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Antifungal effect of *Alhagi maurorum* phenolic extract

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ABSTRACT: *Alhagi maurorum* (Family: Fabaceae) contained many bioactive metabolites including phenolic compounds, flavonoids, fatty acids, coumarins, glycosides, sterols, steroids, resins, vitamins, alkaloids, carbohydrates, tannins, unsaturated sterols and triterpenes. It possessed antibacterial, anti-inflammatory, antipyretic, analgesic, antioxidant, gastrointestinal, cardiovascular, diuretic, dermatological and many other effects. This study was carried out to investigate the antifungal effect of *Alhagi maurorum* phenolic extract against *Alternaria alternata*, *Candida albicans*, *Cladosporium cladosporoides*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes*. The extract possessed antifungal activity against all the tested fungi. The least MIC was recorded against *Trichophyton mentagrophytes* which was l.5 µg/ml, while the highest MIC was recorded against *Cladosporium cladosporoides* which was 6.2 µg/ml.

Keywords: antifungal, Alhagi maurorum, phenolic extract

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I. INTRODUCTION

Alhagi maurorum (Family: Fabaceae) was used in folk medicine as a remedy for rheumatic pains, bilharziasis, liver disorders, various types of gastrointestinal discomfort, general tonic, anthelmintic, to treat constipation, jaundice, and arthritis. It also used as diuretic, blood purifier, antimicrobial, for treatment of dysentery, upper respiratory system problems, wounds, hemorrhoids and uterine problems. The roots were used as aphrodisiac[(1-2)]. It contained many bioactive metabolites including phenolic compounds, flavonoids, fatty acids, coumarins, glycosides, sterols, steroids, resins, vitamins, alkaloids, carbohydrates, tannins, unsaturated sterols and triterpenes. Many phenolic compounds were isolated from *Alhagi maurorum* included, tamarixtin 3-O-dirhamnoside, isorhamnetin 3-O-glucosylneo-hesperidoside, isorhamnetine 3-O-robinoside, isorhamnetin 3-O-rotinoside, quercetin 3-O-rhamnoside, kampferol 3-O-galactoside O-galactoside, quercetin 3, 7-diglycoside, isorhamnetin 3- rutinoside, daidzein 7, 4 -dihydroxyisoflavone, calycisin 3 -hydroxyformononetin, and isorhamnetin, tamarxtin aglycones, isorhamnetin-3-O-[-alpha-l-rhamnopyranosyl- (1→3)]-beta-D-gluco-pyranoside, 3'-O-methylorobol and quercetin 3-O-beta-d-glucopyranoside[(3-10)]. While, nutrient analysis of the plant showed that it contained protein (6.56±0.02%), fat (4.88±0.01%), fiber (3.33±0.01%), carbohydrate (56.52±0.12%), energy values (330.51±0.01Kcal/100g) and trace elements (Ca: 2234, Mg: 1292, K: 14991, Na: 650, Fe: 105.4, Cu: 14.3, Zn: 8.5, Cr: 2.5, Cd: 0.2, Pb: 0.7, and Ni: 2.5 ppm)[(11)]. The previous pharmacological studies showed that it exerted antibacterial, anti-inflammatory, antipyretic, analgesic, antioxidant, gastrointestinal, cardiovascular, diuretic, dermatological and many other effects[(12)]. The present work was design to investigate the antifungal effects of phenolic extract of Alhagi maurorum.

II. MATERIALS AND METHODS

Collection and identification of plant sample:

The plant of Camels' thorn were collected from area near Tikrit university camp, then it was send to be identified by the Iraqi National Herbarium. The plant was dried in the shadow and grind by electric grinder and the powder was kept in a plastic bag.

Extraction of phenolic content:

The method mentioned by Gayon[(13)] was followed for extraction of the phenolic content of the dried green parts powder.

