

Full Length Research Paper

***In vitro* antibacterial and antifungal effect of some medicinal plants**

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The aim of this study was to evaluate the antimicrobial activity of five species of plants known as medicinal plants and used commonly in folkloric medicine. They are: *Nigella sativa*, *Foeniculum vulgare*, *Black piper*, *Loranthus capitellatus*, *Cassia alata* and *Drynaria qurshiforia*. In this study, we investigated the antibacterial and antifungal effects of these plants on Gram- positive and negative bacterial isolates, such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* sp. and fungi isolates, such as *Candida albicans*, *Candida tropicalis*, *Candida glabrata*; *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Alternaria* sp., *Trichophyton mentagrophytes* and *Microsporum gypseum*. The antimicrobial activity of the plants extracts against isolated pathogenic bacteria and fungi was assayed by well-diffusion method for bacteria and *Candida* and pure plate method for dermatophytes. The minimum inhibitory concentrations (MIC) for bacteria and fungi were 0.625 to 40 mg ml⁻¹ and 2.5 to 40 mg ml⁻¹, respectively. The plant extracts were revealed to be effective in inhibiting the growth of tested bacteria and fungi.

Key words: Antibacterial, antifungal activity, medicinal plants.

INTRODUCTION

Nowadays, due to the misuse of antibiotics, antimicrobial properties of medicinal plants have been evaluated for obtaining safe drugs. Hence, in this study we used five species of plants for medicinal purposes. *Nigella sativa* Linn (family, Ranunculaceae) is commonly known as black cumin or black seed. Its seed or oil is used as carminative, diuretic, lactagogue and vermifuge (Akgul, 1989; Ali and Blunden, 2003). The dried seeds of black cumin are also spread on bread or used for flavoring foods such as cheese and bakery crops (Ustum et al., 1990; Takruri and Daneh, 1998). The antioxidant, antibacterial and antifungal activities of spices have been studied (De et al., 1999; Sagdic et al., 2002, 2003). The antibacterial (Akgul, 1989; Hanafy and Hatem, 1991; Farrag et al., 2000), antifungal (Akgul, 1989; Khan et al.,

2003) and antioxidant (Burits and Bucar, 2000) properties of black seed have been reported. *Foeniculum vulgare* Mill. (Apiaceae family), commonly known as fennel, with aromatic odor is native to Southern Europe and Mediterranean region. Its aromatic fruits are used as a culinary spice and for home-made cures in many countries (Tanira et al., 1996). The chemical compositions of the fennel include fatty acid, phenylpropanoids, monoterpenoids, sesquiterpenes, coumarins, triterpenoids, tannins, flavonoids, cardiac glycosides and saponins (Weiping and Baokang, 2011). Essential oils from the fruits revealed significant antibacterial activity against *Escherichia coli* and *Bacillus megaterium* (Araque et al., 2007; El-Adly et al., 2007). The chloroform extracts of *F. vulgare* showed antimicro-

bial activity against bacteria and fungi. Dillapional, scopoletin, dillapiol, bergapten, imperatorin and psolaren revealed antimicrobial activity of fennel against *B. subtilis*, *A. niger* and *Cladosporium cladosporioides* (Kwon et al., 2002). Essential oils of *F. vulgare* exhibit antifungal activities against *C. cladosporioides*, *Penicillium helianthi* and *Trichophyton mentagrophytes*, compared with a standard mycotoxic bifonazol (Mimica-Duki et al., 2003; Singh et al., 2006).

