Research Article

Proximate and phytochemical constituents of *Ocimum sanctum* in Sudan

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Abstract

Objective: In this study the proximate analysis and phytochemical constituents were carried out using standard reference methods. **Material and methods:** Moisture content, total ash, crude fiber and crude protein of *Ocimum sanctum* leaves and flowers were determined according to AOAC methods. The elemental analysis used Atomic Absorption Spectrometer (AAS) and flame emission for Ca, Fe, Na, K, Pb, Mn and Zn. Phytochemical screening of ethanolic extracts of *Ocimum sanctum* was performed for constituents e.g. carbohydrates, reducing sugars, monosaccharides, proteins, amino acids, tannins, saponins, flavonoids, terpenoids and/or steroids, alkaloids, and anthraquinones. **Results:** The moisture content was found to be 3.68 ± 0.03 flower and 6.51 ± 0.01 leave, ash content 10.69 ± 0.23 flower and 16.21 ± 0.24 leave, crude fiber 35.73 ± 0.05 flower and 10.74 ± 0.06 leave and crude protein 10.64 ± 0.05 flower and 19.39 ± 0.13 leave. The elemental analysis used (AAS) and flame emission for Ca (75.01), Fe (2.826), Na (2.247), K (11.000), Pb (Not Detected), Mn (0.117) and Zn (0.205). Carbohydrates, reducing sugars, amino acids, tannins, saponins, terpenoids and/or steroids, alkaloids, anthraquinones were present in leaves and flowers. **Conclusions:** The results obtained showed was a good source of food and therapeutic.

Keywords: *Ocimum sanctum*, proximate analysis, phytochemical screening, anthraquinones

Introduction

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the system of useful drugs (Amna et al., 2018). Thus, recent medical research has started exploring the complete chemical characterization of plethora of medicinal plants available and analyzing the various classes of phytochemicals such as alkaloids, steroids, flavonoids, phenols, tannins and terpenoids etc. Every year approximately one lakh secondary metabolites are derived from around 50,000 plant species and 4,000 new secondary metabolites are being isolated from a broad range of plant species (Gomez-Galera et al., 2007).

These phytochemicals have been used in human healthcare (as antioxidants, drugs), used in several industries (as dyes, flavors, fragrances), and used for agricultural purposes (as insecticides and pheromones) etc. (Brinda et al., 1981).

Ocimum is one of the most important genera of the family Lamiaceae, native to India, Southern Asia and Middle East. Cultivated extensively in Southern, Central and Eastern Europe, North Africa, and in the USA, particularly California (Kruger, 1992; Singh and Panda, 2005). The typical characteristics of this family are a square stem, opposite and decussate leaves with many gland dots. The flowers are strongly zygomorphic with two distinct lips. Many species of the family are strongly aromatic due to essential oils which consist of monoterpenes, sesquiterpenes and phenylpropanoids (Nahak et al., 2011).

Ocimum sanctum is a wonder Ayurveda herb which is known for its tremendous medicinal properties both in traditional folklore as well as pharmacological system of medicines. Every part of the plant finds its use in one form or the other. Traditional use has attributed a number of properties to *Ocimum sanctum*. These include rejuvenating, tonic, and vitalizing properties that would contribute to longevity and a healthy life, as well as antiseptic, antiallergic, and anticancer effects (Reen et al., 1996; Samuelsson, 1999). It shows a number of medicinal activities it used in anticancer, antifertility, antidiabetic and

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various other diseases (Prakash and Neelu, 2005).

Material and Methods

Plant Material

The Plant *Ocimum sanctum*, leave and flower were collected in the April, 2019 from Khartoum state. The plant material was taxonomically identified by Prof. Maha Kordofani University of Khartoum, Department of Botany.

Proximate Analysis

Moisture content, total ash, crude fiber and crude protein, of the *Ocimum sanctum* leave and flower were determined according to (AOAC, 1990) methods.