Fungal isolates:

Five fungal and four bacterial species were tested for the antimicrobial activity of *Alhagi maurorum*. All the fungal isolates used in this study were isolated from clinical cases, included *Alternaria alternata, Candida albicans, Cladosporium cladosporoides, Cryptococcus neoformans* and *Trichophyton mentagrophytes*.

In vitro testing of the antifungal activity of phenolic extract:

Preparation of fungal inoculum:

Fungal inocula were prepared according to the method of McGinnis [14]:

1-Normal saline solution was prepared by dissolving 0.89 gm of NaC1 in 100 ml distilled water and distributed in test tubes (5 ml in each) sterilized in the autoclave at12l C^o and l5 ($lb/lnch^2$) for 15 min, and left to cool to 25C^o.

2-The fungal isolates were reactivated by growing on Sabouroud Dextrose Agar (SDA) medium at 25 C^o. Fungal growth of 2- 5 days o1d for yeasts and of 2 weeks old for dermatophytes were taken by loop and transferred to test tubes containing sterile normal saline and shacked for short time.

3-Fungal inoculum of $l0^6$ conidia/ml was prepared using hemocytometer. In addition, the optical density was measured using a spectrophotometer (Cecil, England) at 540 nanometer.

4-Test tubes were labeled and stored in a refrigerator at 4C^o"until use.

Preparation of agar plates to test the different concentrations of phenolic extract:

Agar well diffusion method[15] was used by pouring 20 ml of Sabouroud Dextrose Agar (SDA) in a Petri dish (9 cm diameter). The medium was inoculated with 10^6 conidia/ ml by spreading the plates and were left for 30 minutes, then four wells (8 mm diameter) were made by corkborer;100 µl of the phenolic extract was added to each well by micropipette . The plates were incubated at 25C^o . The results were examined after 2-5 days of incubation by measuring the diameter of inhibition zone. Different dilutions of the extract were used .The extract and the standard antifungals were dissolved in a (100%) dimethyl sulfoxide (DM SO) a biologically inert substance, which was also used as a negative control.

Determination of MIC for the phenolic extract on the growth of fungi:

Minimal inhibitory concentrations were determined according to the method of Nostro el al., [10] by mixing 2m1 of each concentration (2.0, l.0, 0.5, 0.25, 0.l25, 0.062, 0.03l, 0.0l5, 0.007, 0.003 mg/ml) with 18 ml of cooled SDA medium .Then pouring in Petri dishes, one Petri dish without extract was used as a control; 0.l ml of the inoculum (10^6 conidia/ml) was cultured as small spot on SDA medium as mentioned previously. The plates were incubated at 25 C^o after the appearance of the first growth in the control Petri dish and the results were recorded.

III. RESULTS

The results revealed that the effect of the phenolic extract of *A. maurorum* on 5 fungal species depend on the concentration of the extract and the fungal species. Table l showed that the effect of phenolic extract in a concentration of 0.25-2 mg/ml on Altenaria alternata was similar to that of nystatin in a concentration of 0.25 mg/ml, while its effect in a concentration of 128 mg/ml was significantly better than nystatin and clotrimazole in a concentration of 0.25 mg/ml ($p<0.01$). The effect of *A. maurorum* phenolic extract in a concentration of 0.25-l mg/ml against *Candida albicans* was similar to that of clotrimazole in a concentration of 0.25 mg/ml. While, the effect in a concentration of 2-128 mg/ml was significantly better than nystatin and clotrimazole in a concentration of 0.25 mg/ml ($p<0.0l$). The effect of phenolic extract in a concentration of 0.25-l6 mg/ml was similar to that of clotrimazole in a concentration of 0.25 mg/ml against *Cladosporium cladosporoides*, while the effect of the extract in all concentrations was significantly better than nystatin in a concentration of 0.25mg/ml against the same fungus. The effect of phenolic extract in a concentration of 0.25-2 mg/m1 against *Cryptococcus neoformans* was similar to that possessed by the two standard antifungals at a concentration of 0.25 mg/ml. The effect of phenolic extract in a concentration of 0.5-8 mg/ml against Trichophyton metagrophytes was similar to that of clotrimazole in a concentration of 0.25 mg/ml, while it's effect in a concentration of 4-l28 mg/ml was significantly better than that of nystatin in concelntration of 0.25 mg/ml ($p<0.00l$). However, as appeared in table 1, the antifungal effects of all concentrations of the phenolic extract of *A. maurorum* were significantly better than the effect of negative control, dimethyl sulfoxide ($p<0.0001$) against a1l the tested fungi. Table (2) showed the minimum inhibitory concentrations (MICs) of the extract against the growth of the tested pathogenic fungi. The least MIC was recorded against *Trichophyton mentagrophytes* which was l.5 µg/ml, while the highest MIC was recorded against *Cladosporium cladosporoides* which was 6.2 µg/ml.