Dichloromethane extracts and essential oils from *F. vulgare* revealed antifungal activity against *Candida albicans*. They could be responsible for a new antifungal factor such as candidiasis and other mycotic diseases (Park and Seong, 2010). *Cassia alata* Linn. (Caesalpinaceae) it contains chrysophenol, rhein (1.8 dihydroxy anthraquinone-3 carboxylic acid), glucose and rhamnase sugar (Hauptmann et al., 1950). The biological activity of the alcoholic extracts of *C. alata* leaves (Bungi and Plana, 1960) has been reported to have fungistatic activity against some mycotic organisms such as *T. mentagrophytes*, *Microsporum gypseum* and *Epidermophyton floccosum*. Shah et al. (1968) reported that the efficacy of some *Cassia* species in skin disease might be attributed to their anthraquinone derivatives. *Drynaria quercifolia* (L.) J. Smith (Polypodiaceae family) is found to be growing in rain forest of Western Ghats of Maharashtra, India. The rhizome paste is applied for the treatment of diarrhoea, typhoid, cholera, chronic jaundice, fever, headache and skin diseases (Viswanathan, 1987). From the methanolic extracts of *D. quercifolia*, dried rhizomes isolated include epifriedelinol, beta-amyrin, beta-sitosterol, beta-sitosterol 3-beta-D-glucopyranoside, naringin, comarin and triterpene. *Loranthus capitellatus* Wall. (Loranthaceae) is found to be growing in high rainfall locality. No literature is available on its chemical constituents and antidermatophyte activity. But alkaloids were reported from other species, that is *Loranthus micranthus*, and these chemicals may be responsible for their antimicrobial activity (Osadebe et al., 2004). *Black piper* (Piperaceae family): the pungent and aromatic fruits of some species of *Piper* are used as spices and most of them are used traditionally as insecticides (Yang et al., 2002), antivirals (Lohezic et al., 2002), antimicrobials (Pessini et al., 2003) and antifungal (Ngoni et al., 2003). These biological properties have been attributed to the presence of lignans and/or amides, such as alkyl or olefinic isobutylamides (Freixa et al., 2001), flavonoids, kawa-lactones, butenolides and cyclohexane epoxides, (Sengupta, 1987).

MATERIALS AND METHODS

Collection and preparation of plant materials

The plant materials (*Loranthus capitellatus*, *Cassia alata* and *Drynaria quercifolia*) were collected from various places of Western

Ghats regions of Maharashtra State, India and authenticated by Botanical Survey of India. Also, *N. sativa* and *F. vulgare* were purchased from the local market in Iran. The healthy plants' parts were separated and dried in shade in order to avoid the decomposition of their chemical constituents. These were powdered in grinder and stored in clean and dry airtight containers for future studies.

Ethanolic extracts

Aliquots (10 g) of each dry powdered selected plants were soaked in 80% ethanol, plugged with cotton and kept on a rotary shaker for 3 × 24 h at room temperature. The extracts were filtered using Whatman filter paper no.1, and the crude ethanol extracts were evaporated to dryness in room temperature. Dried extracts were stored in labeled sterile screw capped bottles at -20°C for next work.

Microorganisms and inoculum preparation

The microorganisms used include *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* sp. and fungi isolates include *C. albicans*, *Candida tropicalis*, *Candida glabrata*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Alternaria* sp., *T. mentagrophytes* and *M. gypseum*. All microorganisms were clinical isolates, obtained from the Medical Microbiology and Mycology laboratories at the Department of Medical Mycology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Stock fungi were kept on Sabouraud dextrose agar (SDA; Hi Media- India Merck, 5438) and the bacteria were kept on Muller-Hinton agar (MHA; Hi Media-India) at 4°C. A loop of cells from the stock cultures was removed to the test tubes of Mueller-Hinton broth (MHB) for bacteria, and Sabouraud dextrose broth (SDB) for fungi; and were incubated overnight at 37 and 25°C, respectively. Subsequently, the cultures were diluted in sterile distilled water to obtain 10⁵ spore/ml for fungal strains and 10⁶ colony forming units (CFU/ml) for bacteria strain standardized with the turbidity of 0.5 McFarland (Wright, 1983).