Elemental Determination

Five (5) ml of concentrated HNO₃ was added to 1g of the dried extract in a conical flask and few anti bombing granules were added to it. The mixture was placed in a fume cupboard and gradually heated on a heating mantle with addition of more of the acid until a light colored solution was obtained. The solution was then cooled and filtered in a 100ml volumetric flask and made to the mark with deionized water. The mixture was then analyzed for (Ca, Fe, Na, K, Pb, Mn and Zn) using Pye Unicam 969, Atomic Absorption Spectrophotometer (AAS). The Na and K was determined using flame emission photometer. The principle of this procedure relies on the fact that excitation of a metal in a flame gives rise to the emission of characteristics color.

Preparation of extract

50gm plant sample *Ocimum sanctum*, were placed in 500ml conical flask and then 300ml 70% Ethanol were added. The conical flask was placed in the water bath and was allowed to stand for 1 hour. The time was measured after boiling start (after appearance of the first bubble). The mixture (powder and ethanol) was filtered using filter paper while it is hot by using another 500 ml conical flask (Harborne, 1973).

Qualitative phytochemical Analysis

Phytochemical screening for the identification of major groups of chemical constituents using standard procedures (Harborne, 1973; Sofowora, 2008). The phytochemical components analyzed were, carbohydrates, reducing sugars, monosacharides, protein, amino acids, tannins, saponins, flavonoids, terpenoids and/or Steroids, Alkaloids, Anthraquinones. Phytochemical analyses to test for the presence or absence of various phytoconstituents by the following tests:

Test for Carbohydrates (Molisch's Test)

To the extract 1ml of the Molisch's reagent was added then along the walls of the test tube carefully conc H₂SO₄ was added formation of brown ring at the junction of two liquids was observed.

Test for reducing sugars (Fehling's Test)

The extract was taken in a test tube, and the 1ml of the Fehling's solution (A and B) was added and boiled on the water bath. The solution was observed for the red reaction.

Test for monosacharides (Barfoed's Test)

To the extract in a test tube 1ml of Barfoed's reagent was added and boiled on the water bath the solution was observed for colour change reaction.

Test for protein (Biuret Test)

To 0.5ml of the extract 2ml of Biuret Reagent was added and the reaction mixture observed for the formation of violet colour solution.

Test for Amino Acids (Ninhydrin Test)

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) were added to 2 ml of ethanolic extract. A characteristic purple colour indicated the presence of amino acid.

Test for Tannins (Ferric chloride)

0.5ml of the extract was boiled with 10ml of distilled water in a test tube and then, few drops of 0.1 % ferric Chloride Solution were added and the reaction mixture was observed for blue greenish black Colour change.

Test for Saponins (Frothing Test)

0.5ml the Extract was added to 5ml of distilled water in test Tube. The Solution was shaken vigorously and observed for The Stable persistent froth. Frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Flavonoid

To The 0.5ml of the extract 5ml of distilled water was added and then a piece of magnesium ribbon and 2ml of Concentrated HCL was added. The reaction mixture was observed for the pink or red colour solution

Test for terpenoids and/or steroids

To 0.5ml each of the extract 2ml of chloroform was added and then 3ml of the concentrated H₂SO₄ Was Carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoid and steroids.

Test for Alkaloid

Three different tests were used Identification of alkaloids:

(A) Mayer Test

To 0.5ml of the extract 2ml of Mayer's reagent (K₂HgI₄) was added and the reaction mixture was observed for formation of creamy white precipitate.

(B)Wagner test:

To 0.5ml of the extract 2ml of wagner's reagent (dilute iodine solution) was added and the reaction mixture is observed for the formation of reddish brown precipitate.

(C) Dragendorffs

To 0.5ml of the extract 2ml of dragendorff's reagent was added, the reaction mixture observed the orange or orange red precipitate indicates the presence of alkaloids.

