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Ameliorative effect of *Ocimum sanctum* (Tulsi) extract with spectific reference to phytochemical properties

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Abstract

Objective of the present study was to carry out the phytochemical standardization of *Ocimum sanctum* leaves to develop the standard phytochemical parameters of this valuable medicinal plant. Many standardization parameters of *Ocimum sanctum* were analyzed and Standard method was adopted for the preliminary phytochemicals screening. Analysis of pesticides residues, aflatoxin & heavy metals were also performed. The sections of *Ocimum sanctum* leaves were prepared for quantitative microscopic parameters. The air dried powdered plant material was subjected for determination of physicochemical standardizations like ash value, Extractive value and fluorescence nature of the powder drug using light of short and long wavelength of 254nm and 366nm respectively. Phytochemical screening was performed for the identification of phytoconstituents in the plant which was helpful in the development of analytical profile. Preliminary phytochemical screening showed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins and lipids in the drug extract and flourescence nature of drug was confirmed by fluorescence analysis in different solvent. Concentrations of heavy metals,ash value and extractive value were determined and found within acceptable Pharmacopoeial limits. Pesticides residues and aflatoxins were also determined but not detected in the tested samples. The physicochemical and phytochemical standards which are outcome of this research may be utilized as substantial data for identification and standardization of *Ocimum sanctum* leaves.

Keywords: *Ocimum sanctum*; Tulsi; Aflatoxins; Pesticides Residue; Extractive Value; Heavy Metal.

1. Introduction

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical inter mediates and chemical entities for synthetic drugs (Anonymous 1999). Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Eddouks et al 2005)· Over 50% of all modern clinical drugs are of natural product origin (Maghrani et al 2005)) and natural products play on important role in drug development programmes in the pharmaceutical industry . Tulsi extracts were active against many infectious human pathogenic bacteria that cause many dangerous diseases such as vomiting, diarrhea , urinary nfections, gastroenteritis etc (Maier et al., 2002).In view of the above the present investigation was carried out to perform the phytochemical screening of *Ocimum sanctum* (Tulsi) methanolic extracts.

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2. Material and methods

2.1. Collection of plants

The leaves of *Ocimum sanctum* was collected from the Herbal garden of Vivek college of Technical education, Bijnor and shade dried, powdered.

2.2. Preparation of extracts

Shade dried powder extracted in soxhlet apparatus successively with methanol due to their nature of polarity. After extraction, methanolic extracts were filtered through Whatman No.1 filter paper and stored for further use.

2.3. Macroscopical and microscopical evaluation

The plant material was subjected to macroscopical and microscopical evaluation. The leaf of *Ocimum sanctum* observed carefully and preliminary observations were recorded. The seeds were powdered with the help of grinder and stained with different staining reagent to ascertain the presence of particular type of microscopical characters.

2.4. Physicochemical standardization

2.4.1 Determination of extractive value

It is the amount of soluble constituents extracted with different solvents from a given amount of medicinal plant material. (Harborne1992; Mukherjee2002)

Cold Extraction

The air-dried coarse drug powder (10 gm) was macerated with solvent (Petroleum ether, chloroform, methanol and water) of volume 100 mL in a closed flask for 24 hours, shaking frequently during six hours and allowing standing for 24 hours. It is filtered rapidly, taking precaution against loss of solvent, the filtrate evaporated to dryness in a tarred flat bottom dish and dried at 105oC, to constant weight and calculated percentage yield.

Hot Extraction

The powdered material of the drug (10gm) was packed in a Soxhlet apparatus separately for each solvent like petroleum ether, chloroform, methanol and water. Each extract was evaporated to dryness and constant extractive value was recorded.

Successive Extraction

The dried and coarsely powdered material (10gm) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform and methanol. The extracts were evaporated to dryness and their constant extractive values were recorded.

2.4.2 Determination of ash values

Ash values

This constraint can be used for the determination of inorganic materials, such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthly materials. The water-soluble ash is used to estimate the amount of inorganic elements

2.4.3 Determination of total ash values

Ignition of medicinal plant material yields total ash constituting both physiological (from the plant tissue) and non-physiological (extraneous matter adhering to the plant) ash. The ground drug was incinerated in a silica crucible at a temperature not exceeding 450oC until free from carbon. It was then cooled and weighed to get the total ash content.

2.4.4 Determination of Acid insoluble ash values

Acid insoluble ash represents sand and siliceous earth. Ash is boiled with 25 mL dilute HCl (6N) for five minutes. The insoluble matter collected on an ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450oC to a constant weight.