Screening for antimicrobial activities

Aliquots (1000 mg) of dried plant extracts were dissolved in 2.5 ml 100% dimethyl sulfoxide (DMSO) and the final concentration of each extract was adjusted to 400 mg ml⁻¹. The serial twofold dilutions of plant extracts were prepared in a concentration range of 40 to 0.156 mg/ml. The minimum inhibitory concentrations (MIC) of the ethanolic extracts of selected plant species were assayed using agar well diffusion method (Perez et al., 1990). 0.1 ml of diluted inoculum (10⁵ and 10⁶ CFU: ml) of test organism was used on Mueller-Hinton agar for bacteria and Sabouraud dextrose agar Petri plates media for fungi; and was spread on plates by using sterile spreader in Petri plates media uniformly. Subsequently, the wells of 7 mm diameter were punched onto SDA and MHA agar medium by using a sterile borer. Each well was filled with 0.1 ml of serial dilutions of test plant extracts. Sterile DMSO used as negative control and standard antibiotics such as penicillin (10 IU) for *S. aureus*; gentamicin (10 IU) for *E. coli*; *Klebsiella* sp., *P. aeruginosa*, clotrimazole (10 µg/disc) for *Candida* sp.; amphotericin B (0.4 mg/disc) for saprophytic fungi were used as positive controls. The plates were kept 1 h for diffusion and then incubated at 37°C for 18 to 24 h for bacteria, 24 to 48 h for yeast and 25°C for 5 to 15 days (up to 15 days for dermatophyte strains). Inhibition zones make around the well were measured in millimeter. Each assay was

Table 1. The minimum inhibitory concentration (^aMIC) mg m⁻¹ of selected medicinal plants.

Plant parts used for extract	Ca. al.	Ca. Ca tr. gl.	Sta. aru.	Esh. col.	Psu. are.	Kel. sp.	As. na.	As. flv.	As. te.	Al. sp.	T. M. men. gyp.
<i>Black piper</i> seed	10	20 20	0.625	N.I	N.I	20	40	20	N.I	N.T	N.I 2.5
<i>Nigella sativa</i> seed	40	20 10	10	N.I	40	N.I	N.I	N.I	N.I	N.T	N.I N.I
<i>Foeniculum vulgare</i> fruit	10	20 20	2.5	N.I	20	NI	40	N.I	40	N.T	10 N.I
<i>Loranthus capitellatus</i> leaf	10	20 40	N.T	N.T	N.T	N.T	N.I	20	20	10	10 10
<i>Cassia alata</i> leaf	N.I	N.I N.I	N.T	N.T	N.T	N.T	N.I	20	20	N.I	20 10
<i>Drynaria qurshiforia</i> rhizome	N.I	N.I N.I	N.T	N.T	N.T	N.T	N.I	10	20	5.0	N.I 40

^aValuse are the mean of three replicates; N.I: No inhibition was absorbed ; N.T: not tested; Abbrevatins: *Sta. aru.*: *Staphylococcus aureus* ; *Esh. Col.*: *Scherichia coli*; *Psu. Are.*: *Pseudomonas aeruginosa*; *Kel. sp.*: *Klebsiella* sp., *Ca. al.*: *Candida albicans*; *Ca. tr.*: *Candida tropicalis*; *Ca. gl.*: *Candida glabrata*; *As. na.*: *Aspergillus niger*; *As. flv.*: *Aspergillus flavus*; *As. te.*: *Aspergillus terrus*; *Al. sp.*: *Alternaria* sp., *T. men.*: *Trichophyton mentagrophytes*; *M. gyp.*: *Microsporium gypseum*.

Table 2. The inhibition zone (mm)^b of selected medicinal plants and standard antibiotics.

Plant parts used for extract	Ca. al.	Ca. Ca tr. gl.	Sta. aru.	Esh. col.	Psu. are.	Kel. sp.	As. na.	As. flv.	As. te.	Al. sp.	T. M. men. gyp.
<i>Black piper</i> seed	16	18 10	12	N.I	N.I	7.0	20	17	N.I	N.T	N.I 2.5
<i>Nigella sativa</i> seed	15	13 24	10	N.I	10	N.I	N.I	N.I	N.I	N.T	N.I N.I
<i>Foeniculum vulgare</i> fruit	18	17 15	12	N.I	15	NI	15	N.I	20	N.T	10 10
<i>Loranthus capitellatus</i> leaf	13	12 15	N.T	N.T	N.T	N.T	N.I	12	12	12	10 10
<i>Cassia alata</i> leaf	N.I	N.I N.I	N.T	N.T	N.T	N.T	N.I	11	10	N.I	20 10
<i>Drynaria qurshiforia</i> rhizome	N.I	N.I N.I	N.T	N.T	N.T	N.T	N.I	12	15	7.0	N.I 40
Positive control											
Clotrimazole 20 mm											
Gentamicin 22 mm											
Penicillin 15 mm											
Amphotericin B 17 mm											