Test for anthraquinones (Borntrager's Test)

To 0.5ml of the extract 5-10ml dilute HCL was added and boiled on water bath for 10 minutes and filtered then the filtrate was extracted with carbon Tetra chloride and the equal amount of ammonia was added. After shaking the reaction mixture was observed for the formation of pink red colour in the ammonia layer.

Results and Discussion

Medicinal plants are being used as valuable sources of food and medicine for the prevention of illness and maintenance of human health. In Sudan many indigenous plants are widely consumed as food or home remedies especially in the treatment or management of common diseases.

Proximate composition

The results obtained shows the *O. sanctum* leaves have more moisture content of 6.51 ± 0.01 than the 3.68 ± 0.03 flowers. The high moisture content of leaves indicates that the leaves content not be stored favorably for a long period of time because of deterioration. The stability and susceptibility of microbial contamination of plant material is determined by its moisture content (Scott, 1980). The result also shows that the leaves of *O. sanctum* contain higher level of ash content (16.21 ± 0.24) than flowers (10.69 ± 0.23). The ash content is a reflection of its mineral content. The ash content of the

leaves compared to the flowers presents that the minerals are more concentrated in the leaves than in the flowers (Mustafa and El-kamali, 2019).

The results show that the *O. sanctum* flowers have more crude fibre content with value $35.73 \pm 0.05\%$ than the leaves with value $10.74 \pm 0.06\%$. Crude fibre provides roughages that aids digestion and reduces the accumulation of carcinogen in the body (Robinson, 1978). It is also used to detect adulteration. The crude protein composition shows that the leaves of *O. sanctum* contain a higher protein level of $19.39 \pm 0.13\%$ than the flowers which contains $10.64 \pm 0.05\%$. The basic function of protein in nutrition is to supply adequate amounts of required amino acids (Pugalenth, 2004). Protein deficiency causes growth retardation, muscle wasting, and abnormal swelling of the belly and collection of fluids in the body (Zarkada and Voldeng 1997).

Elemental composition of the aerial parts studied of *Ocimum sanctum*

The concentration of calcium, iron, sodium, potassium, lead, manganese and zinc in plant samples obtained *Ocimum sanctum* was performed using Atomic absorption (Table 2). The elements found in the smallest amount of the investigated plant *O. sanctum* Ca, K, Na and Fe, high concentration was detected for table 3. The results of the calcium content shows that the aerial parts of *O. sanctum* have more calcium ($75.01 \text{ mg}/100\text{g}$). Calcium is required in the body for human growth of bones and teeth (Robinson, 1978). It is an important mineral required for bone formation and neurological function of the body. The recommended daily calcium allowance for children and adult is $360\text{--}1200 \text{ mg}/100\text{g}$.

The result of the manganese content shows that the *O. sanctum* contains $0.117 \text{ mg}/100\text{g}$ which is higher than the other species. The results of sodium shows high level of

Table 1. Proximate composition of the flowers and leaves of the *Ocimum* species (%)

Plant species	Part used	Moisture content (%)	Total Ash (%)	Crude Fiber (%)	Crude Protein (%)
<i>Ocimum</i>	Flowers	3.68 ± 0.03	10.69 ± 0.23	35.73 ± 0.05	10.64 ± 0.05
<i>sanctum</i>	Leaves	6.51 ± 0.01	16.21 ± 0.24	10.74 ± 0.06	19.39 ± 0.13

Mean $\pm$ standard deviation of triplicate determinations

Table 2. Elemental composition (mg/100g) of candidate plant species using Atomic Absorption Spectrometer (AAS)

Plant species	Ca (mg/100g)	Fe (mg/100g)	Na (mg/100g)	K (mg/100g)	Pb (mg/100g)	Mn (mg/100g)	Zn (mg/100g)
<i>Ocimum sanctum</i>	75.01	2.826	2.247	11.000	ND	0.117	0.205