2.4.5 Determination of Water-soluble ash values

Ash was dissolved in distilled water and the insoluble part collected on an ash less filter paper and ignited at 450°C to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash was obtained.

2.4.6 Florescence analysis

Many herbs fluorescence when cut surface or powder is exposed to UV light and this can help in their identification method. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (255 and 366 nm) and after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride etc. (Chase1949; Kokoshi1958)

2.4.7 Phytochemical screening

The Petroleum ether extract, Chloroform extract, Methanolic extract, aqueous extract of the plant material were subjected to preliminary phytochemical investigation for the detection of secondary metabolites. (Mukherjee2002). The screening was performed for Alkaloids, Carbohydrates, Phenolic compounds, Flavonoids, Protein, Saponins,mucilage, resins and lipids or fats etc.

2.4.8 Heavy Metal Residues

Residues of heavy metals (Cd, Pb, As and Hg) in the extracts were determined according to the American Organization of Analytical Chemists (AOAC) official method of analysis (Scott2001) Lead, cadmium, arsenic and mercury are the most common toxic metals that have become a matter of concern due to the reports of their contamination in various herbal preparations and herbal ingredients. (Mukherjee2002). Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration. Acute or chronic exposure of cadmium causes respiratory distress, lung and breast cancers, hemorrhagic injuries, anemia and cardiovascular disorders. Kokosni(1958).Arsenic is reported to cause hypertension, peripheral arteriosclerosis, skin diseases and neurotoxicity. Determination of heavy metals (Cadmium, Lead, Arsenic and Mercury) analysis was carried out in the leaf extractsof *Ocimum sanctum* on Atomic Absorption Spectrophotometer (AAS). All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines. (Anonymous 2006)

2.4.9 Pesticide Residues

Pesticides (organochlorines, organophosphates and pyrethroids) residues in the extracts were determined by GC-MS according to AOAC guidelines. Pesticides are the toxic substances; the drugs should be free from these substances. (Anonymous 2001)

2.4.10 Aflatoxin Analysis

Aflatoxins were analyses in leaf extracts of *Ocimum sanctum* by HPLC method as described by Scott (AOAC method 980.20-ITEM-I) (Anonymous 2006) Mycotoxins are secondary metabolites produced by fungi that develop naturally in food products. These toxins may lead to a great variety of toxic effects in vertebrates, including humans. Toxigenic fungi may contaminate herbal products at different phases of production and processing, mainly in favorable humidity and temperature conditions. Many mycotoxins also have significant chemical stability, which enables their persistence in products even after the removal of the fungi by means of the usual manufacturing and packaging processes. The most common toxigenic fungi found in plants include species from the genera Aspergillus and Fusarium, mainly *Aspergillus flavus, Aspergillus parasiticus and Fusarium verticillioides*. Aspergillus species produce aflatoxins B1, B2, G1 and G2 which are considered to be involved in the etiology of human liver cancer. (Rodriguez 2001).

3. Results and discussion

The macroscopical study of the *Ocimum sanctum* leaf was done. The leaf are green in colour, 2-3 mm in size, oval in shape with smooth surface & characteristic odour (Table-1). Themicroscopic examination of powdered material was performed to detect and established various identifying microscopic characters which will be help full in differentiation

of the substitute of the drug supplied in the form of dried powder. The photomicrographs of the identifying features of the plant material are shown in (Fig 1-3).

Table 1 Macroscopical characters of *Ocimum sanctum*

Sr. No	Macroscopic Characters	Observation
1	External Colour	Green
2	Size	2-3 mm
3	Shape	Oval
4	Surface	Smooth
5	Odour	Characteristic
6	Taste	Bitter