^bValuse are the mean of three replicates; N.I: No inhibition was absorbed ; N.T: not tested; Abbrevatins: *Sta. aru.*: *Staphylococcus aureus* ; *Esh. Col.*: *Scherichia coli*; *Psu. Are.*: *Pseudomonas aeruginosa*; *Kel. sp.*: *Klebsiella* sp., *Ca. al.*: *Candida albicans*; *Ca. tr.*: *Candida tropicalis*; *Ca. gl.*: *Candida glabrata*; *As. na.*: *Aspergillus niger*; *As. flv.*: *Aspergillus flavus*; *As. te.*: *Aspergillus terrus*; *Al. sp.*: *Alternaria* sp. *T. men.*: *Trichophyton mentagrophytes*; *M. gyp.*: *Microsporium gypseum*. penicillin (10 IU), gentamicin (10 IU), amphotricin B (0.4mg/ml) and clotrimazole (10 µg) are used as positive control.

repeated three times.

RESULTS AND DISCUSSION

The results of the antimicrobial screening of selected plant extracts are shown in Tables 1 and 2. Alcohol solvent was used for the extraction of selected plants, since alcohol was detected to be a better solvent for the extraction of antimicrobial activity compared to water and other solvents (Ahmad et al., 1998). The most active plant was *Black piper*, whereas the least active plant was *cassia alata* (Tables 1 and 2). In this study, *Cassia alata*

and *Drynaria qurshifori* were inactive against *Candida* species whereas, *Black piper*, *F. vulgare* and *Loranthus capitellatus* were active plant extracts against this yeast with MIC at the concentration of 10 to 40 mg ml⁻¹. *Black piper*, *N. sativa* and *F. vulgare* were most active against Gram-positive bacteria (MIC = 0.62 to 10 mg ml⁻¹) than Gram-negative (MIC = 10 to 20 mg ml⁻¹) bacteria. This is in agreement with previous reports by several workers (Buwa and van Staden, 2006; Parekh et al., 2005). The ethanolic extract of *B. piper* was most active against *S. aureus*, as the minimum inhibitory concentration was 0.625 mg ml⁻¹ while the minimum inhibitory concentration for

Klebsiella sp. was 20 mg ml⁻¹. Also, *Syzygium cumini* showed good activity against *S. aureus* according to Rajakaruna et al. (2002)'s report. Review literature reported leaves, flowers, root and stem barks of *Cassia alata* revealed activity against several bacteria and protozoa (Khan et al., 2001). In the present study, the leaves of *Cassia alata* revealed low activity against filamentous fungi such as *A. terreus*, *A. flavus*, *T. mentagrophytes* and *M. gypseum* and it was inactive against *Aspergillus niger* and *Alternaria* sp. This is according to previous reports by a review of literature (Radhakrishnan et al., 1976) that showed *C. alata* leaf extract was active against *T. mentagrophytes*, *M. gypseum*.