ND=Not Detected

2.247 mg/100g for the *O. sanctum* while the potassium content of the *O. sanctum* was 11.000 mg/100mg. Potassium is however, required by plants in fairly large amounts and occurs primarily as soluble inorganic salts and occasionally as salts of organic acids. Potassium plays an important role in photosynthesis and helps in the building up of carbohydrate supplies in plants. Potassium acts as an activator for a number of complex enzyme systems and encourages root development in plants. Potassium plays a significant role in the regulation of transpiration and water conditions in the plant cell. It is specially required for opening of stomata by light; no other physiological ion can substitute for potassium in this crucial role. In fact, plant life would not be possible without this element. In medicine, high potassium content in plant samples has diuretic properties (Mustafa and El-kamali, 2019). The result of iron content shows that the *O. sanctum* has 2.826 mg/100g. The result obtained shows that *O. sanctum* contain 0.205 mg/100g Zn more than others two species. The result of Pb shows that this element was not found in plant.

Phytochemical screening

Phytochemical screening of ethanolic extracts of studied plants was performed for constituents: carbohydrates, reducing sugars, monosaccharides, proteins, amino acids, tannins, saponins, flavonoids, terpenoids and/or steroids, alkaloids, anthraquinones (Table 3). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). All studied plant extracts showed that ethanolic extracts were consisted of carbohydrates, reducing sugars, amino acids, tannins, saponins, terpenoids and/or steroids, alkaloids, anthraquinones positive in *O. sanctum* leaves. *O. sanctum* leave gave negative test for monosaccharides, protein and flavonoids. While *O. sanctum*

flower all tests positive except protein.

In this investigation, *O. sanctum* flowers have maximum studied chemical groups. Flavonoids show anti-allergic, anti-inflammatory and reported to have like some Coumadin's to inhibit giant cell formation in HIV-infected cell cultures. Saponins have long employed for their detergent properties. In medicine, it is used in hyper glycaemia and for weight loss. Those saponins have anti-carcinogens properties, immune modulatory activity and cholesterol lowering activity. It is also been reported to have antifungal properties. Tannins are known to exhibit antiviral, antibacterial and anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication (Mustafa and El-kamali, 2019).

Tannins and saponins were also detected in Irish potato tubers. Tannins are known to "tan" the outermost layer of the mucosa and render it less permeable and more resistant to chemical and mechanical injury or irritation (Bahramsoltani et al., 2014). Recently, it has been discovered that these compounds play important roles in human health when ingested into the body. Dietary phytochemicals are ubiquitous in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000). Phytochemicals can be found in different parts of the plants but vary in concentrations from plant to plant depending on the variety, growth conditions etc. These compounds are mainly plants' secondary metabolites (Airaodion et al., 2019).

Conclusions

From the result of proximate analysis, it is quite interesting that *Ocimum sanctum* has more moisture content, Ash

Table 3. Phytochemical screening of ethanolic extracts of three *Ocimum sanctum*

Phytochemical screening	Reagents Used	<i>Ocimum sanctum</i>	
		Leaves	Flowers
Carbohydrates	Molichs	+	+
Reducing sugars	Fehlings	+	+
Monosaccharides	Barfoeds	—	+
Proteins	Biuret	—	—
Amino acids	Ninhydrin	+	+
Tannins	Ferric Chloride	+	+
Saponins	Frothing	+	+
Flavonoids	Magnesium +HCL	—	+
Terpenoids and steroids	Chloroform+H ₂ SO ₄ Con	+	+
Alkaloids	Mayers, Wagners, Dragendorff's	+	+
Anthraquinones		+	+

Key: + = present; — = Absent

content, crude fiber and also the presence of high content crude protein in leave *Ocimum sanctum*. Tannin, flavonoid and saponin is an indication that if further research can be done on the sample, novel bioactive compounds can be derived from it after isolating the compounds and characterizing them using various spectroscopic techniques. In order to establish therapeutic uses of *O. sanctum* in modern medicine, scientists and researchers must study the pharmacological effects of different extracts on different body systems.

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