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Pharmacognostical and biochemical investigation of *Ocimum kilimandscharicum* plants available in western Himalayan region

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ABSTRACT

India has a rich heritage of plants as medicines; Indian systems of medicines utilize 80 percent of the material derived from the plants. Himalaya are a rich repository of tradition, culture and heritage. The diversified topography, soil and microclimatic zones of Himalayan regions have resulted occurrence of several valuable and economically important medicinal and aromatic plants of great therapeutic value. The use of plants as sources of medicines are human substance has been in vogue since antiquity. Large numbers of plants are utilized in various systems of medicine practiced in India and local health traditions for the treatment of human diseases since time immemorial. Ocimum kilimandscharicum Gurke. is used for thousands of years in Ayurveda for its diverse healing properties. Tulsi is the legendary 'Incomparable one' of India, is one of the holiest and most cherished of the many healing and healthy giving herbs of the orient. The paper comprises to determine the pharmacognostical parameters and elemental evaluation along with nutritional components and preliminary phytochemical investigation of the Ocimum kilimandscharicum available in western Himalayan region. It was concluded from the study that the aerial parts of Ocimum basilicum from western Himalaya is very good source of minerals and other phytochemicals, which are biologically active substances responsible for various therapeutic potential.

Keywords: Himalaya, Topography, *Ocimum kilimandscharic*um, microelements.

INTRODUCTION

Medicinal plants being as an important natural resource and potentially safe drugs can play an important role in assuaging human health by contributing herbal medicines. The high cost of allopathic medicine and their potential side effects, encouraged the people to use the traditional medicine [1]**.** The increasing demand of plant extracts to be use in the cosmetic, food and pharmaceutical industries suggests that systematic studies of medicinal plants are very important in order to find active compounds and their use as a medicine for curing various diseases [2].

It has been widely observed in developing countries that, the use of traditional medicines are common to the maintenance of the health [3]. In the developing countries, for the treatment of minor ailments, and cost for personal health maintenance, herbal medicines have become more popular [4]. In addition, the use of medicinal plants in the developed societies have been recognized which can be seen by the extraction and development of several drugs and chemotherapeutics from plants and traditionally used herbal remedies [5].

The use of plants as source of medicines and human sustenance has been in vogue since antiquity. India has a rich heritage of plants as medicines; Indian systems of medicines utilize 80 percent of the material derived from the plants. There are over 2500 plant species in India having documented medicinal value, WHO has listed about 20,000 plant species in the world which are used in manufacturing different medicines [6]. The global demand for herbal medicine is not only large but is growing as per the increasing of the population. Factors contributing to the growth in demand for traditional medicine include the increasing of allopathic medicine in developing countries.

Himalaya are a rich repository of tradition, culture and heritage. The diversified topography, soil and microclimatic zones of Himalayan regions have resulted occurrence of several valuable and economically important medicinal and aromatic plants of great therapeutic value, the Western Himalaya are extremely rich in flora and fauna. Different aspects of ethnobotany in the Himalaya have been studied in depth. The Uttarakhand Himalayas have a rich heritage of medicinal and aromatic flora. Several herbs possess odoriferous principles and are used as raw materials for pharmaceuticals, perfumery, foods, condiments and flavors based industries [7,8,9].

The *Ocimum* (Basil) comprises some of the most popular herbs in the world. It belongs to the family *Lamiaceae*, sub family ocimoideae and includes more than 150 different species and varieties distributed in the tropical regions of Asia, Africa, Central and South Africa considered as one of the largest genera of the *Lamiaceae* family. The name Tulsi is derived from 'Sanskrit', which means "matchless one" [10,11]. Among the plants known for medicinal value, the plants of genus *Ocimum* are very important for their therapeutic potentials. Because of its popularity basil is often referred to as King of herbs, being widely utilized due to its economic, nutritional, industrial and medicinal properties [12,13, 14].