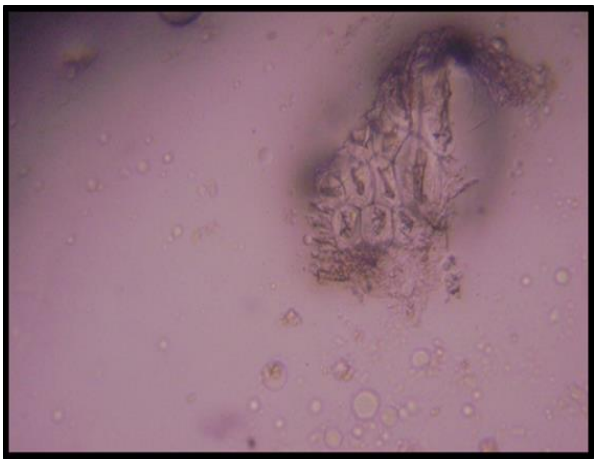

Figure 1 Cells of endosperm in surface view

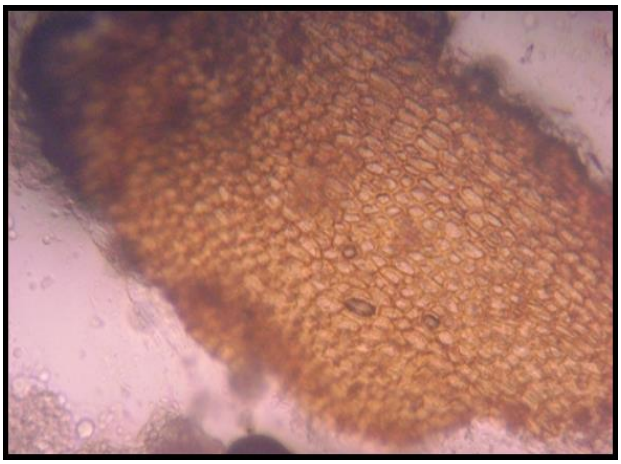

Figure 2 Cells of testa in surface view

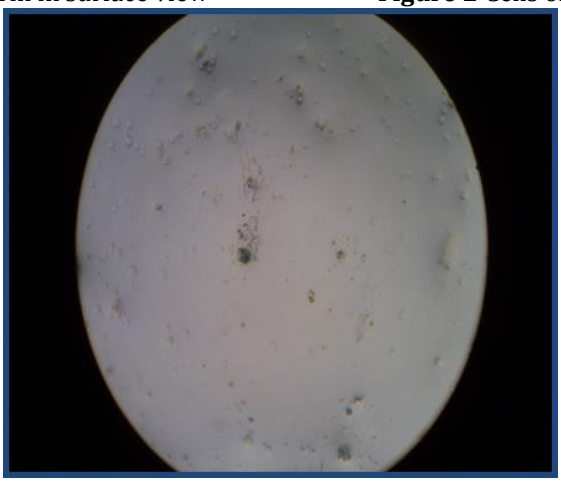

Figure 3 Starch granules

The cells of endosperm were seen and the cells are polygonal in shape and contain alerone grains and oil droplet. The cells of testa are longitudinal, elongated and they are closely packed and contain yellow colouring matter. The starch grains were also present which are oval an rounded in shape. The values of thephysical constant like ash values, loss on drying, extractive value were determined. Physochemical parmeter like Extractive value and colour of extract was investigated (Table-2). Fluorescence nature of the powder drug & different solvent extracted drug with different chemicals was analyze using short light wavelength (254 nm)and longer light wavelength (366nm) and the observation

were reported in Table -3.Preliminary qualitative phytochemical screening shown that presence of alkaloids, carbohydrates, flavonoids and saponins, Presence or absence of particular types of phytoconstituents in the plant of the interest may be helpful, partly in the development of analytical profile and in the differentiation of contravention plants.Theextracts were subjected to preliminary chemical tests to detect the presence and absence of various phytoconstituents. The results of the studies performed are tabulated in (Table- 4). Determination of heavy metals (Cadmium, Lead, Arsenic and Mercury) analysis was carried out in the extracts of *Ocimum sanctum* on Atomic Absorption Spectrophotometer (AAS). All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines. Cd was found to be highest in *Ocimum sanctum* sample (0.24 ± 0.05 mg/kg) but It was below the permissible limit of 0.3 mg/kg as prescribed by WHO in all the samples. Pb ranged from 0.16 ± 0.06 mg/kg to 0.43 ± 0.05mg/kg in the drug samples and was far below the permissible limit of 10 mg/kg as prescribed by WHO inthe *Ocimum sanctum* samples. Hg in *Ocimum sanctum* was found to be below the permissible limit. As and Hg was detected in all samples. Both metals were found to be within permissible limits of 0.5 ppm and 1.0 ppm respectively (Table-5).