Table 1: The effect of the phenolic extract of *Alhagi maurorum* against the growth of the tested fungi

Concentration mg/ml	Growth inhibitory zone (mm) (mean ±SD)				
	Alternaria alternata	*Candida albicans*	*Cladosporium cladosporoides*	*Cryptococcus neoformans*	*Trichophyton mentagrophytes*
128	19.0±1.0 a	25.0±0.5 a	15.0±1.1 a	23.0±0.5 a	27.0±0.5 a
64	17.0±1.1 ab	24.0±0.5 a	12. 3±0.5 b	18.3±0.5 b	19.0±0.5 b
32	15.3±0.5 bc	19.0±1.0 b	11.6±0.5 bc	16.0±1.0 bc	18.0±1.0 b
16	14.0 +0.5 bc	17.6±0.5 bc	11.0±0.5 bcd	15.0±0.5 bc	17.6±1.0 bc
8	13.6 ±1.0 cd	15.0±0.5 c	10.6±1.0 bcd	13.6±0.5 cd	14.6±0.5 cd
4	13.0±1.1 cd	14.6±1.1 cd	10.3±1.0 bcd	12.3±1.0 d	14.0±0.5 cd
22	12.6±0.5 cde	12±1.0 cd	10.0±0.5 cd	12.0±0.5 de	13.3±0.5 de
1	12.0±0.5 cde	11.0±0.5 de	9.6±1.1 cd	11.3±0.5 de	13.0±1.0 de
0.5	11.3±1.0 de	9.6±0.5 e	9.3±0.5 cd	10.6 ±0.5 e	12.6±0.5 de
0.25	11.0±1.0 de	9.0±0.5 e	9.0±0.5 d	9.6±1.0 e	11.0±1.0 e
Clotrimazole 0.25 mg/ml	14.0±0.5 bc	9.5±0.5 e	8.6±1.0 d	9.6±1.0 e	15.0±0.5 cd
Nystatin 0.25 mg/ml	10.0±0.5 e	7.5±1.0 f	0.0±0.0 e	10.6±0.5 e	10.0±0.5 e
DMSO	0.0±0.0				

Vertically: similar letter means not significant

Table 2: MIC (µg/ml) of the phenolic extract of *Alhagi maurorum* against the tested fungi

Fungal spesies	MIC (µg/ml) of the phenolic extract
Alternaria alternata	3.1
Candida albicans	3.1
Cladosporium cladosporoides	6.2
Cryptococcus neoformans	3.1
Trichophyton mentagrophytes	1.5

