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Evaluation of Some Chemical Composition, Minerals Fatty Acid Profiles, antioxidant and Antimicrobial Activities of Tulsi (*Ocimum sanctum*) from India

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Abstract The holy Tulsi, also known as the "queen of herbs" is the most sacred of all the herbs found in India. This sacred plant was found in almost every Indian household. The proximate, minerals and preliminary phytochemical analysis of *Ocimum sanctum* leaves were studied. The nutritional analysis of *Ocimum sanctum* shown high level of ascorbic acid and total carbohydrate i.e., 65.41 mg/100g and 39.58% in their leaves, Whereas the total phenol was found to be maximum (1.88 mg/g) in leaves. Leaves in present investigation, contains major nutrient like N (3.30%), P (1.10%), K (6.62 %), S (1.55 %) and Na (0.74%). Dry weight basis contains 20.64% total protein and 3.60% total fat. The oil of leaves also contains comparable amount of antioxidant as ascorbic acid, flavonoid and total phenol as well as linolenic acid, polyunsaturated fatty acid which was very good for health. According to our results, most of the identified compounds were biologically important. Further the *Oscimum sanctum* leaf possesses certain characteristics that can be ascribed to cultivation on a domestic plantation**.** Antimicrobial activity of Tulsi leaves extract was evaluated and the results shown that *E. coli*, *E. faecalis* were mostly susceptible to methanol extract than *S. aureus* and *A. hydrophila*. It can be suggested that *S. aureus* was the most resistant organisms to the concentrations of 20 and 40 mg/ml of the methanol extract of *Ocimum.* The results of this study indicated the possibility of using the leave extract of Tulsi (*Ocimum sanctum*) as a source of antibacterial compounds for treatment of infections caused by multi-drug resistant bacterial pathogens.

Keywords: *Ocimum sanctum, heavy metal, phytochemicals, antioxidant, antimicrobial, GC-MS*

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1. 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 [1]. *Ocimum sanctum* has been described as of two types i.e., vanya (wild) and gramya (grown in homes). *Ocimum sanctum* has been used in India for around 5000 years and is acclaimed for its healing properties of the mind, body and spirit [2,3]. The use of plants as sources of medicines are human substance has been in vogue since antiquity [4]. 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 [5,6]. 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. Traditionally, Tulsi is taken in many forms: as an herbal tea, dried powder, fresh leaf, or mixed with honey or ghee. For centuries, the dried leaves of tulsi have been mixed with stored grains to repel insects [7]. Many research and studies suggest that tulsi may be a COX_{-2} inhibitor, like many modern painkillers, due to its significant amount of eugenol [4,5,8]. The chemical composition of Tulsi is highly complex, containing many nutrients and other biological active compounds [9,10]. These constituents significantly vary with time, cultivation process and storage. The nutritional and pharmacological properties of the whole herb in natural form, result from synergistic interaction of many different active phytochemicals, consequently, the overall effects of tulsi cannot be fully duplicated with isolated compound or extracts. Recent studies have shown new promising pharmacologically active chemical constituents from this ancient phytomedicine [4,5,11]. The present review summarizes the comprehensive information concerning the traditional use, ayurvedic properties and phytochemistry of tulsi.

It is to be found that the various *Ocimum* species are very much distinguished from each other. All the species are possessing different pharmacological activities science

the huge variation in the chemical composition is there. The literature will serve as a guideline for the researchers in future work related to the complex phytochemistry of the genus *Ocimum*.

Heavy metals have bio-importance as trace elements but the biotoxic effects of many of them in human biochemistry are of great concern. They enter our bodies via food, drinking water and air [12]. Iron, zinc and copper are essential metals whereas cadmium, lead and mercury have no bio-importance [13].

The present study was achieved to evaluate the heavy metal deposition and phytochemical characterization, fatty acid profiles and antimicrobial activities of the leaves extract of *Ocimum sanctum* plant.

