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STUDIES ON PRELIMINARY PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *OCIMUM TENUIFLORUM* L. LEAVES

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ABSTRACT

Phytochemical screening of the plant leaves reveals the presence of saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols and tannins. Hexane, Acetone and ethanol extracts of leaves of *Ocimum tenuiflorum* L. were prepared and antimicrobial activity were studied by disc diffusion method against certain Gram-positive and Gram-negative bacterial pathogens and some fungus. The acetone extracts had wide range of antibacterial activity against bacterial and fungal pathogens than the hexane extract, where as ethanol extract were slightly lower antimicrobial activity than acetone extract. Antimicrobial activity of various extract of leaves of *Ocimum tenuiflorum* was carried in attempt to develop a new pharmaceutical drug from natural origin for prevention of pathogenic microbes.

INTRODUCTION

Among all families of the plant kingdom, members of the family Lamiaceae have been used for centuries in folk medicine. A number of phenolic compounds with strong antioxidant and antimicrobial activities have been identified in plants, especially in those belonging to the Lamiaceae family. The area of interest to food manufactures as consumers move towards functional foods with specific health effects (Ozkan *et al*., 2003).

Ocimum belongs to the family Lamiaceae, sub-family Napetoideae of the tribe Ocimeae. Previous studies show that there are large numbers of species and varieties falls in this genus(Labra *et al*., 2004; Mondello *et al*., 2002; Klimankova *et al*., 2008; Miele *et al*., 2001). Characterizations of each species in this genus (family Lamiaceae) are based on the leaves and habitat (Grayer *et al*., 2002). *Ocimum* constitutes between 50-200 species of aromatic annual and perennial herbs and shrubs (Simon *et al*., 1999) from the tropical region of Asia, Africa, Central and South America (Suppakul *et al*., 2003; Darrah, 1980). Basil originated from Iran, India and other tropical regions of Asia, having been cultivated there for more than 5,000 years. Basil folklore is as complex as its flavour and aroma. They are heat and drought tolerant (i.e. they do not require high humidity). Basil is a source of essential oils and aromatic compounds, culinary herb, and an attractive fragrant ornamental. The seeds also contain edible oils (Morales and Simon, 1996).

The genus *Ocimum* or Mint, collectively called Basil, has long been acclaimed for its diversity. This genus is characterized by a great variability in its morphology and chemotypes (Lawrence, 1988). The genus *Ocimum* is ranked high among some of the astonishing herbs for having enormous medicinal potentialities. This species has a long history as culinary herbs, thanks to its foliage adding a distinctive flavor to many foods. It is also a source of aroma compounds and essential oils containing biologically active constituents that possess insecticidal and nematicidal properties (Deshpande and Tipnis, 1997; Chaterjee *et al*., 1982). The essential oil, leaves, seeds, flowers and roots of basils are used as medicine.

The major phenolic compounds founds in plants are secondary metabolites possessing high antioxidant activity and it is wide spread in the species of Lamiaceae (Gang *et al*., 2001). Craveiro *et al*. (1981) and Janine de Aquino *et al*. (2005) reported some chemical compounds and active ingredients found in these plants such as eugenol, linaol, methyl cinnamate, camphor and thymol. Several species and varieties of plants of the genus *Ocimum* leaves have been reported to yield oil of diverse nature, commonly known as basilica oils. Various species of *Ocimum* for example *O. viride, O. suave, O. basilicum* and *O. canum*. have been reported for their numerous medical uses (Mshana *et al*., 2000). Several *Ocimum* species (Lamiaceae) are used to treat central nervous system (CNS) disorders in various parts of the world and its antidepressive activity is frequently reported (Correa, 1984). Leaves from *Ocimum* species release a pleasing odour when squashed between the fingers and could be used as a culinary

condiment (Makinen and Paakkonen, 1999) and for insect control (Holm, 1999). Various effects of Ocimum sp., including bactericidal, anti-inflammatory, antioxidative, antiulcer, antidiarrheal, chemopreventive, blood-sugar lowering, nervous system stimulation and radiation protection have been reported by Prakash Gupta (2000) and Umadevi (2001). The antimicrobial activity of *O. basilicum* against many kinds of bacteria and fungi was described by Masada (1976) and (Morales and Simon, 1996).

