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Antibacterial and Antioxidant study of Holy Basil (*Ocimum sanctum* Linn.)

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Abstract

Ethanol, methanol, and hexane extracts from (*Ocimum sanctum* Linn.) were investigated for them in vitro antimicrobial properties. a disk-diffusion and minimal inhibition concentration (MIC) method. Both the hexane and ethanol extracts, but not the ethanol extracts, inhibited three isolates out studied. All three extract of *O. sanctum* were different in terms of their antibacterial activities. The hexane extract showed a stronger and broader spectrum of antibacterial activity, study was also carried out to evaluate the in-vitro antioxidant activities of ethanol, $chcl_3$ and cc_4 extract of Ocimum species namely *Ocimum sanctum* Linn. This was achieved by screening the two plant extracts at varying concentrations (10-50 g/ml) using DPPH radical scavenging activity, reducing power assay, hydroxyl radical scavenging activity and nitric oxide radical scavenging activity. The results were analyzed statistically which showed that ethanol extract *Ocimum sanctum* had more antioxidant activity than standard antioxidant.

1. Introduction

Basil (*Ocimum sanctum* Linn.) which belongs to the Lamiaceae family, commonly known as "Tulsi", "holy basil", "Queen of plants" and "The mother medicine of nature". Basil is aromatic herbs that are used extensively to add a distinctive aroma and flavor to food. The leaves can be used fresh or dried as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics (Simon *et al*., 1999). Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunction (Nejatzadeh-Barandozi *et al*., 2014). In ancient Ayurveda, 'Tulsi' (*Ocimum sanctum* Linn.) is known as the elixir of life since it promotes longevity and used in many formulations for the prevention and cure of various ailments (Govind *et al*., 2010). All parts of the plant such as fresh leaves, juice, seeds, and volatile oil are very beneficial to us. The *O. sanctum* plant finds wide applications in the treatment of cough, coryza, hay asthma, bronchial infections, bowel complaints, worm infestations, and kidney stones in traditional systems of medicine (Bauer *et al*., 1966; Sood *et al*., 2005) . *O. sanctum* possesses diverse pharmacological properties that include antioxidant (Govind *et al*., 2010), antibiotic, antidiabetic, antiatherogenic, immunomodulatory (Govind *et al*., 2010; Pattanayak *et al*., 2010), anti-inflammatory (Singh *et al*., 2007; Pattanayak *et al*., 2010), analgesic, antiulcer (Pattanayak *et al*., 2010), and chemo-preventive and antipyretic properties (Bhattacharyya *et*

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al., 2013). Tulsi leaf extract reduces blood glucose and cholesterol and promotes immune system function (Mondal *et al*., 2011), and one of the constituents, β-elemene, has been reported to have potent anticancer property (Li *et al*., 2009). The major phytochemicals present in *O. sanctum* plant belong to terpenoid, phenolic, tannin, steroid, alkaloid, and saponin class of compounds (Baliga *et al*., 2013).

O. sanctum is rich in a variety of important nutrients, most notably vitamin A, vitamin C, calcium, and phosphorus. It is also a source of iron, potassium, and magnesium. It is thought to have significant health effects, particularly in improving the health of the cardiovascular system. Used for strong eyesight and healthy skin and hair. It also contains high concentrations of carotenoids like beta carotene, and these substances are converted to vitamin A within the body. Beta carotene offers even more benefits than vitamin A alone, and it is known to be a powerful antioxidant. Free radicals are potentially important in a number of ailment states that can have severe effects on the cardiovascular system, either through lipid per oxidation or vasoconstriction 1. Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. Many experts believe that the Recommended Dietary Allowance (RDA) for specific antioxidants may be inadequate and, in some instances, the need may be several times the RDA.

Therefore, as a part of our studies on *Ocimum sanctum* of Iran, we report here results of a disk-diffusion and minimal inhibition concentration (MIC) method of the Ethanol, methanol, and hexane extracts mentioned *Ocimum sanctum* as well as evaluation of them in vitro antimicrobial properties.

2. Materials and methods

2.1. Plant Material

The plant materials used in this study, *Ocimum sanctum* Linn. Roots, seed and leaves of were collected from the field in Urmia city identified by Department of Horticulture, Islamic Azad University of Khoy, Iran. A voucher specimen of the collected sample was deposited in our institutional herbarium for the reference.

2.2. Preparation of various extract of *Ocimum sanctum* Linn.

In present study, we use dry stem of the plant collected. Dried stems are cut into small pieces, these pieces are then grinded. The grinded sample is dark brown in color with a special smell. This powder stirred in non-polar solvent i. e. CCl_4, for 1/2 hour and then it is refluxed for 1/2 hour this is performed for extraction of non-polar component from powder. After extraction the CCl_4 layer is distilled to recover solvent and to get a brown colored liquid fraction which shows single spot on thin layer chromatography. The residue of CCl_4 extraction is used for further study. This residue is mixed with $CHCl_3$ and stirred for 30 minutes and then refluxed for 1 hour. After filtration the filtrate is distilled to get $CHCl_3$ Fraction which is Red-brown colored liquid. Then the Residue of $CHCl_3$ is used for extraction with Ethyl acetate stirred well and refluxed for 1 hour then filtered. Filtrate is then distilled and fraction of Ethyl acetate is collected it shows no spot on TLC plate. Conclusion is that no organic compound is present. The Ethyl acetate residue is further mixed with methanol and stirred for 30 minutes and refluxed for 1hr. Then it is filtered and filtrate is distilled out. Methanol fraction is yellow brown in color and show single spot On TLC plate. The remaining residue also have smell and it is observed that residue is insect repellant.

