Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 10 (7): 365-369, 2011
ISSN 1682-3974 / DOI: 10.3923/ajps.2011.365.369

Pharmacognostic and Preliminary Phytochemical Study of *Ocimum gratissimum* Linn. (Family: Lamiaceae)

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Abstract: The aim of this research was to study the pharmacognostic parameters of leaves of *Ocimum gratissimum* L. (family: Lamiaceae). Macroscopic study showed that the leaves of this plant possess dark green color with characteristic aromatic odour and bitter taste. Powder microscopy showed the presence of numerous glandular simple trichomes of average length 101 μm and anomocytic stomata were also present, whereas, T.S of leaf showed that the leaf has a pot shape midrib and a thin lamina with uneven lower epidermis attached at the lateral sides of its upper side leaving a concave central dorsal depression. Both upper and lower epidermis showed simple, covering, uniseriate trichomes as well as sessile short stalked glandular trichomes. Quantitative microscopy viz., veinlet termination number, vein islet number, stomatal number, stomatal index; physico-chemical parameters viz., ash values, extractive values, Thin layer chromatography (TLC); florescence analysis of *Ocimum gratissimum* L. were also been determined. TLC studies showed the presence of various phytochemicals in the extracts. Qualitative phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, terpenoids (methanolic, ethanolic extracts); alkaloids, flavonoids and terpenoids (petroleum ether, chloroform extracts) and carbohydrates in alcoholic extract. This is the first such study on standardization of *Occimum gratissimum* L. leaf which would serve as a standard reference for identification and distinguishing the plant from its adulterants.

Key words: Fever plant, pharmacognostic, standardization, Phytochemical, *Ocimum gratissimum* L.

INTRODUCTION

A herbal medicine has been practiced worldwide and is now recognized by World Health Organization (WHO) as an essential building block for primary healthcare (Onayade *et al*., 1990). According to the WHO, more than 80% of the world populations still rely on herbal medicines as their prime source of health care (Adeyemi *et al*., 2009). Herbal based medicines play an important role in healthcare management system. Almost in all the traditional system of medicine, the medicinal plants play a key role and constitute the backbone of the same. In order to make sure the position of herbal medicines within the herbal care system and safe use of these medicines, a necessary first step is the establishment of standards of quality, safety and efficacy of the herbal drugs. Keeping this objective in mind, pharmacognostical evaluation of *Ocimum gratissimum* L. used as traditional medicines in various diseases were drawn up according to WHO guidelines (Bhatia *et al*., 2008).

The plant *Ocimum gratissimum* L. belonging to Lamiaceae family, is a perennial, woody shrub that is distributed throughout India, often cultivated, Ceylon, Java, tropical Africa, South America, Nigeria and Asia. It is also found in some states of North India like Jammu, Punjab, Haryana and also cultivated in Kerala and commonly known as Ramtulsi (Hindi); Shrubby basil, Fever plant, Fever leaf (English); Banjere (Punjabi); Bantulsi, Ramtulsi (Bengali); Avachibavachi, Ramtulsi (Gujrati); Rama tulsi (Malyalam); Nimma tulsi, Ramtulsi (Kannad) and Elumicham tulasi (Tamil). Leaves are 6.3-12.5 cm long, elliptic-lanceolate, acute, coarsely crenate-serrate, pubescent on sides, gland dotted, base cuneate petioles 2.5-6.3 cm long, slender, more or less pubescent. Flowers are simple or branched rather short racemes, in tolerably close whorls; rhachis quadrangular, softly pubescent; bractas sessile, longer than the calax, acuminate from broad ovate base, decussate and squarrose in the young inflorescence, cilate, pedicels shorter than the calyx, softly pubescent. Calyx 3 mm long

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in flower, becoming twice as long in fruit, pubescent and glandular, upper lip rounded, veined, scarcely mucronate, curved upwards in fruit, longer than lower, lower lip strongly nerved, the 2 central teeth short, subulate, the lateral teeth shorter and broader, lanceolate. Corolla 4mm. long, pale greenish yellow, pubescent outside; upper 3 mm. broad with 4 rounded teeth; lower lip longer than upper, 1.25 mm broad. Stamens exerted upper filaments with a bearded tooth at the base. Nutlets are 1.5 mm in diameter subglobose, rugose and brown (Kritikar and Basu, 1993; Rastogi and Mehrotra, 2002).