2. Materials and Methods

2.1. phytochemical Analysis of *Ocimum sanctum:*

The leaves was collected from three different places from local market of Junagadh and treated as replication. The amount of reducing sugar, total carbohydrates, true protein, Anthrone reagent and Folin-Phenol reagent was estimated. [14]. The phenol content was determined using methanolic extract [15]. Standard graph was prepared for quantification using gallic acid as a standard. Results of total phenol were expressed as mg of gallic acid equivalents per gm of fresh weight of sample. Total Ascorbic acid was quantified [16]. Total ascorbic acid was ex-pressed in mg per 100 gm leaves sample. Total flavonoid was estimated using 1 ml of methanolic extract in which 0.5 ml of 2% w/v $AlCl_3$ in methanol and 0.5 ml potassium acetate (120 mM) were added and incubated at room temperature for 30 minutes. Absorbance was read at 415 nm. Quercetin was used as a standard and the results were expressed as mg of quercetin equivalents per gm of fresh weight sample [17].

The dry leaves of *Ocimum sanctum* was used for oil estimated [18]. Oil was extracted from dry leaves by soxhlet extraction in hexane as solvent. The extracted oils were dried under reduce pressure in rotary evaporator to make free from solvent. Oils were stored at -20°C until prior to use for fatty acid profile. The nutritional data were expressed on dry weight basis.

2.2. Determination of Major Elements in *Ocimum sanctum:*

Plant samples were analysed for N by micro-Kjeldahj using Automatic Digestion, Distillation System (Vap 50s Gerhardt), Total P by vanado molybdo phosphoric yellow color method using spectrometer method and K by mix acid (perchloric acid and nitric acid) digestion and flame photometry [19].

2.3. Fatty Acid Profile of *Ocimum sanctum* Leaves Oil

The fatty acids profiles were determined by GC-MS. Fatty acid methyl esters were prepared using BF3 methanolic solution and extracted with hexane [20]. GC-MS analyses were performed with some modification [20]. GC-MS analyses were performed by Food Testing Laboratories using a Shimdzu model QP2010 quadruple mass spectrometer detector. The GC column was a DB-5, 30 m, 0.25 µm capillary. The initial column temperature was 60°C. The temperature program was 12°C per minute with one minute hold time when rich at 150°C. A final temperature was 240°C per minutes with hold time at five minutes and the mass spectrometer detector analyses. The ion source temp was 230°C. Interface temp was 240°C and the solvent cut time was 2 minutes. For the identification of the compounds the mass spectra of the samples were compared with those Mass spectral library as well as the fatty acids composition was quantified using appropriate standards.

2.4. Determination of Antimicrobial Activity of *Ocimum sanctum* Leaves Extract

The agar-well diffusion method was employed for determination of antibacterial activities [21]. The dry leaves of *Ocimum sanctum* was used for leaves extract [18]. Leaves extract was extracted from dry leaves by methanol 80 % as solvent. The extracts were dried to make free from solvent. Extracts were stored at 4°C until prior to use for antimicrobial activety (NCCLS, 2002) All bacteria were suspended in sterile water and diluted to~10^6 CFU/ml. 200 µl of the suspension was spread onto the surface of NA medium. Wells (4.6 mm in diameter) were cut from the agar with a sterile borer. The concentrations (20 and 40 mg /ml) of the *O. sanctun* leaves extract were applied. Negative controls were prepared using water. The artificial Streptomycin with a concentration of (0.8 µg/ml) was used as positive reference standards to determine the sensitivity of each microbial species tested and to compare the relative percent of antibacterial activity. The inoculated plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters [22].

2.5. Statistical Analysis

All experiments were repeated three times. The growth turbidity of the three tested strains treated with different concentrations of *O. sanctum* extract was calculated by one way ANOVA for the relationship between the growth turbidity and the concentrations of crude extract Both Microsoft Excel 2007 and SPSS (version 16) were used in such analysis.

3. Results and Discussion

3.1. Phytochemical Analysis of *Ocimum sanctum:*

The chemical composition of the *Ocimum sanctum* leaves and stem are given in (Table 1 and Figure 1). As can be seen, stem of leaves shown high level of nutrition value as compared to the leaves. The average percentage w/w of the ash content and the extractive values were determined. Total acidity was determined by simple titration method which shows the negligible quantity in leaves, while the total carbohydrate in leaves is quite high 39.58%. The nitrogen and protein were analyzed by Auto Kjedahl method and nitrogen and total protein value was

3.30 and 20.64% for leaves. These values were taken in triplicate but there was no change among these values having a very small number of standard deviation. Fat were extracted with 95% n-hexane by Soxhlet apparatus and found 3.60% crude fat in leaves. The reducing sugar, flavonoids, ascorbic acid and total phenol were also quantified and tabulated in Table 2, which shown maximum concentration of ascorbic acid that was 65.41 mg/g for leaves, while minimum concentration of total phenol was found 1.88 mg/g. The presence of phenol or alkaloid indicates that the use of the plants have harmless effect. The flavonoids in leaves 28.38 mg/g. The presence of flavonoids confirms that the plants has high antioxidant value, as well as justify its antimicrobial, anti-inflammatory, anti-mutagenic, antiviral and anti allergic actions these results was agree with those obtained by El-Awady [23].

