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Analysis of the Phytochemical Contents and Anti-microbial Activity of *Ocimum basilicum* L.

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ABSTRACT

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and large number of diverse types of plants grows in different parts of the country. In this study the *in vitro* antimicrobial activity of crude ethanolic, methanolic and water extracts of the stem bark of *O.basilicum* were investigated. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 5 to 12, 8 to 20 and 0 to 8 mm for ethanol, methanol and water extracts, respectively. The minimum inhibitory concentration (MIC) of the ethanol extract was between 0.5 and 6.25 mg/ml while that of methanol extract ranged from 0.5 to 10 mg/ml. The minimum bactericidal concentration (MBC) for ethanol extract ranged between 2.0 and 12.50 mg/ml, while this value for methanol ranged from 2.0 to 20 mg/ml. All the extracts exhibited appreciable activity against *Candida albicans*. The zones of inhibition exhibited by the extracts against *C. albicans* ranged between 15 and 18, 15 and 20 and 5 and 10 mm for ethanol, methanol and water extracts, respectively. Primary phytochemical screening revealed the presence of saponin, steroids, tannins, glycosides, alkaloids and flavonoids in the extracts. The ability of the crude stem extracts of *O. basilicum* to inhibit the growth of bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections. It is also concluded that *O. basilicum* stem bark could be a potential source of active antimicrobial agents, and future works will be concentrated on the *in vivo* potencies and toxicological profiles.

1. Introduction

Plants are the richest resource of drugs of traditional, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500 - 1600 B.C. and is supposed to be the

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oldest repository of human knowledge (Sharfkandy, 1367). It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi and Mehrotra, 2002). Medicinal plants are a source of great economic value all over the world. Nature has given us a very rich botanical wealth and large number of diverse types of plants grows in different parts of the country. About 1500 plants like Ayurveda, Unani and Siddha are systematically used in indigenous system of medicine. However, the ethnopharmacologists, botanists, microbiologists and natural-product chemists are trying to find the medicinal efficacy of plants and their phytochemical compositions.

The drugs which are already in use to treat infectious diseases are of concern as drug safety remains an enormous global issue. It is estimated that 2.22 million hospitalized patients had serious adverse drug reactions (ADR) and 106,000 died in one year in the USA. This herbal and natural product have been used in traditional medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals. For this reason and their lower cost it is recommended to research more on the traditional medicine as an alternative to synthetic drugs (Nair et al., 2005). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena and Sharma, 1999). The World Health Organization (WHO) estimated that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (World Health Organization 1993).

Iranian people have a tradition of using a number of plant species for the treatment of infectious diseases (Omidbeigi, 2000). The genus *Ocimum* L. (Lamiaceae) is an important group of aromatic and medicinal flora which yield many essential oils and aroma chemicals and has been used in the perfumery and cosmetic industries as well as in indigenous systems of medicine. Basil (*Ocimum basilicum* L.), a member of the Lamiaceae family, has been used both as a culinary and ornamental herb. The genus *Ocimum* contains 50 to 150 species of herbs and shrubs found in the tropical regions of Asia, Africa, and central and South America. Traditionally basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions. Basil can also be used as an ointment for insect bites, and its oil is applied directly to the skin to treat acne. Natural components from basil have long been used to flavor foods. In Iran, basils are being used to treat fevers, throat congestions, and stomachache.

Rosmarinic acid (RA) (R-O-caffeoyl-3-4-dihydroxyphenyllactic acid) is one of the most abundant caffeic acid esters present in *Ocimum* spp. (Ozcan and Chalchat, 2002; Adiguzel et al., 2005). RA and its derivatives have been reported to have antioxidant, anti-HIV, and anti-inflammatory or cyclooxygenase inhibitory activity, comparable to ibuprofen, naproxen, and aspirin (Ntezurubanza, 1984; Keita, 2000). Similar to RA, lithospermic acid B (LAB) is known to be a common phenolic constituent in most members of the Lamiaceae family and exhibits endothelium-dependent vasodilator and hypotensive effects. Among the various *Ocimum* species, *O. basilicum* L. (sweet basil) is commercially and extensively cultivated for essential oil production. Earlier studies reveal that the essential oils of these species have been the subject of several studies with different chemical composition and antimicrobial activities (Yayi, 2001; Tada, 1996; Purkayastha and Nath, 2006; Almeida, 2007; Matasyoh, 2008; Ahonkhai, 2009; Koba, 2009). The essential oil composition of these three *Ocimum* species were assigned some highly valuable compounds including methyl chavicol, linalool, eugenol, thymol, methyl eugenol and camphor (Pino et al., 2009; Akujobi, 2010). In this study we investigated the phytochemical screening and antibacterial activity of *Ocimum basilicum* stem bark extracts.