This species is used as a traditional medicine in South America and Africa in treating bacterial infections, diarrhea, diabetes (Aguiyi *et al*., 2000). It has been reported that extracts of *Ocimum gratissimum* Linn. exerted anti-diarrheal effects in experimental animals (Onajobi, 1986; Offiah and Chikwendu, 1999), showed high antiviral indices against HIV-1 and HIV-2 (Ayissi and Nyadedzor, 2003).

Various phytoconstituents viz. monoterpenes, sesquiterpenes, aromatic compounds, oxygen containing aromatic compounds have been reported from the volatile oil of the plant leaves (Sanda *et al*., 1998). The various monoterpenes e.g., α-thujene, α-pinene, β-pinene, camphene, sabinene, myrcene, α-phellandrene, D-3-carene, α-terpinene, limonene, 1,8-cineole, β-ocimene, terpinolene. The various oxygen-containing monoterpenes, (Z)-sabinene hydrate, linalol, borneol, terpin-4-ol, (E)-ocimenone were also been isolated from the volatile oil of leaf of plant. The various documented sesquiterpenes are α-copaene, β-elemene, α-caryophyllene, α-bergamotene, α-caryophyllene, germacrene-D, β-selinene, bicyclogermacrene from the leaf of the plant have been displayed. The other aromatic compounds p-cymene, estragole, thymol, carvacrol have also been reported from the volatile oil of leaf of plant. The plant exhibited various biological activities including antidiabetic (Aguiyi *et al*., 2000), muscle relaxant (Madeira *et al*., 2002), anthelmintic (Pessoa *et al*., 2002), antinociceptive (Rabelo *et al*., 2003), antihypotensive (Interaminense *et al*., 2007), antileishmanial (Ueda-Nakamura *et al*., 2006), antioxidant activity (Ifesan *et al*., 2006), anticonvulsant (Freire *et al*., 2006).

A literature review showed that no pharmacognostical work has been done so far which can be helpful in characterization and standardization of this plant. Therefore, an attempt has been made to study the pharmacognostic parameters of *Ocimum gratissimum* L. leaves in both whole and powdered form.

MATERIALS AND METHODS

Procurement and authentication of plant material: The leaves of *Ocimum gratissimum* Linn. were collected from District Kaithal, Haryana, India in the month of September and were authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Material Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi (NISCAIR/RHMD/Consult/-2009-10/1265/69). The voucher specimen (TT/YK/2010) has been deposited in Pharmacognosy Research Laboratory-02, Department of Pharmacognosy, ASBASJSM College of Pharmacy, Bela (Ropar), Punjab for further reference. The leaves were shade dried, coarsely powdered and stored in tightly closed container for further studies.

Extraction: Shade dried and powdered leaves of *O. gratissimum* L. were successively extracted with petroleum ether, chloroform, methanol and aqueous solvent using Soxhlet apparatus. The extracts were concentrated to dryness at 40°C in Rotary evaporator (Heidolph, model-Heizbad Hei-VAP, USA). The yield of each extract was calculated and stored in refrigerator.

Morphological parameters: Morphological study of the plant leaves was carried out as per the reported methods (Khandelwal, 2003).

Microscopical characters: Microscopic characteristic were studied in powdered form. Transverse Section (TS) of leaf was studied with compound microscope.

Transverse section (TS): Transverse sections of fresh leaves were prepared with the help of sharp blade. The lignified tissues were distinguished by using saffranin stain. For this free hand sections of leaf were placed for two minutes in the saffranin solution in a petri dish and washed in other petri dish containing distilled water. Then the sections were mounted on clean glass slide with help of glycerin water and covered by glass cover slip. Then slides were observed under microscope (Khandelwal, 2003).

Powder microscopy: A small amount of powder was taken and stained with phloroglucinol solution for few minutes and followed by concentrated hydrochloric acid (1:1) in watch glass. It was mixed well and allowed to stand for about 3 min. It was then mounted in glycerin (50%) and observed under microscope. Similarly, the powder was also stained with weak iodine solution for the identification of starch grains. Powder was treated with concentrated H_2SO_4 for the identification of calcium oxalate crystals. The powder microscopic characters were observed (Fahn, 1997).

Length of trichomes: Calibrate the eyepiece micrometer using stage micrometer. Boil the little quantity of powder drug with chloral hydrate solution mount a little powder

sample in glycerin water and measure the length of 25 trichomes. Multiply the values by the calibration factor for the exact dimension of trichomes. Calculate the average value (Khandelwal, 2003).

Leaf constants: The various leaf constants like stomatal number, stomatal index, vein islet number, veinlet termination number etc. were carried according to the reported methods (Khandelwal, 2003).