The chemical composition of basil oil has been the subject of considerable studies. There is extensive diversity in the constituents of the basil oils and several chemo-types have been established from various phytochemicals investigations. The chemical composition of Tulsi is highly complex, containing many nutrients and other biological active compounds. These constituents significantly vary with time, cultivation process and storage. The nutritional and pharmacological properties of the whole herb in natural form, as it has been traditionally used, result from synergistic interaction of many different active phytochemicals, consequently, the overall effects of Tulsi cannot be fully duplicated with isolated compound or extracts. Due to its inherent botanical and biochemical complexity, standardization of the active components of Tulsi so far is very complex. The plant itself purifies the air around any dwelling near which it is planted it has been shown to produce ozone by modern researchers. There are some problem with the traditional ethno-medical systems is that, even the medicinal plants that seem to work have no sufficient data to prescribe the dosage required. There is therefore the need for the traditional and orthodox medicine practitioners to work together in order to standardize the phytochemicals contents of the plants for safety, seeing that medicinal plants are rich sources of these chemicals, coupled with the fact that they are cheaper and more accessible to the wide population of world .

It is well known that the environmental condition and agricultural practices may significantly modify productivity, oil content, and composition of basil as well as due to cold stress of Himalayan region the secondary metabolite may also interfered. The aim of this paper is to determine the pharmacognostical parameters and elemental evaluation along with nutritional components and preliminary phytochemical investigation of the *Ocimum kilimandscharicum* Gurke plant available in western Himalayan region.

MATERIALS AND METHODS

1. Procurement and authentication of plant material

The mature aerial part of *Ocimum kilimandscharicum* was grown to the flowering stage at Defence Institute of Bio-Energy Research (DIBER) field station Pithoragarh district of Uttarakhand India which is situated at 5500 feet altitude in 29°35'N 80°13'E in the western Himalayan region. The plant was authenticated from Botanical Survey of India Dehradun, the voucher specimen were deposited to the herbarium of BSI with reference number BSI/NRC/Tech(ident.)/2011-12/549, dehydrated in dehydration chamber below 40^{o} C, powdered with a mechanical grinder and stored in an air-tight container for present study.

2. Macroscopic Examination

Morphological study of the plant was carried out as per the reported method [15, 16] for its morphological characters such as colour, odour ,taste, shape, size etc.

3. Physico-chemical evaluation
The various physico-chemical parameters like ash values (total ash, acid insoluble ash values),determination of ph of solution(10%),extractive values (ethanol soluble and water soluble)were carried out according to the reported methods[17] .

4. Biochemical composition
The moisture content of the leaves and stems were determined by drying 5 g of the aerial part (in triplicate) in a hot air oven at 105^0 C until constant weight was obtained. The chlorophyll content in dry leaves powder was estimated by reported method [18]. Dry aerial powder (0.5 g) was extracted with 20 ml 80 percent acetone (pre-chilled), centrifuged at 5000 rpm for 5 min. and transferred the supernatant to a 100 ml volumetric flask. The residue was ground with 20 ml of 80 percent acetone, centrifuged and collected supernatant to same volumetric flask. This procedure was repeated until the residue was colourless. The volume was made up to 100 ml with 80 percent acetone and the absorbance was measured at 645 and 663 nm. Total carbohydrate contents in plants were estimated by the Phenol - sulphuric acid method [19] The carbohydrate present in leaves powder was first hydrolyzed into simple sugar by acid hydrolysis (5 ml of 2.5 N HCl) for 3 hours which resulted monosaccharides. It is neutralized with solid sodium carbonate until the effervescence ceases, then centrifuged and made volume to 100 ml. After that 0.5 to 1 ml supernatant were pipetted-out for analysis. 1 ml of 5 % phenol solution and 5 ml of 96 % sulphuric acid was added. Similarly, from stock solution (100mg/liter) working standards of different concentrations viz. 0.2, 0.4, 0.6, 0.8 and 1.0 ml of glucose were prepared in different test tubes. The absorbance was measured at 490 nm after 10 min. Total carbohydrates percent in sample solution was calculated using the standard graph. The crude fibre was also estimated according to reported method [20]

5. Estimation of Ursolic acid
The ursolic acid in the *Ocimum kilimandscharicum* aerial powder was estimated by using reported chemical method [21]. 5g sample in 25 ml 50%v/v methanol was heated to complete dissolution, 75 ml of water was added and contents were mixed thoroughly and transferred to round bottom flask and 10g H_2SO_4 and reflux for 6-8 hours. Content was cooled and transferred into separating funnel than 25 ml of chloroform was added and the layer was allowed to separate, the chloroform layer was transferred to another separator and aqueous acidic layer once again washed with 25 ml chloroform. The separated chloroform layer is mixed with earlier washings. Both chloroform washings (50 ml) are washed with water till acid free, the acid free chloroform layer was dried over anhydrous sodium sulphate and after filtration, chloroform is evaporated to dryness in a pre-weighed beaker. The residue in beaker is finally dried at 80^0C under vacuum to constant weight. This gives the quantity of total triterpenic acids (major one is ursolic acid) for calculating the % of ursolic acid.