According to previous studies, it is also observed that *C. albicans* was inhibited by both the ethanol and water extracts of bark of *C. alata* in a concentration of 15-30 µg/µl, but *C. albicans* was resistant to both the ethanol and water extracts of leaves of *C. alata* (Somchit and Mutalib, 2003). Neither water nor ethanol extracts of the bark and leaf of *C. alata* inhibited the growth of *A. fumigatus* and *M. canis*. In contrast, Ibrahim and Osman (1995) reported that *C. alata* leaf from Malaysia at 500 mgml⁻¹ concentration of ethanolic extract showed antifungal activities against fungi especially *Trichophyton* sp., *Microsporum* sp., *Aspergillus* sp. and *Penicillium* sp. but was inactive against yeast sp. such as *C. albicans* and *C. neoformans*. Khan et al. (2001) reported that the methanolic extracts of leaf, flower, bark and root of *C. alata* at a concentration of 4 mgml⁻¹ inhibited bacterial sp. such as *E. coli* and *S. aureus* but not active in the fungi including *C. albicans*, *A. niger* and *T. mentagrophytes*.

D. qurshifolia was highly sensitivity against *Alternaria* sp. with MIC in concentration of 5.0 mg ml⁻¹. Since the ethyle acetate extract of *D. quercifolia* rhizome contained triterpene and coumarin soluble in semipolar Di-ethyl ether solvent, it could be that these compounds are responsible for antidermatophytic activity of this plant. Also, Ramesh et al. (2001) reported that the methanolic extract of *Drynaria quercifolia* rhizome showed inhibitory activity by the agar-well diffusion method against all tested bacteria such as *Klebsiella pneumoniae*, *Salmonella typhi*, *Vibrio cholerae*, *S. aureus* and *Bacillus subtilis* but did not show inhibitory activity against *Aspergillus flavus*, *A. niger* and *Candida albicans*.

In the present study, the ethanolic extract of *D. quercifolia* rhizome was active against *A. flavus*, *A. terreus* and *Alternaria* sp. while it was inactive against *A. niger*, *C. glabrata*, *C. albicans* and *C. tropicalis*. In the current study, the ethanolic extracts of *F. vulgare* (fennel) exhibited good antimicrobial activity against bacteria and fungi including *S. aureus*, *A. flavus*, *A. terreus* and *Alternaria* sp. while it was inactive against *A. niger*, *A. flavus*, *A. terreus*, *Alternaria* sp. *C. glabrata*, *C. albicans* and *C. tropicalis*. Kwon et al. (2002) reported that the chloroform extracts of the stems of *F. vulgare* (fennel)

revealed antimicrobial activity against *B. subtilis* and Weiping and Baokang (2011) reported that dichloromethane extracts and essential oils from *F. vulgare* exhibited antifungal activity against *C. albicans*. They could be used as new antifungal drug for candidiasis and other fungal diseases (Park and Seong, 2010). Weiping and Baokang (2011) reported the chemical compositions of the fennel including coumarins, triterpenoids, tannins, flavonoids, cardiac glycosides and saponins.

In previous studies, it was reported that chemical compositions such as triterpenes and coumarins are effective against pathogenic fungi (Cowan, 1999). Hence, coumarins and triterpenoids in the fennel might be responsible for the antifungal activity of this plant which gave a positive test results by *in-vitro* assay. In the present study the ethanol extract of *N. sativa* seeds inhibited the growth of *C. glabrata*, *C. albicans*, *C. tropicalis* and the bacteria such as *S. aureus*, *P. aeruginosa* while was inactive against saprophytic fungi and dermatophytes. Farrag et al. (2000) reported that the oil of black cummin showed inhibitory effect against gram positive such as *S. aureus* and *B. cereus* and Gram negative bacteria. Ozcan (1998), De et al. (1999) and Khan et al. (2003) reported that the extract of *N. sativa* seeds had antifungal activity against *A. parasiticus*, *C. albicans* and *S. cerevisiae*, respectively. Also, the ethanolic extract of *F. vulgare* (fennel) exhibited good antimicrobial activity against bacteria and fungi including *S. aureus*, *flavus*, *A. terreus* and *Alternaria* sp. while it was inactive against *A. niger*, *A. flavus*, *A. terreus*, *Alternaria* sp. *C. glabrata*, *C. albicans* and *C. tropicalis*. In conclusion, appearance of multi-drug resistance in pathogenic bacteria and unpleasant side effects of these antibiotics led to the necessary search for new antimicrobial drugs from herbal medicines.

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