Physico-chemical parameters: The various physico-chemical parameters like ash values (total ash, acid insoluble ash and water-soluble ash values), Loss on drying, swelling index, foaming index, determination of pH of solution (1 and 10%), extractive values (etahnol soluble and water soluble) were carried out according to the reported methods (CCRUM, 1987; WHO, 1998; Mukherjee, 2002; Gupta, 2003).

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 (254 and 366 nm) and after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine and ferric chloride (Chase and Pratt, 1949; Kokoshi *et al*., 1958).

Preliminary phytochemical screening: The different extracts like petroleum ether extract (OGPE), chloroform extract (OGCE), methanol extract (OGME) and aqueous extract (OGAE) were subjected to qualitative tests for the identification of various phytochemical constituents like Alkaloids, Steroids, Terpenoids, Flavonoids, Tannins, Saponins, Carbohydrates etc. as per the standard procedure (Evans, 2006).

Thin Layer Chromatography (TLC) Profile: TLC studies of Petroleum ether, chloroform, methanol and ethanol extracts were carried out in various solvents at room temperature using Silica gel Gas an adsorbent (Stahl, 2005).

RESULTS

Extraction: The yield of various extracts of leaves of *Ocimum gratissimum* L. was found to be 2.14, 4.32, 8.88, 6.72% w/w in petroleum ether, chloroform, methanol and aqueous extract, respectively.

Morphological parameters: The leaf of *Ocimum gratissimum* L. was observed as dark green in colour, aromatic, bitter taste, dentate margin, possess sparsely hair, cuneate leaf base, opposite arrangement, lanceolate shape and size 2-7 cm long, 1-3 cm wide.

Microscopical characters: T.S. of leaf had a pot shape midrib and a thin lamina with uneven lower epidermis attached at the lateral sides of its upper side leaving a concave central dorsal depression. Midrib consists of a radiating arc of xylem and phloem. Both upper and lower epidermis showed simple, covering, uniseriate trichomes as well as sessile short stalked, glandular trichomes. Powder microscopy: Powder of the air dried leaves of this plant was observed under the microscope. The numerous glandular simple trichomes of average length 101 μm were observed.

Leaf constants: The leaf of Ramatulsi exhibited anomocytic type of stomata. The various leaf constants *viz.* stomatal number, stomatal index, vein islet number, veinlet termination number were also been determined (Table 1).

Physico-chemical constants: The physico-chemical constants of the plant leaves were carried out as per the reported methods and the results have been shown in Table 1.

Florescence analysis: Florescence analysis of leaf powder has been shown in Table 2.

Preliminary phytochemical screening: Alkaloids, flavonoids, terpenoids were detected in most of the

Table 1: Various leaf constants and physico chemical parameters of *Ocimum gratissimum* L.

Parameters	Results	Parameters	Results
Type of stomata	Anomocytic		
Stomatal number	8	Acid insoluble ash (% w/w)	1.53
Stomatal index	25.8	Water soluble ash (% w/w)	6.57
Vein islet number	10 mm^{-2}	Loss on drying (% w/w)	1.49
Veinlet termination number	12 mm^{-2}	Ethanol soluble extractives (% w/w)	8.53*, 7.20**
Type of trichomes	Covering	Water soluble	24.98*, 22.5**
Length of trichomes	101 μm	extractives (% w/w)	
Swelling Index	Nil	pH (1 and 10% Solution)	7.4 and 6.65
Total ash (% w/w)	8.94	Foaming index	100

All values are average of three observations, *cold extraction, **hot extraction

Table 2: Florescence analysis of *Ocimum gratissimum* L. leaf powder treatment

Treatment	Day light	U.V. light
Powder as such	Green	Green
Powder+1 mL NaOH	Green	Dark green
Powder+1 mL Picric acid	Yellow	Dark yellow
Powder+1 mL Acetic acid	Greenish brown	Greenish brown
Powder+1 mL Hcl	Dark green	Dark green
Powder+1 mL HNO_3	Light green	Dark green
Powder+1 mL Iodine	Brown	Dark brown
Powder+1 mL $FeCl_3$	Greenish	Dark green

Table 3: Preliminary phytochemical analysis of various extracts of *Ocimum gratissimum* L. leaves

Chemical test	OGPE	OGCE	OGME	OGAE
Alkaloids				
Mayer's reagent	+	+	+	+
Dragendroff's reagent	+	+	+	+
Wagner's reagent	+	+	+	+
Hager's reagent	+	+	+	+
Saponins				
Froth test	-	-	+	-
Tannins				
Lead acetate solution	-	-	+	+
$FeCl_3$ solution	-	-	+	+
Flavonoids				
Lead acetate solution	+	+	+	+
$FeCl_3$ solution	+	+	+	+
Terpenoids				
Liebermann-Burchard Reaction	+	+	+	+
Salkowski test	+	-	-	+
Carbohydrates				
Molisch's test	-	-	-	+
Fehling's test	-	-	-	+
Benedict's test	-	-	-	+

+ : Present, - : Absent

Table 4: TLC profile of various extracts of *Ocimum gratissimum* L.