6. Mineral analysis
Macro and micronutrients were estimated by wet digestion method.0.5 g of dried plant material (3 samples each) followed by application of 10 ml of triple acid mixture (HNO_3, $HclO_4$ and H_2SO_4, 10:4:2 v/v) heated at 200^0 C and reduce to 1ml. The digested residue was dissolved in double distilled water, filtered and diluted to 100 ml. The solution was used for the estimation of minerals. Macro minerals viz. Na, K, Ca and Li were estimated by AIMIL flame photometer, while microelements viz. Fe, Cu, Mn, Zn and Co were estimated by Atomic absorption spectrophotometer Model 4129,electronic corporation of India Ltd.

7. Preliminary Phytochemical evaluation
Freshly prepared aqueous extract of leaves were tested for the presence of phytochemical constituents by using reported methods [15,16, 22].

RESULTS AND DISCUSSION

1. Morphological evaluation
The evaluation of a crude drug is a vital part for establishing its exact identity and quality, before inclusion of a crude drug in a herbal pharmacopoeia, pharmacognostical parameters and standards must be established. Therefore an attempt was made to identify and differentiate the plant of *Ocimum kilimandscharicum* from the other crude drugs or adulterants by some diagnostic features. Morphological studies of *Ocimum kilimandscharicum* revealed that it is a perennial evergreen shrub, pale green leaves with opposite arrangements and average leaf length 3.16 cm having pubescent leaf surface, vertical inflorescence 15.2 cm, brownish green stems, flowers were purplish white in simple or much branched racemes, odour camphor like, aromatic, pleasant, taste pungent, astringent.

2. Physico-chemical analysis

The ash values are useful to determine the quality and purity of the crude drug. Ash contains inorganic radicals like phosphate, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Extractive values are useful for evaluation of crude drugs. It gives an idea about the nature of the chemical constituents present in the crude drug. The physico-chemical constants of the plant were carried out as per reported methods and the results have been shown in table 1.

3. Biochemical Composition

The biochemical composition of the aerial part of *Ocimum kilimandscharicum* was shown in Table 2. The moisture content was found 80.55%, Chlorophyll a, Chlorophyll b and total chlorophyll were found 96.57mg/100g, 97.4mg/100g and 195.7mg/100g respectively. Total soluble sugar (TSS) and crude fiber was found 4.0 and 2.0 %. Carbohydrates are the human body's key source of energy, providing 4 calories energy per gram and the plant contains 40.23% carbohydrate which suggest its high energy providing capacity

4. Estimation of ursolic acid

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) is an ursane type triterpene found in all parts of plants, but mainly in leaves and presenting several important biological activities. These include anti-inflammatory, antioxidant and antitumor properties, being effective in reducing growth of a variety of cancer cell lines in vitro, the anti-inflammatory activity in *Salvia officinalis* L. leaves were attributed to Ursolic acid and this compound showed activity two times greater than indomethcin. Ursolic acid is one of the most important non volatile chemical constituent of the *Ocimum*. Ursolic acid was previously identified and reported in *Ocimum basilicum*. The ursolic acid was found 9.82% (crude extract). Ursolic acid is also capable of inhibiting various types of cancer cells by inhibiting the STAT3 activation pathway and human fibrosarcoma cells by reducing the expression of matrix metalloproteinase-9 by acting through the glucocorticoid receptor. Ursolic acid can serve as a starting material for synthesis of more potent bioactive derivatives, such as anti-tumor agents. It has been found to reduce muscle atrophy and to stimulate muscle growth in mice.

5. Mineral analysis

The result of mineral composition clearly reveals that *Ocimum kilimandscharicum* aerial part constitutes the rich source of mineral elements. It reveals that the *Ocimum kilimandscharicum* plant of the western Himalayan region shows appreciable quantities of nutritional components. It contains macro and micro nutrients indicated that the aerial part serve as source of supplement for the mineral supplements. The results are shown in table 3.