Basil is one of the oldest herbs/spices within the Ocimum genus in the lamiaceae family and well known for its medicinal value, it is also popular as a kitchen herb. Basil has many uses including culinary, ornamental, aromatic and medicinal. It is grown as a perennial in tropical and subtropical regions of Asia, Africa, Central and South Africa (Suppakul *et al*., 2003). Basil is a rich source of essential oil and has been used in confectionaries, condiments, sausages and meats, salad dressings, nonalcoholic beverages and ice cream. The various parts of the basil plant namely leaves, flowers and stems are being used in the treatment various disorders such as skin diseases, cold, cough, fever, vomiting, swelling etc. Into this, basil is reported to have anti-allergic, anti-cancer (Hakkim *et al*., 2007), antimicrobial, antiseptic, antispasmodic (Suppakul *et al*., 2003) antifungal, antiviral, anti-inflammatory, analgesic and immuno-stimulatory properties (Umadevi, 2001).

MATERIALS AND METHODS

Plant materials

Fresh plant of *Ocimum tenuiflorum* were collected from local region of Theni District in Tamilnadu. The leaves were identified by Mr. G.V.S. Murthy, Join Director, Botanical survey of India (BSI), Coimbatore, Tamilnadu. India.

Preparation of extracts

50gm of the plant material in each batch was exhaustively extracted by soxhlet extraction method using Hexane, Acetone and Ethanol. The solvent used in each batch was recovered under pressure until dry extracts were obtained and then labeled and stored separately at 4°C in amber colored airtight bottles.

Phytochemical Screening of Plant materials

The presence of saponins, tannins, alkaloids, flavonoids, glycosides, phenols, steroids, and alkaloids, were detected by simple qualitative methods (Khandelwal, 2011).

Antimicrobial activity

Antimicrobial assays described in the literature include measurement of:

(i) The radius or diameter of the zone of inhibition of bacterial and fungal growth around paper discs impregnated with (or wells containing) an antimicrobial compound on media.

(ii) The inhibition of organisms' growth on a medium with the antimicrobial compound diffused in the agar.

(iii) The minimum inhibitory concentration (MIC) of the antimicrobial compound in liquid media.

(iv) The changes in optical density or impedance in a liquid growth medium containing the antimicrobial compound.

To screen the antimicrobial activity of 'Unknown' compounds, the second methodology mentioned above is considered to be the simplest where the results are obtained rapidly.

Target Microorganisms

The antimicrobial activity of the extracts (Hexane, Acetone and Ethanol) of *Ocimum tenuiflorum* were investigated against six Gram-positive, nine Gram-negative bacteria and eleven fungi. The clinical isolates were received from the Department of Mycology, University of Madras, Chennai.

Gram positive bacteria

Streptococcus faecalis, Streptococcus pyogenes, Enterococcus faecalis, Bacillus subtilis, Bacillus thuringiensis, Staphylococcus aureus and *Serratia marcescens.*

Gram negative bacteria

Klebsiella pneumoniae, Proteus vulgaris, Proteus mirabilis, Salmonella paratyphi, Salmonella paratyphi A, Salmonella paratyphi B, Pseudomonas aeruginosa and *Escherichia coli.*

Fungi

Alternaria alternata, Fusarium oxysorum, Curvularia lunata, Rhizoctonia solani, Aspergillus flavus and *Candida albicans*.