2. Materials and methods

2.1. Plants and preparation of the extract

Fresh stem bark of Basil was collected from a local farm in Lahijan City, Guilan Province, Iran in August, 2009 and was identified by the Botany Department of Islamic Azad University of Lahijan (IAUL), Iran. The fresh stem bark was air-dried to constant weight, pulverized in a mill (Pye Unicam, Cambridge, England) and stored in an air-tight container for further use. 250 g of the pulverized

plant material was cold extracted in ethanol and methanol separately. Another 250 g of plant material was extracted in water for 4 days with occasional shaking. The separated extracts were then filtered through Whatman's No. 1 filter paper and ethanol and methanol filtrate were separately concentrated for dry in vacuo using a rotary evaporator to remove the ethanol and methanol. The aqueous extract was lyophilized to obtain a dry powder extract.

2.2. The microorganisms

The test microorganisms used in this study including *Staphylococcus aureus*, *Staphylococcus epidermitidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus faecalis*, *Shigella dysenteriae*, *Klebsiella pneumonia*, *Bacillus cereus*, *Bacillus subtilis*, *Proteus vulgaris*, *Serratia marcescens* and *Candida albicans* were obtained from the culture collections of the Faculty of basic science in IAUL. The bacterial isolates were first subcultured in a nutrient broth (Oxoid) and incubated at 37°C for 18 hours while *C. albicans* isolates were subcultured on a Sabouraud dextrose agar (SDA) (Oxoid) for 72 hours at 25°C.

2.3. Phytochemical analysis of the plant extracts

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, steroid, alkaloids and glycolsides in accordance with Trease and Evans (Trease and Evans, 1989) and Harborne (Harborne, 1998) with modification.

2.4. Antibacterial activity

The antibacterial activity of the crude extracts was determined in accordance to the agar-well diffusion method described by Irobi and colleagues (Irobi et al., 1994). The bacterial isolates were first grown in a nutrient broth for 18 hours before use and standardized to 0.5 McFarland standards (10^6 cfu/ml). Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar (Oxoid). The wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µl of the crude extract at 10 mg/ml were introduced into the wells, allowed to stand at room temperature for about 2 hours and then incubated at 37°C. Controls were set up in parallel using the solvents that were used to reconstitute the extracts. The plates were observed for zones of inhibition after 24 hours. The results were compared with those of streptomycin and ampicillin at a concentration of 1 mg/ml and 10 µg/ml, respectively.

2.5. Antifungal activity

The fungal isolate were allowed to grow on a Sabouraud dextrose agar at 25°C. The harvested fungal and bacterial isolates were standardized to an OD600 nm of 0.1 before use. One hundred microliter of the standardized fungal suspension was evenly spread on the SDA (Oxoid) using a glass spreader. The wells were then bored into the agar media using a sterile 6 mm cork borer and the wells filled with the extracts solutions carefully not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour for proper diffusion of the extract into the media. Plates were incubated at 25°C for 96 hours and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with amphothericin B and miconazole at a concentration of 1 mg/ml.

2.6. Minimum Inhibitory Concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of Akinpelu and Kolawole (Akinpelu and Kolawole, 2004). Two-fold dilutions of the crude extract was prepared and 2 ml aliquots of different concentrations of the solution were added to 18 ml of pre-sterilized molten nutrient agar and SDA for bacteria and yeast, respectively, at 40°C to give final concentration regimes of 0.05 and 10 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar flow before streaking with 18 hours old bacterial and fungal cultures. The plates were then incubated at 37°C for 24 hours and at 25°C for up to 72 hours for bacteria and fungi, respectively. After that they were examined for the presence of the growth. The MIC was taken as the lowest concentration that prevented the growth of the microorganism.

2.7. Minimum bactericidal concentration (MBC)

The MBC of the plant extracts was determined by a modification of the method of Spencer and Spencer (Spencer and Spencer, 2004). Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared nutrient agar and SDA plates, and later incubated at 37°C for 48 hours and 25°C for 72 hours for bacteria and fungi, respectively. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates. The MBC was not determined for water extract since the antibacterial activity was low.

3. Results

All three extracts of the plant tested showed varying degree of antibacterial activities against the bacterial species (Table 1). The antibacterial activities of the ethanol and methanol extracts compared favorably with that of two standard antibiotics (streptomycin and ampicillin) and have appeared to be broad spectrum as its activities were independent on gram reaction. The inhibition zone for *Klebsiella pneumonia* was less (0 - 8 mm) as compared to other bacteria. The methanol extracts (inhibition zone 8 - 20 mm) were found to be more effective than the ethanol extracts (inhibition zone 5 – 12 mm) against all the organisms. The water extract showed low antibacterial activity with inhibition zones ranging between 0 and 8 mm for different bacteria. The minimum inhibitory concentration (MIC) of the ethanol extract for different organisms ranged between 0.5 and 6.25 mg/ml, while that of the methanol extract ranged between 0.5 and 10 mg/ml. The MIC of streptomycin ranged between 0.065 and 0.5 mg/ml (Table 2). The minimum bactericidal activity (MBC) of the extract for different bacteria ranged between 2.0 and 12.50 mg/ml for the ethanol extract while this amount for the methanol extract ranged between 2.0 and 20 mg/ml (Table 2). Water extract was not active against any of the organism at 10 mg/ml which was the highest tested concentrations. In general, the methanol extract was more active than other extracts. This may be attributed to the presence of soluble phenolic and polyphenolic compounds. The inhibitory effect of the extract of *O. basilicum* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development and the treatment of ailments caused by these pathogens. The lack of activity of the water extract against most bacterial strains investigated in this study is in agreement with previous works which show that aqueous extracts of plant generally has little or no antibacterial activities. This is similar to the findings of Obi and Onuoha (Obi and Onuoha, 2000), who reported that alcohol is the best solvent for the extraction of most plants of medical importance.