Potassium has many functions for protein synthesis, action of many enzymes, stimulation of the movement of the intestinal tract; it is necessary for the function of all living cells and is thus present in all plant and animal tissues. Epidemiological studies and studies in animals subject to hypertension indicate that, diets high in potassium can reduce the risk of hypertension and possibly stroke. Calcium is essential for nerve impulse conduction and activates some enzymes, which generates neurotransmitters; it is an important component of a healthy diet and a mineral necessary for life. It plays an important role in building strong and dense as well as in the keeping of healthy bones and teeths. Copper has a number of important functions in the human body. It helps to produce red and white blood cells and triggers the release of iron to form hemoglobin- the substance that carries oxygen around the body a deficiency of copper may cause hypertension, antibiotic sensitivity, hyperglycemia, manic disorders, insomnia, allergies and osteoporosis. Iron makes up part of many proteins in the body. It plays a vital role in many metabolic reactions. which indicated that the leaves of this plants are good sources of iron compared to the RDA of iron which are 10 mg/day for adult male and children (7-10 years), 13 mg/day for pregnant and lactating mothers and 15 mg/day for adult female (NRC, 1989). Manganese plays an important role in number of physiologic processes as a constituent of some enzymes and an activator of other enzymes. Different enzyme systems in the body require mineral zinc as cofactor. These enzyme systems are responsible for every major physiological function that necessitates catalytic activity from enzyme at the molecular level.

6. Preliminary Phytochemical screening

The preliminary Phytochemical screening with the various qualitative chemical tests revealed the presence of pharmacologically useful classes of compounds tannins was reported to possess physiological astringent properties, which hasten wound healing and ameliorate inflamed mucus membrane and also have haemostatic properties. Saponin has expectorant action, which is very useful in the management of upper respiratory tract inflammation; saponin present in plant is cardiotonic in nature. Alkaloids are reported to have analgesic ,anti-inflammatory and adaptogenic activities which help to alleviate pains, develop resistance against disease and endurance against stress. carbohydrate, phenolic compounds, flavanoids, protein and amino acids, tannins , alkaloid , glycoside, saponin glycoside and steroids were present. According to India forestry outlook study by the ministry of environment and forest government of India. The growth rate % in the trade growing demand of O*cimum sanctum* was found 17.9% which indicate that the cultivation of the plant species can be very beneficial in the Himalayan region which can provide economic benefits to the poor people of Himalayas.

Table. 1 Physico-chemical parameters of *Ocimum kilimandscharicum*

S.No	Total ash	11.25%
1.	Acid soluble ash	10.45%
2.	Acid insoluble ash	0.80%
3.	Water soluble extractives	24%
4.	Alcohol soluble extractives	5.6%
5.	pH (10%)	6.04

Table 2 Biochemical composition of *Ocimum kilimandscharicum*

S.No	Biochemical parameters	Composition
1.	Moisture	80.55%
2.	Total carbohydrate	40.23±2.28 mg/g
3.	Total chlorophyll	195.7 mg/100g
4.	Chlorophyll a	96.57 mg/100g
5.	Chlorophyll b	97.4 mg/100g
6.	Crude fiber	2 %
7.	Total soluble sugar	4

** All values are presented in triplicate*

Table 3. Elemental evaluation of *Ocimum kilimandscharicum*

Mineral	Composition mg/100g
Sodium(Na)	25.58±1.26
Potassium (K)	596.35±7.57
Calcium (Ca)	1144.96±0.5
Lithium(Li)	8.0±0.01
Iron(Fe)	111.6±0.96
Copper (Cu)	1.6±0.04
Manganese(Mn)	7.86±1.98
Cobalt (Co)	0.03±0.07
Zinc (Zn)	20.83±1.37

**all values are expressed in mean ±SD*

Table 4. Qualitative Phytochemical screening of *Ocimum kilimandscharicum*

S.no	Phytochemical	Inference
1	Alkaloids	+++
2	Glycosides	+++
3	Saponin glycosides	+++
4	Flavanoids	+++
5	Phenols	+++
6	Carbohydrates	+++
7	Protein & amino acids	+++
8	Steroids	+++

+++ *present,--- absent*

CONCLUSION

It is concluded from the study that the aerial parts of *Ocimum kilimandscharicum* from western Himalayas is very good source of minerals and other phytochemicals, which are biologically active substances responsible for various therapeutic potential, due to the presence of Ursolic acid it can be very good anticancer agent. The physical constants and preliminary phytochemical screening provide useful information about its correct identification. There is a need of quantitative phytochemical investigation and isolation of the pharmacologically active compounds for its medicinal uses. Due to the high growth rate the plant species is said to be economically valuable and can provide the benefit to the poor people of western Himalaya.

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