Disc Diffusion Assay

Antimicrobial activity of leaf extracts was tested against the above Gram-positive, Gram-negative bacteria and fungal strains using Disc Diffusion method (Berghe and Vlietinck, 1991; Cappuccino and Sherman, 1998). These bacteria and fungi were grown in Nutrient Agar Medium and Potato Dextrose Agar Medium (PDA). 20ml of medium was poured into the plates to obtain uniform depth and allowed to solidify. The standard inoculum suspension (10^6 CFU/ml) was streaked over the surface of the media using sterile cotton swab to ensure confluent growth of the organisms. 6mm diameter discs were prepared with Whatmann No.1 paper and used for the study. 10µl of extracts was diluted with two volumes of 5% Dimethyl Sulfoxide (DMSO) and impregnated on the filter paper discs, and placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Ampicillin, Kanamycin and Erythromycin were used as positive reference standard and 5% DMSO was used as blind control. Finally, the inoculated plates were incubated at 37ºC for 24hrs and the inhibition zones were observed, including the diameter of the disc (mm). All the experiments were done in triplicates.

RESULTS

Phytochemical screening of *Ocimum* extracts in this study showed that flavonoids, saponins, cardiac glycosides, alkaloids, phenols and tannins constituents were present. Detection of antimicrobial properties in plant extracts is really an indication of the presence of some of these constituents (Table 1).

Table1. Preliminary phytochemical constituents of *Ocimum tenuiflorum*.

Phytochemical constituents		Hexane	Acetone	Ethanol
Saponins		+	+	+
Flavonoids		-	-	+
Steroids		-	-	+
Cardiac glycosides		-	-	+
Alkaloids	Dragendroff'sreagent	-	+	+
	Mayers test	-	+	+
Phenols	$FeCl_3$	-	+	+
	Elagic acid test	-	+	+

Tannins	$FeCl_3$	-	+	+
	Lead acetate test	-	+	+

- Absent, + Present

In our present study, a wide range of human pathogenic microorganisms were examined, including not only gram positive and gram negative bacteria, but also fungi. The Disc diffusion assay results of *Ocimum tenuiflorum* extracts of leaves with the inhibition zones formed by standard antibiotics discs showed in Tables 2 and 3. The antimicrobial assay showed that hexane, acetone and ethanol extracts of *Ocimum* leaves exhibited *in vitro* antibacterial activity against Gram-positive and Gram negative bacteria where as significant activity was not observed with hexane extract. As shown in Table 2 the extracts from *Ocimum tenuiflorum* displayed antibacterial activity against all of the tested gram positive and gram negative bacterial with the diameters of zone inhibition ranging between 8mm and 12mm. The most active acetone extract was that obtained *Ocimum tenuiflorum* leaves inhibited the growth of all the bacterial strains tested, specifically *Enterococcus faecalis, Serratia marcescens, Salmonella paratyphi A,* and *Salmonella paratyphi B* respectively.

All the three extracts showed activity with the fungal organisms. Further more, the fungi studied *Alternaria alternata* and *Candida albicans* was susceptible to all extracts. In contrast, the inhibition zone of solvent control (negative control) was almost zero. So that it was noted active against all of the tested microorganisms. However, three antibiotics (30µg/ml) of Ampicillin, Erythromycin and Kanamycin, were more effective than any of the extracts from the diameters of zone inhibition, ranging between 12 and 28mm.

This may indicate that the *Ocimum tenuiflorum* leaves extracts have broad inhibitory activity to pathogenic microorganisms and are promising to act as potential antibacterial and antifungal agents from natural plant sources. It is not surprising that these are differences in the antimicrobial activities of plant groups, due to the phytochemical differences. Negative results do not indicate the absence of bioactive constituents, nor is that the plant inactive.