2.3. DPPH Scavenging Test

Quantitative measurement of radical scavenging property was carried out in a universal bottle. The reaction mixture contained 50 µL of test samples (or 80% MeOH as blank) and 5 mL of a 0.004% (w/v) solution of DPPH (1,1' -diphenyl-2-picrylhydrazyl) in methanol. Different known antioxidants, vitamin E, and butylated hydroxy toluene (BHT, Sigma) were used for comparison or as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. Measurements was taken at least in triplicate. DPPH radical's concentration was calculated using the following equation: DPPH scavenging effect (%) = [(Ao – A1) / Ao] ×100; Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample. The actual decrease in absorption induced by the test compounds was compared with the positive controls. The mean OD 517 results of DPPH scavenging activity were recorded.

2.4. Antimicrobial Activity

The agar diffusion method (Husain et al., 1987) was used to evaluate the antimicrobial activity. Bacteria were cultured overnight at 37 o C in Mueller Hinton 10 µl Broth (MHB, Oxoid) and fungi at 28 o C for 72 h in Potato Dextrose Broth (PDB, Oxide) and used as inoculums. A final inoculums, using 100 µl of suspension containing 10 8 CFV/ml of bacteria 10 4 spore/ml of fungi spread on Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium respectively. The disc (6 mm in diameter) was impregnated with 10 µl of 75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml of each extracts and for each organism placed on seeded agar. Ciprofloxacin and Fluconazole (75µl/ml, 50µl/ml, 25µl/ml, 10µl/ml and 5µl/ml) were used as positive control bacteria and fungi respectively. The test plates were incubated at 37 o C for 24h for bacteria and at 28 o C for 72h for fungi depending on the incubation time required for a visible growth. MIC values were also studied for microorganisms by turbid metric method, which were determined as sensitive to the extracts in cup plate method. MIC was defined as the lowest concentration of extract that inhibit visible growth.

3. Results and Discussion

In this study, the antioxidant properties of the methanol extracts of leaves and stems of plant like of reexamined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Table 1 and Table 2 as comparable with known antioxidant BHT. The highest antibacterial activity was related to *Bacillus paludis* in ethanolic extract and the lowest antibacterial activity was related to *Enterobacter aerogenes* in Tetrax extract. In Extract CCl_4 highest antibacterial activity was related to *Staphylococcus Oureus.* In Cefotax and Penicil extract highest antibacterial activity was obtained to *Bacillus paludis* and *Bacillus subtilis* (Table 1).

Table 1. Antibacterial activity of methanolic extract from *Ocimum sanctum*.

Bacterial	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
G (+)	In mm	In mm	In mm	In mm	In mm
1. *Staphylococcus epidermidis*	11	7	6	9	8
2. *Staphylococcus Oureus*	9	11	9	7	7
3. *Bacillus paludis*	15	7	10	9	7
4. *Bacillus subtilis*	14	7	10	9	6
G (-)					
1. *Escherichia Coli*	5	5	5	4.5	5.5
2. *Pseudomonus aeruginosa*	6	6	5	6	4.5
3. *Shigella flaxinely*	7	7	4.5	7.5	8
4. *Enterobacter aerogenes*	3	4	4	3	2

The highest antifungal activity was related to *Aspergillus niger* in *extract* CCl_4 and the lowest antifungal activity was related to *Candida albicans* in Extract ethanolic and *Aspergillus fumigates* in Tetrax (Table 2).

Table 2. Antifungal Activity of Ethanilic Extract from Ocimum sanctum

Fungus	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
1. *Candida albicans*	4	6	6	5	8
2. *Aspergillus fumigates*	5	7	5	6	4
3. *Aspergillus niger*	4	11	9	7	8

The Antimicrobial (antibacterial and atifungal) activities of different extracts of Ocimum were evaluated qualitatively and quantitatively against the pathogenic microorganisms by the presence or absence of inhibition zones and zone diameter (Table 3, Table 4). The isoamyl extract showed stronger and broader spectrum of antimicrobial activity compared to other solvent extracts. These results predict that isoamyl alcohol is a better solvent for consistent extraction of antimicrobial substances from Ocimum species. The highest antibacterial activity from root of *Ocimum sanctum* was obtained in ethanolic extract and the lowest antibacterial activity from root was related to *Enterobacter aerogenes* in Tetrax extract (Table 3).