Table 1. Preliminary phytochemical analysis of *Ocimum sanctum* leaves

Parameter	Concentration*
Total Protein %	20.64±1.47
Total Fat/oil %	3.60±_0.08
Total Carbohydrate %	39.58±2.09
Reducing Sugar %	3.58±0.14
Total phenol mg/g	1.88±0.09
Ascorbic acid mg/100g	65.41 ±0.76
Total Flavanoid mg/g	28.38± 0.58

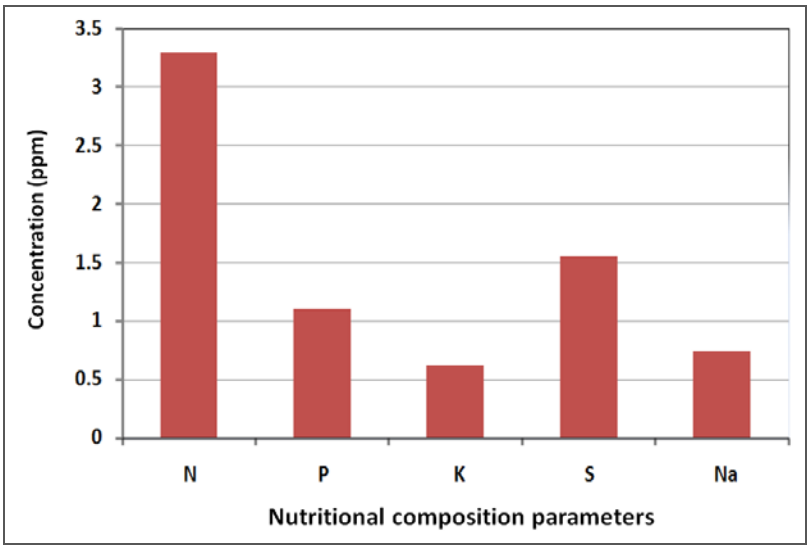

Figure 1. Chemical Nutritional compositions of *Ocimum sanctum* leaves

Table 2. Metal contents and heavy metal contents in medicinal plant *Ocimum sanctum*

Micro-nutrient	Concentration (ppm.)
Mn	61.75±2.06
Zn	32.38±1.42
Cu	14.48±0.72
Mo	0.58±0.21
Ni	5.64±0.37
Li	0.59±0.07
Al	Trace
Mg	Trace
Cd	ND
Cr,	3.67±0.020
Pb	1.17±0.045
As,	3.54±0.304
Hg	0.37±0.051

*data were expressed on dry weight basis.

3.2. Determination of Major and Minor Elements in *Ocimum sanctum:*

The micro (Co, Cu, Fe, Ni, Mn and Zn) and macro (K, Na Ca and Mg) minerals in *Ocimum sanctum* stem and leaves were determined (Table 2). The concentration of these elements reported as ppm on dry weight basis. Among micro minerals Mn and Zn have greater value as compare with other medicinal plants that was 61.75 and 32.38 ppm. while in macro minerals including K, Na, P, Mg of the leaves is same, which is 0.62, 0.74, 1.10 ppm and traces of Mg for leaves, respectively. The amount of heavy metals in the plants was analyzed to show the potential threat of their effects to the animals and human beings who consume them as such or their derived products. This study reports the investigation on the presence of five elements Cd, Cr, Pb, As and Hg (Table 2) in the plant leaves. The leaves were found to contain very high amount of detectable levels of Cr and As. *O. sanctum* leaf digest shown 3.67 and 3.54 ppm of Cr and As.