Table 2. Antibacterial activity of *Ocimum tenuiflorum*

S. No	Microorganisms	Ampicillin	Hexane	Acetone	Ethanol
1	*Streptococcus faecalis*	16	-	10	09
2	*Streptococcus pyogenes*	15	-	10	08
3	*Enterococcus faecalis*	25	-	12	10
4	*Bacillus subtilis*	23	-	11	08
5	*Bacillus thuringiensis*	22	-	11	08
6	*Staphylococcus aureus*	25	-	10	10
7	*Serratia marcescens*	20	-	12	10
8	*Klebsiella pneumoniae*	23	-	10	10
9	*Proteus vulgaris*	25	-	08	10
10	*Proteus mirabilis*	25	-	10	10
11	*Salmonella paratyphi*	28	-	11	12
12	*Salmonella paratyphi. A*	25	-	12	10
13	*Salmonella paratyphi. B*	20	-	12	08
14	*Pseudomonas aeruginosa*	23	-	10	10
15	*Escherichia coli*	25	-	10	10

Table .3 Antifungal activity of *Ocimum tenuiflorum*

S. No	Microorganisms	Kanamycin	Erythromycin	Hexane	Acetone	Ethanol
1	*Rhizoctonia solani*	15	18	-	08	10
2	*Alternaria alternata*	13	25	-	15	13
3	*Curvularia lunata*	15	13	-	12	10
4	*Fusariumoxysporium*	12	13	-	12	10
5	*Aspergillus flavus*	12	13	-	12	10
6	*Candida albicans*	13	14	-	15	10

DISCUSSION

Recently the attention has been directed towards extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in convering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Shadomy *et al*., 1985; Odds,1989).

In general agreement with previously published qualitative analysis of Lamiaceae species (Shan *et al*., 2005), the phytochemical screening of the phytoconstituents of the plant studied showed that leaves were found to have flavonoids, saponins, cardiac glycosides, alkaloids, phenols and tannins. They were known to show medicinal activities well as exhibiting physiological activity (Sofowara, 1993). This is also in agreement with Javanmardi and his co others, who reported that rosmarinic acid is the most abundant component in *O. basilicum* (Javanmardi *et al*., 2002). *Ocimum* species has been extensively reported for its essential oil content (Roberto *et al*., 2003). Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants due to plant adaptation in response to biotic and abiotic stresses (Infection, water stress, cold stress, high visible light) (Pitchersky and Gang, 2001). So far, in the *Ocimum* species the *O. basilicum* and *O. sanctum* leaves have been reported for their secondary metabolite content (Javanmardi *et al*., 2002; Hakkim *et al*., 2007).

Among the three extracts used, acetone extract showed almost equal inhibition towards both the Gram-positive and Gram negative bacteria. The presence of these phytochemicals in

the investigated medicinal plant would be responsible for the antimicrobial activity of the extracts. The literature indicates that the antibacterial activity is due to different chemical agents present in the extract, including essential oils (especially thymol), flavonoids and triterpenoids and other natural phenolic compounds or free hydroxyl groups. These are classified as active antimicrobial compounds (Hasan *et al*., 1994). This is in conformity to Mahato and Chaudary (2005). The alcoholic extract of *Argyria nervosa* was found to be effective against *S. aureus* which is in similarity with the earlier observation of Chansakaow *et al.,* (2003) and Parekh and Chanda (2007).

Acetone extract showed higher antifungal activity in *Candida albicans* and *Alternaria alternata*. There are many reports available that plants have been evaluated *in vitro* for their antibacterial potency against some important human pathogenic fungi (Hiremath *et al.,* 1993; Srivastava and Lal, 1997; Adelakun *et al.*, 2001; Verma and Dohroo, 2003; Singh and Singh, 2005; Patni *et al.,* 2005; Sermakkani *et al*., 2010). Higher plants are rich in active principles, which are used as therapeutic drugs (Evans *et al*., 1986).

The presence of antifungal compounds, in higher plants, has long been recognised as an important factor in disease resistance. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Siva *et al*., 2008). According to Nelson and Onyeagba (2007), the extracts of higher plants have been proved a very good source of antibiotics against various bacterial agents. Plant extracts are quite efficient in the control of several diseases caused by microorganisms.

The result of the antimicrobial assay show promising evidence for the antibacterial and antifungal effect of leaves of *Ocimum tenuiflorum*. From the above evidence, it is clear that plant extracts have great potential as antimicrobial compounds against bacterial and fungal pathogens and they can be used in the treatment of infectious diseases. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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