Extract	Solvent system	Detecting reagent	No. of spots	R_f values
OGPE	a	Vanillin-H_2SO_4	7	0.92, 0.91, 0.90, 0.75, 0.70, 0.55, 0.48
	b	Anisaldehye-H_2SO_4	5	0.58, 0.79, 0.71, 0.65, 0.49
OGCE	a	Vanillin-H_2SO_4	4	0.93, 0.75, 0.46, 0.38
	b	Anisaldehye-H_2SO_4	4	0.76, 0.65, 0.57, 0.40
OGME	a	Vanillin-H_2SO_4	5	0.94, 0.87, 0.69, 0.58, 0.49
	b	Anisaldehye-H_2SO_4	2	0.89, 0.77
OGAE	a	Vanillin-H_2SO_4	1	0.79
	b	Anisaldehye-H_2SO_4	1	0.45

A: Toluene: ethyl acetate (93:7), B: Cyclohexane:ethanol:diethylamine (7:2:1)

extracts. However, tannins were been detected in methanolic and aqueous extract. Details of results have been shown in Table 3.

Thin layer chromatography: TLC of various extracts showed many spots, having different R_f values have been shown in Table 4.

DISCUSSION

The plant *Ocimum gratissimum* L. is widely employed in folk medicine to treat a number of diseases like upper respiratory tract infections, diarrohea, headache, ophthalmic and skin diseases, pneumonia, cough, fever, conjunctivitis, abdominal, oral and gynecological disorders. The plant leaves also used as a condiment and flavouring agent (Onajobi, 1986; Sanda *et al*., 1998; Offiah and Chikwendu, 1999; Ayissi and Nyadedzor, 2003).

The evaluation of a crude drug is a vital part for establishing its exact identity and quality. Before inclusion of a crude drug in an herbal pharmacopoeia, pharmacognostical parameters and standards must be established. Therefore, in the present study, some diagnostic features have been evolved to identify and to differentiate the leaf of *Ocimum gratissimum* L. from other crude drugs and adulterants. Morphological studies of *Ocimum gratissimum* L. revealed that the leaves of this plants are dark in colour; 2-7 cm long, 1-3 cm wide; aromatic odour; bitter taste; Dentate Margin; Sparsely hair; Cuneate leaf base; Opposite arrangement; Lanceolate shape; The physical constants such as total ash value (8.94% w/w), acid insoluble ash (1.53% w/w) and water soluble ash (6.57% w/w) were measured which are specific identification characteristics for this plant. The extractive values of alcohol soluble (8.53% w/w) and water soluble (24.98% w/w) were determined indicating the nature of constituents present. Quantitative microscopical study also gives valuable information regarding specific leaf constants such as vein islet (10 mm^{-2}), vein termination number (12 mm^{-2}), stomatal number (8 mm^{-2}) stomatal index (25.8 mm^{-2}). Leaf powder, when treated with different reagents, showed different fluorescence under UV and visible light, which helps in identifying the drug in powdered form. Preliminary phytochemical study of the plant leaves revealed the presence of alkaloids, tannins, flavonoids, terpenoids in the methanolic and ethanolic extracts; alkaloids, flavonoids and terpenoids in petroleum ether and chloroform extracts; carbohydrates in alcoholic extracts. Thin Layer Chromatography of various extracts showed many spots which have different R_f values. The TLC profiling of different extracts helps in identification of this drug and also gives an idea about the presence of various phytochemical in the different extract. Different R_f value of various spots provide valuable clue regarding their polarity and selection of solvents for separation.

CONCLUSION

In the present study, some diagnostic features have been determined to identify and to differentiate the leaf of *Ocimum gratissimum* Linn. from other crude drugs and adulterants and provides specific parameters of standardization which are useful in correct identification and authentification of this drug.

ACKNOWLEDGMENT

Authors are very thankful to Management and Director, ASBASJSM college of Pharmacy, Bela, for providing the necessary facilities to carry on the work.

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