Middle-East Journal of Scientific Research 13 (2): 236-240, 2013
ISSN 1990-9233
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DOI: 10.5829/idosi.mejsr.2013.13.2.1870

Comparative Analyses of *Ocimum santum* Stem and Leaves for Phytochemicals and Inorganic Constituents

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Abstract: The proximate, minerals and preliminary phytochemical analysis of *Ocimum sanctum* leaves in comparison with stem were studied. The nutritional analysis of *Ocimum sanctum* showed very low level of acidity 0.06% and protein i.e., 1.10% and 0.80% in their leaves and stems. Whereas the crude fiber was found to be maximum (12.20 and 9.80%) in stem and leaves. Results also showed that sugar contents were found to be low i.e. 1.20%. The stem of *Ocimum sanctum* was nutritionally enriched in all respect as compare to leaves. The minerals analysis showed no remarkable change in the results. Although the quantities of Fe^{2+}, Na^{+}, K^{+} were higher in stems than leaves. The results of preliminary phytochemical analysis were same for both the samples and confirm the presence of various phytochemicals viz., alkaloids, glycosides, flavonoids, tannins, terpenoids and saponins. Quantitative analysis revealed maximum amount of saponins i.e., 2.58mg/g in stem in comparison to 2.30mg/g in leaves. These phytochemicals in *Ocimum sanctum* have an important role in traditional medicinal system. Present paper deals with the significant difference between the stem and leaves.

Key words: Secondary plant metabolites · Heavy metals · Proximate analysis.

INTRODUCTION

Ocimum sanctum in English Holy Basil, Tulsi (in Urdu) belongs to plant family Lamiaceae. It has made important contribution to the field of science from ancient times as also to modern research due to its large number of medicinal properties. *Ocimum sanctum* has been described as of two types i.e., vanya (wild) and gramya (grown in homes). Although having identical usage, the former has darker leaves. *Ocimum sanctum* is a popular home remedy for many ailments such as wound, bronchitis, liver diseases, catarrhal fever, lumbago, hiccough, ophthalmia, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and psychosomatic stress disorders [1, 2]. Phytochemicals like flavonoids, tannins, terpenoid, saponins are present in the leaves and stem of most of the wild plants [3, 4]. Similarly the same are also present in *Ocimum sanctum*, while alkaloids are absent. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [5] and their absence in this plant tend to lower the risk of poisoning by the plant.

The presence of tannins make *Ocimum sanctum* as useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer; similar reports were also made by previous researchers [6, 7]. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 [8, 9]. Flavonoids serve as health promoting compound as a results of its anion radicals [10]. These observations support the usefulness of this plant in folklore remedies in the treatment of stress related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores [11-13].

Saponins, in plants have been suggested as possible anti-carcinogens. They possess surface-active characteristics that are due to the amphiphilic nature of their chemical structure. The proposed mechanisms of anticarcinogenic properties of saponins include direct

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cytotoxicity, immune-modulatory effects, bile acid binding and normalization of carcinogen-induced cell proliferation [14]. However, the anticarcinogenic effects of saponins from commonly consumed plant foods have not been studied.

Steroids which are very important compounds especially due to their relationship with compounds such as sex hormone [15, 16]. The presence of these phenolic compounds in this plant contributed to their anti-oxidative properties and thus the usefulness of these plants in herbal medicament. Keeping in view the above importance of medicinal plants the present study was designed to evaluate the Ocimum sanctum for its phytochemical, proximate analysis and minerals studies.

MATERIALS AND METHODS

A. Collection of Samples: *Ocimum sanctum* greenish stem and leaves were collected separately from botanical garden of PCSIR Labs complex Peshawar, brought to the Laboratory, washed thoroughly with tap water and shade dried at room temperature.

Proximate Analysis: The samples were dried in vacuum oven at 50°C, crushed by grinder, sieved and further analyzed physicochemicaly. The proximate parameters i.e., moisture, ash, fiber and pectin were analyzed by manual method of [17]. TSS and pH value was determined by ATAGO RX-1000 Digital Refractometer Japan and WTW -3110 pH meter Germany, while nitrogen and proteins were analyzed by BUCHI AutoKjedahl unit K-370.

Mineral Analysis: The micro and macro minerals in leaves were determine by flame photometer and atomic absorption (Hitachi Z-2000 Japan) by standard operating condition and the result has been recorded as milligram per hundred grams (mg/100gm) on dry weight basis.

