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Antibacterial and Antioxidant study of Ocimum basilicum Labiatae (sweet basil)

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ABSTRACT:
Ethanol, methanol, and hexane extracts from Ocimum basilicum Labiatae (sweet basil) were investigated for their 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. basilicum 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 ,chcl3 and cc4 extract of Ocimum species namely Ocimum basilicum This was achieved by screening the two plant extracts at varying concentrations (10-50ig/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 basilicum had more antioxidant activity than standard antioxidant.
Keywords: Antibacterial, Antioxidant, Ocimum basilicum**,** DPPH, Labiatae (sweet basil).

INTRODUCTION:

The flowers are white, labiate (like lips) are in six blossom, pedicled (part of the flower still attached to the stem), almost sessile (still directly attached), axillary (grown from an axil), false whorls. The calyx (usually green outer whorl) is bilabiate (having two lips), and the corolla (the part of a flower that consists of the separate or fused petals) is 4-lobed. The lower lip is simple with four stamens laying on it.
The entire plant grows from 8 - 20 inches (20 - 50 cm) high, erect and sometimes bushy. It has a downy feel from the base up. The leaves are ovate or oblong, very lightly toothed, shinny, with deep vein markings. They are long petioled (slender stem), acuminate (tapering to a slender point), irregularly dentate (having teeth or pointed parts) or entire-margined. It has a very characteristic scent that once smelled will most

likely not be forgotten One of my favorite uses (and no this isn't a suggestion) is to drink a cup of tea in the evening; 1 teaspoon per cup of water. I do this because of its high content of magnesium and potassium. Magnesium draws water out of the body and into the bowels, potassium helps with muscle cramps (something I have because of my MS). It is said to stimulate digestion, is used for the feeling of being too full and flatulence, is said to kill intestinal parasites, and used as a diuretic. Personally, I just like the way it tastes. It 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.

MATERIALS AND METHODS:

Plant Material:

The plant materials used in this study, Ocimum basilicum Labiatae roots, seed and leaves of were collected from the field in Khandala Tal. Shrirampur, Dist Ahmednagar identified by Dr. A. K. Mohite, R.B.N.B College, Shrirampur, Maharashtra, India. A voucher specimen of the collected sample (voucher sample no 234) was deposited in our institutional herbarium for the reference.

Preparation of various extract of Ocimum basilicum Labiatae

In present study we use dry stem of the plant collected from western ghat region Maharashtra. 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 & 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 & 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 $CHCl_3$ & stirred for 1/2 hour & 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 & refluxed for 1 hour then filtered. Filtrate is then distilled & 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 & stirred for 1/2 hr & refluxed for 1hr. Then it is filtered & filtrate is distilled out. Methanol fraction is yellow brown in color & show single spot On TLC plate.

The remaining residue also have smell & it is observed that residue is insect repellant.

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 in methanol. Different known antioxidants, vitamin E, and butylatedhydroxytoluene (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.

Antimicrobial Activity:

The agar diffusion method [11] 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 72h 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 ° C for 24h for bacteria and at 28 ° 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.

RESULTS AND DISCUSSION:

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 5-7. Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defence 8-9. 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 10-11. Therefore, in this study, the antioxidant properties of the methanol extracts of leaves and stems of plant like of re examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in table 1 & table 2 as comparable with known antioxidant BHT. 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 basilicum displayed the highest activities as antioxidant activity as removal of the stable radical DPPH and the lowest activity were found in CCl4 *extract of stem.* As expected, the overall activity of the raw extracts was lower than that of commercial antioxidant BHT, the reference antioxidant.

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 basilicum Labiatae showed a good inhibition against all the bacterial Strains tested (MIC between 10& 80 ug/ml). The gram (+) bacteria were sensitive with gram (-) bacteria and some common fungi.

Table 1. Antibacterial activity of methanolic extract from extract from Rhizome of Valeriana wallichii

Bacterial	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
G (+)	In mm	In mm	In mm	In mm	In mm
1. Staphylococcus epidermidis	12	8	7	10	9
2. Staphylococcus oureus	10	12	10	8	8
3. Bacillus paludis	16	8	11	10	8
4. Bacillus subtilis	15	8	11	10	7
G (-)					
1. Escherichia Coli	5	5	6	5.5	5.5
2. Pseudomonus aeruginosa	7	7	6	7	4.5
3. Shigella flaxinely	8	8	4.5	8.5	9
4. Enterobacter aero genes	3	4	5	3	2

Table 1 (i). Antifungal Activity of Ethanilic Extract From Extract From Ocimum Basilicum Labiatae

Fungus	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
1. Candida albicans	5	7	7	5	9
2. Aspergillus fumigatus	6	9	5	7	4
3. Aspergillus niger	4	12	10	8	9

Table 1 (ii). Antibacterial Activity of Methanolic Extract From Root Of Ocimum Basilicum Labiatae

Bacteria	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
G (+)	In mm	In mm			
1. Staphylococcus epidermidis	12	11	10	10	10
2. Staphylococcus oureus	12	10	10	8	8
3. Bacillus paludis	10	9	10	11	9
4. Bacillus subtilis	12	5	11	10	9
G (-)					
1. Escherichia Coli	8	9	4	3.5	5.5
2. Pseudomonus aeruginosa	10	9	4	3	3.5
3. Shigella flaxinely	11	9	4.5	3.5	4
4. Enterobacter aero genes	12	4	5	3	2

Table 1 (iii). Antifungal activity of methanolic extract from root of *delonix regia* plant

Fungus	Extract mthanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
1. Candida albicans	13	5	7	5	4
2. Aspergillus fumigatus	10	9	5	6	4
3. Aspergillus niger	11	8	6	4	5

Antioxidant Activity of Leaves

Extract Conc. Mg/ml	BHT	Ethanol	CHCl3	CCl4
0.05	45.1	26.6O	16.53	16.47
0.1	46.91	46.64	22.53	10
0.2	49.24	58.24	28.50	24
0.3	57.57	64.12	50.00	30

Table 2. Antioxidant activity of seed

Extract Conc. Mg/ml	BHT	Ethanol	CHCl3	CCl4
0.05	45.1	13	14	16
0.1	46.91	22	24	21
0.2	49.24	28	26	25
0.3	57.57	45	32	26

Table 3. Antioxidant activity of root

Extract Conc. Mg/ml	BHT	Ethanol	CHCl3	CCl4
0.05	45.1	18	11	11
0.1	46.91	27	10	12
0.2	49.24	24	12	16
0.3	57.57	32	16	15

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