IV. DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. In an approach toward the development of ecofriendly antifungal compounds for controlling major fungal diseases, many medicinal plants possessed wide range of antifungal activities[16-20], some of them were formulated and used clinically nowadays. These included: *Adiantum capillus-veneris*[21], *Allium sativum*[22], *Alpinia galangal*[23], *Ammi majus*[24], *Anchusa strigosa*[25], *Apium graveolens*[26], *Arachis hypogaea*[27], *Arundo donax*[28], *Asclepias curassavica*[29], *Asparagus officinalis*[30], *Avena sativa*[31], *Ballota nigra*[32], *Bellis perenni*[33], *Benincasa hispida*[34], *Betula alba*[35], *Brassica rapa*[36], *Caesalpinia crista*[37], *Calendula officinalis*[38], *Calotropis procera*[39], *Capparis spinosa*[40], *Capsella bursa-pastoris*[41], *Capsicum annum*[42], *Carum carvi*[43], *Cassia occidentalis*[44], *Chenopodium album*[45], *Chrozophora tinctoria*[46], *Cicer arietinum*[47], *Cichorium intybus*[48], *Citrullus colocynthis*[49], Citrus species[50], *Clerodendrum inerme*[51], *Clitoria ternatea*[52], *Corchorus aestuans*[53], *Corchorus capsularis*[54], *Coriandrum sativum*[55], *Cressa cretica*[56], *Crotalaria juncea*[57], *Cyminum cuminum*[58], *Cupressus sempervirens*[59], *Cydonia oblonga*[60], *Cymbopogon schoenanthus*[61], *Cynodon dactylon*[62], *Cyperus rotuntdus*[63], *Dactyloctenium aegyptium*[64], *Datura metel* and *Datura stramonium*[65], *Desmostachya bipinnata*[66], *Dianthus caryophyllus*[67], *Dodonaea viscose*[68], *Dolichos lablab* (*Syn*: *Lablab purpureus*)[69], *Echinochloa crus-galli*[70], *Echium italicum*[71], *Ephedra alata* and *Ephedra foliate*[72], *Erigeron canadensis*[73], *Erodium cicutarium*[74], *Eryngium creticum*[75], *Eucalyptus* species[76], *Eupatorium cannabium*[77], *Euphorbia hirta*[78], *Fagopyrum esculentum*[79], *Ficus carica*[80], *Ficus religiosa*[81], *Foeniculum vulgare*[82], *Fraxinus ornus*[83], *Fumaria officinalis*[84], *Galium aparine*[85], *Galium verum*[86], *Glycyrrhiza glabra*[87], *Gossypium* species[88], *Haplophyllum* species[89], *Hedera helix*[90], *Helianthus tuberosus*[91], *Heliotropium* species[92], *Hibiscus rosa-sinensis*[93], *Hibiscus sabdariffa*[94], *Hyoscyamus* species[95], *Hypericum triquetrifolium*[96], *Inula graveolens*[97], *Jasminum officinale*[98], *Juglans regia*[99], *Jasminum sambac*[100], *Juniperus communis*[101], *Juniperus oxycedrus*[102], *Lythrum salicaria*[103], *Mangifera indica*[104]. In this study, *Alhagi maurorum* flavonoids extract was investigated against *Alternaria alternata*, *Candida albicans*, *Cladosporium cladosporoides*, Cryptococcus neeoformans and *Trichophyton mentagrophytes*. Previous investigation of antifungal activity of *Alhagi maurorum* showed that the methanol extract of *Alhagi maurorum* (at 23 mg/ml concentration) exerted antifungal activity against Aspergillus flavus, Alternaria alternate, Fusarium oxysporum, Fusarium solani, Bipolaris oryzae, Chetomium and Mucor, with a percentage of growth inhibition of 33.4, 89.4, 89.3, 94.6, 91.7, 59.0 and 94.1%[105]. The antifungal effect of

Alhagi maurorum could be attributed to its phenolic content, many medicinal plant phenolic compounds possessed antifungal effects by many mechanisms, they caused membrane disturbance resulting in the loss of membrane integrity, inhibited DNA transcription and reduced the cell populations and inhibited the activity of fungal antioxidant enzymes [105-114]. Therefore, *Alhagi maurorum* phenolic extract could be exerted antifungal effects via all these mechanisms.

V. CONCLUSION

According to the results of the current study, *Alhagi maurorum* possessed strong antifungal activity. It could be conveniently used as a promising alternative source for presently problematic fungal resistance.

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