Phytochemical Analysis: Preliminary phytochemical analyses were carried out by the reported procedure [18] in which powder sample or extracts of samples (freshly prepared) was treated with different reagents and the result shows the presence of targeted compound, while the quantification of alkaloid, flavonoids, tannins and saponins were determined as under.

Determination of Alkaloids: The determination of alkaloids in each sample was carried out by the described method of [19]. A 50g of sample was well mixed with 10% acetic acid solution in pure ethanol and left for 4 hours at room temperature, after that the mixture was filtered and concentrated to one fourth of its original volume by rotary evaporator. Concentrated NH_4OH was added drop wise in concentration till the alkaloid was precipitated. The precipitate was collected on weighted filter paper, washed with 1% ammonia, dried in oven at 80°C.

Determination of Flavonoids: The total flavonoids were also determined by a previous method [19], accordingly 10 g of each sample boiled in 50 ml HCl (2M solution) by reflux condensation for 30 minuets, cooled and filtered. The filtrate was then mixed with equal volume of ethyl acetate. The flavonoids were recovered from the filtrate and the calculated results expressed in mg/g.

Determination of Tannins: Tannins in leaves and stem of *Ocimum sanctum* were determined using 2g of each grinded sample was mixed with 20 ml of 50% methanol, covered with paraffin and placed on water bath at 80°C. After one hour the extract was filtered. 1 ml from extract was taken in volumetric flask, add 20ml distilled water, 10 ml Na_2CO_3 (17%) and 2.5ml Folin denis reagent and made the volume 50ml with distilled water. A bluish green color developed after 20 minuets. The absorption was read at 760nm by Spectrophotometer with the different concentration 0-10ppm of tannic acid standard treated as a sample and tannin concentration was calculated in mg/g [20].

Determination of Saponins: Saponins were determined using 1 g of grinded sample was taken in 250 ml beaker, adds isobutyl alcohol (100ml) and shacked for 5 hours on orbital shaker. After that the mixture was filtered through Whatman No.1 filter paper into beaker, added 20 ml $MgCO_3$ saturated solution and again filtered to obtain colorless solution. Then took 1 ml of solution in 50ml flask, mixed with 2ml $FeCl_3$ (5%solution) and made the volume with distilled water. It was allowed to stand for 30 minuets to develop red color. The absorbance was recorded at 380nm with the different concentration 0-10 ppm standard saponins. The standard solutions were also treated as a sample and the concentration of saponins was also calculated in mg/g [21].

RESULTS AND DISCUSSION

The chemical composition of the *Ocimum sanctum* leaves and stem are given in Table 1. As can be seen, stem of leaves showed high level of nutrition value as compared to the leaves. The average percentage w/w of

Table 1: Proximate analysis of *Ocimum sanctum* stem and leaves

S.No	Parameters	Stem	Leaves
1	Moisture (%)	6.60 ± 0.30*	5.30 ± 0.3
2	Ash(%)	2.60 ± 0.10	2.50 ± 0.08
3	Fat (%)	1.10 ± 0.60	0.90 ± 0.45
4	Pectin (%)	6.50 ± 0.41	8.0 ± 0.21
5	Crude Fiber (%)	12.20 ± 1.40	9.80 ± 0.80
6	Total Sugar (%)	2.20 ± 2.30	2.10 ± 2.80
7	Total acidity (%)	0.06 ± 0.003	0.06 ± 0.001
8	Vitamin-C (mg/100g)	45.0 ± 1.30	31.0 ± 1.90
9	Protein (%)	1.10 ± 0.001	0.80 ± 0.001
10	Nitrogen(%)	1.90 ± 0.00	1.90 ± 0.00
11	pH of 10% Sol	7.50 ± 0.0	7.20 ± 0.0
12	TSS 10% Sol	1.40 ± 0.0	1.01 ± 0.0

* Standard deviation value.