Table 2 Summary of results of physicochemical evaluation of drug (n=3)

S. No	Parameters		% w/w (Mean ± SEM)
1	Moisture content		
2	Ash values	Total Ash	4.8 ± 1.23
		Acid insoluble Ash	1.43± 0.14
		Water soluble Ash	0.72 ± 0.03
3	Successive extraction	Petroleum ether	0.80 ± 0.06
		Chloroform	2.01 ± 0.85
		Methanol	2.65 ± 0.74
		Water: alcohol (50:50)	8.09 ± 1.86

Table 3 Effect of different chemical reagents on the fluorescence behaviour of crude drug powder

S. No.	Treatment	Day light	UV light 254 nm	UV light 366 nm
1.	Powder as such	Brownish red	Brownish red	Brown
2.	Powder treated with distilled water	Brownish red	Dark brown	Dark brown
3.	Powder treated with 1N NaOH in water	Brownish red	Brownish red	Brown
5.	Powder treated with HNO_3	Light brown	Dark green	Dark violet
6.	Powder treated with H_2SO_4	Green	Black	Blue
7.	Powder treated with iodine	Green	Blue	Dark brown
8.	Powder treated with conc. HCl	Dark green	Radish brown	Greenish black
9.	Powder treated with ammonia	Light green	Dark green	Greenish brown
10.	Powder treated with ferric chloride	Green	Radish black	Greenish brown
11.	Powder treated with Iodine	Dark brown	Brown	Blue
12.	Powder treated with Glacial acetic acid	Yellow	Dark yellow	Yellow
13.	Powder treated with Picric acid	Dark yellow	Yellow	Dark yellow
14.	Powder treated with Petroleum ether	Dark green	Pale yellow	Dark Brown
15.	Powder treated with Chloroform	Dark green	Dark brown	Dark green

Table 4 Results of Phytochemical screening

Constituents	Extracts			
	Petroleum ether	Chloroform	Alcoholic	Aqueous
Alkaloids	-	+	+	+
Carbohydrates	-	-	-	+
Phenolic compounds	-	+	+	-
Flavonoid	-	+	+	+
Proteins and amino- acids	-	-	+	+
Saponins	-	-	+	+
Mucilage	-	-	+	-
Resins	+	+	+	-
Lipids / Fats	+	-	-	-

(-: Absent, +: Present)

Table 5 Determination of heavy metal residues

S. No	Heavy Metals	Concentration
1	Cadmium (Cd)	0.24 ± 0.02
2	Lead (Pb)	0.42 ± 0.14
3	Arsenic (As)	0.48 ± 0.06
4	Mercury (Hg)	0.38 ± 0.06

Determination of pesticide residue was carried out in extracts of *Ocimum sanctum* by standard methods as described in AOAC guidelines. Total 35 pesticides were tested in all the samples, none of the pesticides was found in samples of the extracts (Table-6).Mycotoxins are secondary metabolites produced by fungi that develop naturally in food products. These toxins may lead to a great variety of toxic effects in vertebrates, including human.Toxigenic fungi may contaminate herbal products at different phases of production and processing, mainly in favorable humidity and temperature conditions. Many mycotoxins also have significant chemical stability, which enables their persistence in products even after the removal of the fungi by means of the usual manufacturing and packaging processes.

Table 6 Determination of pesticide residue

S. No	Pesticide	Test method	Results	MDL
1	α -BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
2	β-BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
3	γ-BHC(Lindanee)	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
4	δ-BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
5	Heptachlor	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
6	Heptachlor_Epoxide	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
7	α -Chlordane	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
8	α -Endoulfan	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
9	β -Chlordane	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
10	Endrin	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
11	Total DDE	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
12	Total DDD	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
13	Total DDT	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
14	β -Endoulfan	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
15	Endrin_Aldehyde	AOAC970.52/EPA525.5	Not detected	0.01mg/kg

The most common toxigenic fungi found in plants include species from the genera *Aspergillus* and *Fusarium,* mainly *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides* Aspergillus species produce aflatoxins B_1, B_2, G_1 and G_2, which are considered to be involved in the etiology of human liver cancer. Aflatoxins B_1, B_2, G_1 and G_2 were determined in the extract of *Ocimum sanctum*. No aflatoxin was detected in drug sample of the extract. (Table 7). The outcome of this study might prove beneficial in herbal industries for identification, purification and standardization of *Ocimum sanctum* leaf extract.

Table 7 Determination of aflatoxin residues

S. No	Test parameter	Test method	Results	MDL
1	AflatoxinB1	AOAC 990.332	Not detected	1.0µg/kg
2	AflatoxinB2	AOAC 990.33	Not detected	1.0µg/kg
3	AflatoxinG1	AOAC 990.33	Not detected	1.0µg/kg
4	AflatoxinG2	AOAC 990.33	Not detected	1.0µg/kg

4. Conclusion

The generated data of this study will be used to establish its quality and purity and may be utilised to develop pharmacopoeial monograph of this plant. *Ocimum sanctum* leaves have wide range of medicinal values. The outcome of this study might prove beneficial in herbal industries for identification, purification and standardization of *Ocimum sanctum* leaves extracts.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

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