3.3. Fatty Acid Profile of *Ocimum sanctum* Leaves Oil

The major chemical compounds that were found in *O. Sanctum* extract using GC-MS analysis (shown in Figure 2 and Table 3) are Lauric acid, methyl ester (14.60 ppm), Myristic acid, methyl ester (54.64ppm), Palmitic acid, methyl ester (619.21 ppm), Palmitoleic acid, methyl ester (14.59 ppm), Stearic acid, methyl ester (279.03 ppm), Oleic acid, methyl ester (325.88 ppm), Linoleic acid, methyl ester (585.64 ppm), Linolenic acid, methyl ester (29.57), Arachidic acid methyl ester (102.84 ppm), and Behenic acid, methyl ester (77.84 ppm). The suitable extracts for respective compounds can be chosen on the basis of GC-MS analysis [21]. Eugenol is reported to possess Antimycotic, Antiviral, Desinsection, Antiparasitic, Antioxidant, Anticancer, and Antiinsect activities [24,25].

The current work provides preliminary information and methodologies for rapid quantifiable screening of chemical components and antioxidant potentials of *Ocimum sanctum*[1]. It is, therefore, important to study the total antioxidant properties and chemical profiling of *Ocimum sanctum*. Overall growth of the *Ocimum* species was found to be retarded by longer UV-B exposures. Slight enhancements in the contents of enzymatic and non-enzymatic antioxidants due to the low duration exposures may be the sign of recovery from oxidative stress condition. Antioxidants as Flavonoids, proline and ascorbate contents shown usually enhancements proving their potential for scavenging reactive oxygen species [7,9,10]. Methyl-Isoeugenol has the property of Antifungal activity, Nematicidal activity and Anti feedant activity [26,27]. Caryophyllene is well-known for its anti-inflammatory, cytotoxicity and antifungal activity [3,28,29]. *In vitro* screening of antioxidant activities in organ systems shown varied responses that might be explained on the basis of variable enzymatic contents in different organs. *O. sanctum* oil was proved to be better antioxidant for liver than muscle systems in oxidative stresses [25]. It was observed that there was an intimate relationship among the contents of natural antioxidant and recovery potential of plants from oxidative stress conditions in terms of antioxidant index [4,5,11].

Table 3. Chemical components of the essential oils distilled from *Ocimum sanctum* leaves.

Peak	R. Time	Tulsi (ppm)	Name
1	10.90	14.60	Lauric acid, methyl ester
2	14.30	54.64	Myristic acid, methyl ester
3	17.96	619.21	Palmitic acid, methyl ester
4	18.31	14.59	Palmitoleic acid, methyl ester
5	21.56	279.03	Stearic acid, methyl ester
6	21.91	325.88	Oleic acid, methyl ester
7	22.72	585.64	Linoleic acid, methyl ester
8	23.73	29.57	Linolenic acid, methyl ester
9	25.00	102.84	Arachidic acid methyl ester
10	28.45	77.84	Behenic acid, methyl ester