Table 2: Minerals composition of *Ocimum sanctum* leaves and stem as mg/100gm

	Micro Minerals					Macro Minerals			
Sample	Co	Cu	Fe	Ni	Zn	K	Na	Ca	Mg
Stem	3.8	3.0	36.6	10.2	74.7	180.0	156.0	45.0	21.0
Leaves	3.7	3.1	35.4	10.4	71.3	181.0	154.0	45.0	21.0

Table 3: Preliminary phytochemical analysis of *Ocimum sanctum* stem and leaves

S.No	Name of the Test	Procedure	Observation	Stem	Leaves
1	Alkaloids	Mayer's reagent	White ppt.		
		Hager's reagent	Yellow ppt.	-ve	-ve
2	Glycosides	Anthrone + H_2SO_4+ Heat	Purple or green	+ve	+ve
3	Carbohydrates	Drug + Molishs reagent+conc. H_2SO_4	Purple color		
		Fehling's solution A and B	Brick red color	+ve	+ve
4	Phytosterols/triterpenoids	Liebermann Test	Bluish green		
		Salkowski Test	Red and fluorescent		
		Noller's test	Pink color	+ve	+ve
5	Proteins and Amino acids	Xanthoprotein test	Orange color		
		Millon's reagent test	White ppt		
		Lead acetate test	White ppt	-ve	-ve
6	Saponins	Drug + water + shaking	Formation of honey	+ve	+ve
7	Flavonoids	Shinodaw's Test	Red color	+ve	+ve
8	Fixed oils and Fats	Spot test	Stains appear	-ve	-ve
9	Phenolics/Tannins	$FeCl_3$	Intense color		
		Drug + lead acetate + water	Formation of white ppt	+ve	+ve

Table 4: Quantitative phytochemical estimation of *Ocimum sanctum* stems and leaves as mg/g

Sample	Alkaloid	Flavonoids	Tannins	Saponins
Stem	0.08 ± 0.02	0.50 ± 0.08	0.72± 0.06	0.58± 0.11
Leaves	0.10± 0.06	0.60± 0.08	0.52 ± 0.12	0.30± 0.02

* Standard deviation value.

the ash content and the extractive values were determined. The moisture content in stem was quite large 6.6% than the leaves 5.3%. Total acidity was determined by simple titration method which shows the negligible quantity in stem as well as in leaves, while the fiber content in stem and leaves is quite high, which is 12.2% in stem and 9.8% in leaves. The nitrogen and protein were analyzed by AutoKjedahl the nitrogen and protein value was 1.1 and 1.9% for stem, while 0.8 and 1.9% for leaves. These values were taken in triplicate but there was no change among these values having a very small number of standard deviation both for stem and leaves. Fat were extracted with 95% n-hexane by Soxhlet apparatus and found 1.1% crude fat in stem and 0.9% in leaves.

The micro (Co, Cu, Fe, Ni and Zn) and macro (K, Na, Ca and Mg) minerals in *Ocimum sanctum* stem and leaves were determined and tabulated in Table 2. The concentration of these elements reported as milligram per hundred grams (mg/100gm) on dry weight basis. Among micro minerals Zn and Fe have greater value as compare with other medicinal plants that was 74.7 and 36.6mg/100gm. These micro minerals have also a quite little difference between stem and leaves, while in macro minerals including K, Na, Ca, Mg of the stem and leaves is same, which is 180, 156, 45, 21mg/100gm for stem and 181, 154, 45, 21mg/100gm for leaves, respectively.

The preliminary phytochemical parameters were studied not only in search of bioactive agents but also for starting products which uses in the synthesis of useful drugs [22]. The *Ocimum sanctum* plant was broadly used for the treatment of different diseases in third world countries, in the latest research on *Ocimum sanctum* found that it may have natural bioactive compounds which provide protection to animal against different diseases [12]. Table 3, showed same results for leaves and stem, the glycosides, carbohydrates, phytosterols/ triterpenoids, saponins, flavonoids and phenolic/tannins were present in both samples, while fixed oil and amino acids were absent. The alkaloids, flavonoids, tannins and saponins were also quantified and tabulated in Table 4, which showed maximum concentration of saponins that was 1.58 and 1.30mg/g for stem and leaves, while minimum concentration of alkaloid was found 0.08 and 0.10mg/g. The presence of alkaloid indicates that the use of the plants have harmless effect. The flavonoids in leaves 0.50mg/g and in stem 0.60mg/g which was also greater than stem. The presence of flavonoids confirms that the plant has high antioxidant value, as well as justify its antimicrobial, anti inflammatory, antimutagenic, antiviral and anti allergic actions.

CONCLUSIONS

Both parts of this plant have almost same nutritional, minerals and phytochemical values. Therefore both the leaves and stem can be used in traditional medicine system for different types of ailments.

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