Antifungal effect of some medicinal plants: *In vitro* antibacterial

Babhi Kumari, Kapoor Randi and Rohit Sharma

Department of Parasitology and Mycology, School of Medicine, Tamil University, P O Box 1549, Thanjavur-10, India. * Email: Kumar_b@yahoo.com

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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 terrus*, *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 psoralen revealed antimicrobial activity of fenell 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. (Caesalpiniaceae) it contains chrysophenol, rhein (1.8 dihydroxy anthraquinone-3 carboxylic acid), glucose and rhamnose 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 (Ngono 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 x 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 terrus, 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 Sabauaurd 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 obtain10^{-5} spore/ml for fungal strains and 10^6 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 (10^{-5} and 10^{-6} 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 mg/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
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 quishforia were inactive against Candida species whereas, Black piper, F. vulgare and Loranthus capillatus 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. areus, as the minimum inhibitory concentration was 0.625 mg ml$^{-1}$ while the minimum inhibitory concentration for

### Table 1. The minimum inhibitory concentration (MIC) mg m$^{-1}$ of selected medicinal plants.

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<tr>
<td>Black piper seed</td>
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<td>20</td>
<td>20</td>
<td>0.625</td>
<td>N.I</td>
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<td>N.I 2.5</td>
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<td>Nigella sativa seed</td>
<td>40</td>
<td>20</td>
<td>10</td>
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<td>N.I</td>
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<tr>
<td>Foeniculum vulgare fruit</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>2.5</td>
<td>N.I</td>
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<td>Loranthus capitellatus leaf</td>
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<td>20</td>
<td>40</td>
<td>5</td>
<td>N.T</td>
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<td>Cassia alata leaf</td>
<td>N.I</td>
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<tr>
<td>Drynaria quishforia rhizome</td>
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<td>10 20 5.0</td>
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Value are the mean of three replicates; N.I: No inhibition was absorbed; N.T: not tested; Abbrevatins: Sta. aru.: Staphylococcus areus; 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.: Microsporum gypseum.

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

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<tr>
<td>Black piper seed</td>
<td>16</td>
<td>18</td>
<td>10</td>
<td>12</td>
<td>N.I</td>
<td>15</td>
<td>N.I 2.5</td>
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<tr>
<td>Nigella sativa seed</td>
<td>15</td>
<td>13</td>
<td>24</td>
<td>10</td>
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<td>Foeniculum vulgare fruit</td>
<td>18</td>
<td>17</td>
<td>15</td>
<td>12</td>
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<td>10 10</td>
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<tr>
<td>Loranthus capitellatus leaf</td>
<td>12</td>
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<td>15</td>
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<td>N.T</td>
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<td>10 10</td>
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<td>Cassia alata leaf</td>
<td>N.I</td>
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<td>Drynaria quishforia rhizome</td>
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<td>Positive control</td>
<td>Clotrimazole 20 mm</td>
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<td>Gentamicin 22 mm</td>
<td>Penicillin 15 mm</td>
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<td>Ampicillin B 17 mm</td>
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Value are the mean of three replicates; N.I: No inhibition was absorbed; N.T: not tested; Abbrevatins: Sta. aru.: Staphylococcus areus; 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.: Microsporum gypseum. penicillin (10 IU), gentamicin (10 IU), ampicillin B (0.4mg/ml) and clotrimazole (10 µg) are used as positive control.
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. terrus, 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 mg ml \(^{-1}\) 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 mg ml \(^{-1}\) 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. quyrshioria 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. terrus and Alternaria sp. while it was inactive against A. niger, C. glarata, 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. areus, A. flavus, A. terrus and Alternaria sp. while it was inactive against A. niger, A. flavus, A. terrus, Alternaria sp. C. glarata, 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 Weeping 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). Weeping 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. glarata, C. albicans, C. tropicalis and the bacteria such as S. areus, P. aeruginosa while was inactive against saprophytic fungi and dermatophytes. Farrag et al. (2000) reported that the oil of black cumin showed inhibitory effect against gram positive such as S. areus 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. areus, flavus, A. terrus and Alternaria sp. while it was inactive against A. niger, A. flavus, A. terrus, Alternaria sp. C. glarata, 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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REFERENCES