3.1. Antifungal activity

The three extracts showed broad antimycotic activity against the tested fungal isolates at a concentration of 10 mg/ml (Table 3) and the performance of the three extracts were similar to the antibacterial activity. The susceptibility of these fungi to *O. basilicum* extracts is significant, as most of these fungi have recently been implicated in cases of immuno-compromised patients who frequently develop opportunistic infections (Portillo, 2001). In general the methanol extract had the highest activity against both bacterial and fungal isolates. This was followed by the ethanol extract and the least was observed in the water extract. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of *O. basilicum*, which makes the plant a candidate for bioprospecting for antibiotic and antifungal drugs.

3.2. Phytochemical screening

Investigations on the phytochemical screening of *O. basilicum* stem bark extracts revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids (Table 3). These compounds are known to be biologically active and therefore aid the antimicrobial activities of *O. basilicum*. Some researches have also attributed to their observed antimicrobial effect of plant extracts to the presence of these secondary plant metabolites (Nweze, 2004)

Table1. Screening of primary antimicrobial activity of *Ocimum basilicum* L. Stem bark extracts on selected microbial isolates (mm)

Microorganism	Miconazole mg/ml	AMP µg/ml	ST (mg/ml)	Water	Methanol	Ethanol	Amphotericin (mg/ml)
S. aureus	–	0	20	5	20	10	–
P.aeruginosa	–	0	20	4	16	12	–
E.coli	–	15	0	7	14	11	–
S.faecalis	–	28	R	R	R	R	–
S.epidermidis	–	0	22	2	15	11	–
S.dysenteriae	–	0	25	4	16	12	–
K.pneumonia	–	0	0	0	8	5	–
B.cereus	–	0	20	5	16	10	–
B.subtilis	–	0	22	5	14	12	–
P.vulgaris	–	22	18	7	14	14	–
S.marcescens	–	15	25	3	15	11	–
C.albicans	30	–	–	10	6	3	28

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration of *Ocimum basilicum* L. Stem bark extracts (mg/ml)

Extracts	Ethanol		Methanol		Water		ST
Microorganism	MIC	MBC	MIC	MBC	MIC	MBC	MIC
S.aureus	5	10	5	10	Na		0.065
P.aeruginosa	5	10	5	10	Na		0.5
E.coli	5	10	5	10	Na		Nd
S.faecalis	2.5	5	5	10	Na		0.065
S.epidermidis	0.5	2	0,5	2	Na		0.065
S.dysenteriae	5	10	10	20	Na		0.25
K.pneumonia	5	10	10	20	Na		Nd
B.cereus	0.5	2	10	20	Na		0.065
B.subtilis	5	10	6.25	12	Na		0.065
P.vulgaris	5	10	5	10	Na		0.25
S.marcescens	6.25	12.5	10	20	Na		0.25

ST- Streptomycin; MIC- minimum inhibitory concentration; MBC- minimum bactericidal concentration; Nd- not determined; Na- not active at 10 mg/ml, the highest concentration.

Table 3. Preliminary phytochemical analysis of *Ocimum basilicum* L. stem bark extracts

Solvents / Phytoconstituents	Ethanol	Methanol	Water
Carbohydrate	+	+	++
Steroids	++	++	+
Tannins	+	+	+
Alkaloids	-	+	-
Saponins	-	+	-
Cardiac Glycosides	-	+	++
Terpenoids	+	++	+
Flavonoids	+	++	+

4. Discussion

The secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with proline rich proteins (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (Parekh and Chanda, 2007) reported that tannins react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treatment of intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). These observations therefore support the use of *O. basilicum* in herbal cure remedies. Li and Wang reviewed the bio-logical activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *O. basilicum* has potential as a source of important bioactive molecules for the treatment and prevention of cancer (Li and Wang, 2003). The presence of tannins in Basil supports the traditional medicinal use of this plant in the treatment of different ailments. Another secondary metabolite compound observed in the stem bark extract of Basil was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori, 1994). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Quinlan and colleagues worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates (Quinlan et al, 2000). Neumann and colleagues also confirmed the antiviral property of steroids (Neumann et al., 2004). Flavonoids which is another constituent of Basil stem bark extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002). Just and colleagues revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in Basil extracts and has supported the usefulness of this plant in managing inflammation. Steroidal compounds present in O. basilicum extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Just et al., 1998). Although we did not observed any considerable activity of the different extracts against C. albicans but the results of this study shows that the extracts have antimicrobial activities. The results of this study will contribute to knowledge about the Phytochemistry of Basil extracts in general, and will shed some light on antimicrobial activity in particular as a source of natural products with potential use in the pharmaceutical industry. It is concluded that *O. basilicum* stem bark could be a potential source of active antimicrobial agents, and future works will be concentrated on the *in vivo* potencies and toxicological profiles.

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