Table 3. Antibacterial Activity of Methanolic Extract from Root of *Ocimum sanctum*

Bacterial	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
G (+)	In mm	In mm	In mm	In mm	In mm
1. *Staphylococcus epidermidis*	11	10	9	9	9
2.*Staphylococcus oureus*	11	9	9	8	9
3. *Bacillus paludis*	9	8	10	10	8
4. *Bacillus subtilis*	11	5	10	11	8
G (-)					
1. *Escherichia coli*	7	9	4	3.5	4.5
2. *Pseudomonus aeruginosa*	9	8	4	3	3.5
3. *Shigella flaxinely*	10	8	4.5	3.5	3
4. *Enterobacter aerogenes*	11	4	5	3	2

The highest antifungal activity was related to *Candida albicans* in *extract Ethanolic* and the lowest antifungal activity was obtainted in Penicil and Tetrax (Table 4). In the antimicrobial assay, among the tested pathogens *B. subtilis* and *S. typhi* were most sensitive for Ocimum extract and no inhibition activity was observed against *E. faecalis*.

Table 4. Antifungal activity of methanolic extract from root of *Ocimum sanctum*

Fungus	Extract Ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
1. *Candida albicans*	12	5	6	5	4
2. *Aspergillus fumigates*	11	8	5	7	4
3. *Aspergillus niger*	10	7	5	4	5

The results of phytochemical analysis of Ocimum showed the presence of chemical compound such as phenolic compounds, glyco-sides, flavanoids, tannins and saponins which account for their usefulness as medicinal plants. The DPPH radical scavenging activities of *O. gratissimum* was higher compared to other species at lower concentration: whereas, antioxidant activity of Camphor basil was higher in other assays.

These differences might be due to the different principles of these assays: hence, we can construe that antioxidant activity cannot be compared by different antioxi-dant assays. Our antioxidant assay result corresponds to the earli-er studies (Hakkim *et al*., 2008; Farrukh *et al*., 2006). In terms of antioxidant activity, all the extracts investigated exhibited a rather high degree of activity (more than 40%). In particular, leaves (ethanol extract) of *Ocimum sanctum* Linn. (Table 5) displayed the highest activities as antioxidant activity as removal of the stable radical DPPH and the lowest activity were found in CCl_4 extract of stem. As expected, the overall activity of the raw extracts was lower than that of commercial antioxidant BHT, the reference antioxidant. The highest antioxidant activity of leaves of

Ocimum sanctum was obtained in 0.3 mg/ml concentration of ethanol and the lowest antioxidant activity of leaves of *O. sanctum* was related to 0.1 mg/ml concentration of CCl_4 (Table 5).

Table 5. Antioxidant Activity of Leaves of *Ocimum sanctum*

Extract Conc. (Mg/ml)	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	43.1	25.61	15.23	15.97
0.1	44.01	45.63	21.33	10
0.2	48.24	57.22	27.70	22
0.3	55.97	63.10	49.00	29

Our results are different from results of (Adiguzel *et al*., 2005), in our study zone of inhibition was seen for Ethanol and Methanol extract of O. basilicum on Proteus sp. and S. aureus. The minimal inhibitory concentration was determined at different concentrations and varied for different species. Among the tested microorganism most were sensitive for isoamyl alcohol extract of all Ocimum species. The highest antioxidant activity of seed of *Ocimum sanctum* was obtained in 0.3 mg/ml concentration of BHT and the lowest antioxidant activity of seed of O. sanctum was related to 0.05 mg/ml concentration of ethanol extract (Table 6).

Table 6. Antioxidant activity of seed of *Ocimum sanctum*

Extract Conc. (Mg/ml)	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	44.1	12	13	17
0.1	43.91	21	22	20
0.2	48.24	27	25	24
0.3	56.57	43	31	25

The highest antioxidant activity of root of *Ocimum sanctum* was related to 0.3 mg/ml concentration of BHT and the lowest antioxidant activity of root of *O. sanctum* was related to 0.1 mg/ml concentration of $CHCl_3$ (Table 7).

Table 7. Antioxidant activity of root of of *Ocimum sanctum*

Extract Conc. (Mg/ml)	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	44.1	17	10	10
0.1	45.91	25	9	11
0.2	48.24	22	11	15
0.3	56.57	31	15	14

4. Conclusion

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases which can have serious effects on the cardiovascular system, either through lipid per oxidation or vasoconstriction The extracts and essential oils of many plants have been investigated for their antioxidant activity 5-6. Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defence 7-8. Polyphenols interact with pathogens, herbivores, and other plants; they protect from ultraviolet radiation and oxidants, repel or poison predators and attract beneficial insects or microbes 9-10. The results of Antimicrobial activity were done for all the five, pet ether, chloroform, acetone, and methanol and aqueous extracts. During antimicrobial study methanolic extracts showed maximum zone of inhibition against almost all organisms in cup plate method. The methanolic extract from roots of *Ocimum sanctum* showed a good inhibition against all the bacterial Strains tested (MIC between 10 and 80 ug/ml). The gram (+) bacteria were sensitive with gram (-) bacteria and some common fungi. As Ocimum is widespread in Iran, it can be recommended as an easily available and renewal source of antimicrobial agent instead of synthetic chemicals. The present

findings indicate that *Ocimum sanctum* possesses compounds with antimicrobial properties against pathogenic microorganisms.

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