Journal of Advances and Scholarly Researches in Allied Education
Vol. 18, Issue No. 7, December-2021, ISSN 2230-7540

Studies on Phytochemicals and Inorganic Constituents of Ocimum Sanctum (Lamiaceae) Stem and Leaves

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Abstract – The preliminary phytochemical analysis of ocimum sanctum (LAMIACEAE) leaves/stem were studied. The nutritional analysis of this plant showed very low level of acidity (0.06%) and protein 1.10% in its leaves and stem. The crude fibres were found to be maximum 12.20% and 9.80% in stem and leaves. As such its stem was nutritionally enriched in all respect with respect to leaves. The minerals analysis showed no remarkable change in the result. Although the quantities of Fe, Na and K were higher in stems than leaves. The results of phytochemical analysis were same in both stem and leaves which confirm the presence of phytochemicals like alkaloids, glycosides, flavonoids, tannins, terpenoids and saponins which have important role in traditional medicinal drug preparations.

Keywords – Ocimum Sanctum (LAMIACEAE); Phytochemicals Analysis.

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INTRODUCTION

Ocimum sancticm belongs to family LAMIACEAE. The stem, leaves and inflorescence of this plant form a home remedy for many oilments such as bronchitis gastric disorders, genitourinary disorders, catarrhal fever and liver diseases (Ashoka *et al.* 2009' Udupa *et al.* 2006). Phytochemicals like floavonoids, tannins, terpenoids, saponins are present in the leaves and stem of this plant (Kayani *et al.* 2007; Achakzai *et al.* 2009). Alkaloids have been associated with medicinal values due to cytotoxicity and their absence in this plant tend tower the risk of poisoning (Suthar Singh *et al;* 2011). The presence of tannins make this plant as useful in the treatment of inflamed (=ulcerated) tissues (Hannan *et al;* 2006; Grover *et al.* 2005). 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 A_2 (Trevisan *et al.* 2006; Maluventhan *et al.* 2012). Flavonoids also serve as health promoting compound as a results of its anoin radicals (Bhartiya, *et al.* 2006). These observations strogly support the usefulness of this plant among folklore to treat stress related ailments as well as dressing for wounds particularly cuts sores and bruises. Saponins present in this plant bear anti-carcinogens which possess surface-active values due to its amphiphilic nature in chemical structure. The proposed mechanisms of anti-carcinogenic properties of saponins inclide direct cytotaxocity, immume-modulatory effects, bile acid binding and normalization of caricinogen-incluced cell proliferation (Nimisha *et al;* 2012). Sterioids which are very important compounds especially due to their relationship with sex hormone (Das *et al.* 2008; Juss *et al.* 2012). The presence of these phenolic compounds in Ocimum sanctum contributed to its anti-oxidative properties and thus the usefulness of this plant in herbal medicament all over the world is very pertinent.

MATERIALS AND METHODS

Materials:

- Leaves and stem of Ocimum sanctum (LAMIACEAE)
- ATAGO RX-1000 Digital Refractometer.
- WTW -3110 $_P$H meter
- BUCHI Auto Kjedahl unit K-370
- Flame photometer
- Acetic acid
- Ethanol
- Rotary evaporator
- NH_4OH
- Filter paper

- Ammonia
- Oven
- HCL
- Ethyl acetate
- Methanol
- Water bath
- Volumetric flask
- Distiled water
- Na_2CO_3
- Folin denis
- Spectrophotometer
- Tannic acid
- Beaker 250ml.
- Isobutyl alcohol
- Orbital shaker
- Whatman filter paper No. 1
- $M_g\ CO_3$
- $Fecl_3$ 5%

Methods:

The collected plant samples were dried in accum oven at 50°C, crushed by grinder, sieved and further analyzed physicochemically. The moisture, ash, fiber and pectin were analyzed by manual method (AOAC, 1998). TSS and p^H value were determined by ATAGO RX-1000 Digital Refractometer and WTA-3110 p^H meter, while Nitrogen and protein were analyzed by BUCHI AutoKjedahl unit K-370.

Mineral Analysis:

The micro and macro minerals present in leaves were determined by flame photometer and atomic absorption by standard operating condition on dry weight basis.

Phytochemical Analysis:

Phytochemical analysis was carried out by chapman and hall (Harborne, JB, 1973) to screened out alkaloids, flavonoids, tannins and saponin.