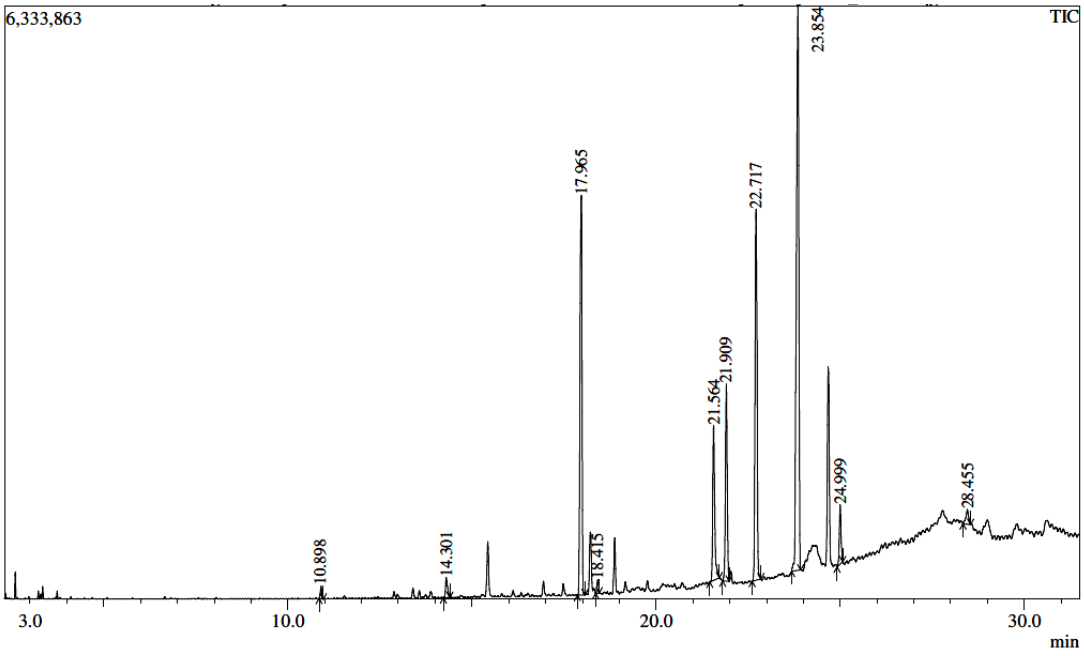

Figure 2. A typical gas chromatogram of the constituents of *Ocimum sanctum* oil

3.4. Antimicrobial activity of *Ocimum sanctun* extracts

Based on the growth inhibition zone diameter obtained by 20 and 40 mg/ml *Ocimum* methanol extract concentration, bacterial strains were tested. This method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms. The antimicrobial activity of extracts of *O. sanctum* was used against four pathogenic organisms, *Escherichia coli*, *Staphylococcus aureus*, *Aeromonas hydrophila*, and *Enterococcus faecalis*, the data are presented in Table 4.

The results shown that the *Ocimum* extracts at final concentrations of 40 mg/ml was active against the four types of micro-organism, while, at the concentration 20 mg/ml for the methanol extracts was not efficient to kill some types of treated microorganism. On the other hand, the final concentration of 40 mg/ml methanol extract of *Ocimum* was effective to kill the treated microorganism. The results also indicated that the methanol was the best solvent for extracting antimicrobial substances from the leaves of this plant [23,30]. Interestingly, *E. coli*, *E. faecalis* were mostly susceptible to extracts than *S. aureus*, *A. hydrophila*. It can be suggested that *S. aureus* was the most resistant organisms to the concentrations of 20 and 40 mg/ml of the methanol extract of *Ocimum* (Table 4). Maximum activity of methanol extract was seen against *E. coli* (25 mm) at concentration 40 mg/ml. The plant extracts compared favorably with the standard antibiotic Streptomycin at a final concentration of 0.8 µg/ml. Similar reports of synergistic role of microbial inhibition on combined usage of plant extracts were also reported by many earlier workers [31,32,33]. Essential oil present in most of the *Ocimum* species is responsible for its antifungal, antibacterial and antiviral properties. Microorganisms develop resistance against various

antibiotics and due to this an immense clinical problem develops in treatment of infectious diseases. Medicinal plants can be used to overcome this problem. Tulsi leaves have been reported to show strong antimicrobial activities against the *Klebisella*, *E. coli*, *Proteus*, *S. aureus*, *Shigella*, *P. aeruginosa* and *Vibrio cholera* [21,33].

Table 4. Diameter of inhibition zone (DIZ) in mm of four bacterial strains which caused by leaves extract of *Ocimum sanctum*

Isolates	DIZ of Streptomycin	DIZ of Methanol extract	
		20 (mg/ml)	40 (mg/ml)
E. coli	19.0+0.51	20.0±0.52	25.0±0.61
S. aureus	24.0+0.49	15.5±0.49	18.0±0.51
A. hydrophila	21.5+0.53	16.0±0.46	18.5±0.48
E. faecalis	16.5+0.42	19.0±0.51	21.0±0.54

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 [33]. 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 [14].

4. Conclusion

In present study we were achieved to evaluate the heavy metal deposition and phytochemical characterization, fatty acid profiles and antimicrobial activities of the leaves extract of *Ocimum sanctum* plant. We found that the nutritional analysis of *Ocimum sanctum* shown high level of ascorbic acid and total carbohydrate i.e., in their leaves, Whereas the total phenol was found to be low in leaves. Leaves also, contains major nutrient like N , P , K , S , and Na. The oil of leaves also contains comparable amount of antioxidant as ascorbic acid, flavonoid and total phenol as well as linolenic acid, polyunsaturated fatty acid which was very good for health. Antimicrobial activity of Tulsi leaves extract against *E. coli* and *E. faecalis* were mostly susceptible to methanol extract than *S. aureus* and *A. hydrophila*.

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