- The dermination alkaloid present in leaves and stem samples were carried out separately. A 50gm of sample was well mixed with 10% acetic acid solution in pure ethanol and left for 4 hours at room temperature. The mixture solution was then filtered and concentrated to ¼ the of its original volume by rotary evaporator. Concentrated NH_4OH solution was then added drop wise till alkaloid gets precipitated. The resultant precipitate was collected on filter paper, washed with 1% ammonia and dried in oven at 80°C.

- The determination of total flavonoids were determined as above and accordingly 10gm each samples were boiled in 50ml. HCI by reflux condensation for 30 minutes, cooled and filtered. The filterate was then mixed with qual volume of ethyl acetate. The resultant flavonoids were recovered from the filterate.

- The tannins content of leaves and stems were determined. Using 2gm of each grinded sample being mixed with 20ml methanol and covered with paraffin and then placed on water both at 80°C. After one hour, the extract was filtered. Then 1ml extract was taken in volumetric flask, 20ml water was added, 10ml Na_2CO_3 and 2.5ml folin denis reagent to make the volume 50ml with distilled water. A bluish green colour developed after 20minutes. The absorption was read at 760nm by spectrophotometer with different concentrations (i.e. O-10ppm of tannic acid) treated as a sample and tannin concentration was calculated.

- Saponins were determined by using 1gm of grinded sample in 250ml beaker, 100ml isobutyl alcohol was added and shaked for 5 hours on orbital shaker. The mixture was then filtered through whatman filter paper-1 into beaker and 2.0ml $MgCO_3$ solution was added and filtered to obtain colourless solution. The 1ml solution in 50ml flask was taken and mixed with 2ml $FeCl_3$ and made the solurion upto required volume with distilled water. It was allowed to stand for 30 minutes to develop red colour. The absorbance was recorded at 380nm with different concentration (0-10ppm) of saponins.

RESULT AND DISSUSSION

The chemical composition of the Ocimum sanctum leaves and stems are given in table-1. Stem showed high level of nutrition volume as compared to leaves.

Table: 1 proximate analysis of stem and leaves:

Parameters	Stem	Leaves
Moisture	6.60 ± 0.30	5.30 ± 0.30
Ash	2.60 ± 0.10	2.50 ± 0.08
Fat	1.10 ± 0.60	0.90 ± 0.45
Pectin	6.50 ± 0.41	8.00 ± 0.21

Crude fiber	12.20 ± 1.40	9.80 ± 0.80
Total sugar	2.20 ± 0.003	2.10 ± 2.80
Protein	1.10 ± 0.001	0.80 ± 0.001
Nitrogen	1.90 ± 0.00	1.90 ± 0.00
p^H of 10% Sol^n	7.50 ± 0.00	7.20 ± 0.00
TSS 10% Sol^n	1.40 ± 0.00	1.01 ± 0.00

Table: 2 (Showing mineral composition of stem and leaves):

Sample	Micro Minerals								
	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 stem/leaves

Phytochemical	Stem	Leaves
Alkaloids	-ve	-ve
Saponins	+ve	+ve
Flavonoids	+ve	+ve
Tannins	+ve	+ve

Table: 4 (Quantitative phytochemical estimation of stem/leaves)

Sample	Phytochemicals			
	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

The average percentage w/w of ash content and the extractive volues were determined. The moisture content in stem was quite large (6.6%) with respect to leaves (5.3%). Total acidity was also determined by titration method which showed negligible quantity in stem and leaves. The fiber content in stem/leaves are quite high (12.2% and 9.8%). The nitrogen and proteings were analyzed by Autokjedahl where nitrogen and proteins volue for stem was 1.1% and 1.9%. fats were extracted with 95% n-hexance by soxhlet apparatus and found 1.1% crude fat in stem and 0.9% in leaves.

The micro nutrients (= Co, Cu, Ni, and Zn) as well as macro nutrients (= K, Na, Ca and Mg) present in stem and leaves of Ocimum sanctum were tabulated in table-2. Among micro minerals Zn and Fe have got greater value as compared to other medicinal plants that was 74.7 and 36.6 mg per 100mg.

The preliminary phytochemical parameters were studied not only in search of bioactive agents but also for making them to be used in the synthesis of useful drugs (Vimal, RT *et al.* 2012). Table 3, showed same results for leaves and stem containing glycosides, carbohydrates, triterpenoids, saponins flavonoids and tannins. The alkaloids, flavonoids, tannins and saponins were also quanified and tabulated in table-4, which showed maximum concentration of saponins that was 1.58 and 1.30 mg/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 this plant have got harmless effects on our health. The presence flavonoids confirms that this plant has high efficiency of antioxidant value as well as justify its anti-inflammatory and anti-allergic actions.

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