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Antibacterial effect of the phenolic extract of *Alhagi maurorum*

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Abstract: *Alhagi maurorum* is customarily 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, 3-4]. The plant contained flavonoids, fatty acids, coumarins, glycosides, sterols, steroids, resins, vitamins, alkaloids, carbohydrates, tannins, unsaturated sterols and triterpenes**.** The current study was designed to determine the the antibacterial effects of phenolic extract of *Alhagi maurorum* against *E. coli, Klebsiella pneulnoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The effect of the phenolic extract in a concentration of 32-128 mg/ml was similar to that of gentamicin in a concentration of 10 mg/disc against *Escherichia coli*, while the extract in a contration of 0.25- 4 mg/ml possessed the same effect exerted by gentamicin in a concentratioln of l0 µg/disc against *Klebsiella pneumoniae*. The least (MIC) was recorded against S. aureus (3.1 mg/ml),while the highest (MIC) was recorded against *E.coli* (12.5 mg/ml).

I. INTRODUCTION

Alhagi maurorum (belong to the family: Fabaceae) is a deep rooted, rhizomatous, perennial shrub, with roots that can extend six to seven feet into the ground. The spiny, intricately-branched shrub reaches 1.5 to 4 feet in height. The plant, which is grayish green and hairless, has simple, entire leaves that are alternately arranged. The leaf shape is oval to lance-shaped. The small pea-like flowers are pinkish purple to maroon and are borne on short, spine-tipped branches that arise from the leaf axials. The reddish-brown to tan fruits are found between the seeds, with a short narrow beak at the end(1-2). It is native to North Africa, Middle East and South East Europe. *Alhagi maurorum* is customarily 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, 3-4). The plant contained flavonoids, fatty acids, coumarins, glycosides, sterols, steroids, resins, vitamins, alkaloids, carbohydrates, tannins, unsaturated sterols and triterpenes(5-8). The total phenolic and flavonoid contents of the plant were 23.83 mg gallic acid equivalent/g dried-weight and 11.53 mg rutin equivalent/g dried-weight respectively(9). The highest total content for phenolics (mg/g) and flavonoids (mg/g) were observed in leaves extract (50.39±2.67; 39.24±1.54, respectively), followed by flowers extract (32.00±1.62; 18.50±0.80, respectively)(6-7). Many flavonoids 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, 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(5, 10-12). *Alhagi maurorum* possessed antimicrobial activity, the leaves and flowers extracts was tested against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Salmonella typhi-murium. Both extracts showed antibacterial activity. The minimum inhibitory concentrations of the leaves extract were 80.7±4.5, 68.8±4.6, 60.6±8.3 and 58.0±6.3, and that of flowers extract were 84.0±0.0, 65.0±2.7, 65.2±6.2, 62.4±5.0 and 60.4±5.6 µg/ml against the tested microorganisms, respectively(13). The antibacterial activity of methanol extracts (6 mg/ml) of the fresh aerial parts of *Alhagi maurorum* were evaluated against gram positive microorganisms B cereus, C. perfringens, L. innocua, L. ivanovii Li4, L. monocytogensis, S. aureus, and S. epidermis. It showed antibacterial activity against only B cereus, L. ivanovii Li4, and S. aureus diameter of inhibition of 10-20 mm. The extract at the same concentration was also evaluated against gram negative microorganisms E. coli, Y. Enterocolitica, K. oxytoca, K. pneumonia, S. enterica. It showed activity against only K. pneumonia with a diameter of inhibition of 7mm. The antibacterial activity of hexane extracts (6 mg/ml) of the fresh aerial parts of

Alhagi maurorum were evaluated against gram positive microorganisms: B cereus, C. perfringens, L. innocua, L. ivanovii Li4, L. monocytogensis and S. aureus. It showed antibacterial activity against only B cereus, L. ivanovii Li4, L. monocytogensis and S. aureus with diameter of inhibition of 7-15 mm. The extract at the same concentration was also evaluated against gram negative microorganisms: *E. coli, Y. Enterocolitica, K. oxytoca, K. Pneumonia, S. enterica. It showed activity against only E. coli, Y. Enterocolitica, K. oxytoca* and *K. pneumonia* with diameter of inhibition of 12-15(14). The MIC of 90% methanolic extract of the leaves of *Alhagi maurorum*, against *Escherichia coli, Moraxella lacunata, Proteus merabiles, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Micrococcus luteus, Sarcina ventricull, Streptococcus byogenes* and *Saccharomyces cerevisiae* were 3,2,3,3,4,4,4,5, 5 and 5 mg/ml(15). This study was designed to to investigate the antibacterial effects of phenolic extract of Alhagi maurorum.

II. MATERIALS AND METHODS

Collection and identification of plant sample:

Alhagi maurorum was 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[16] was followed for extraction of the phenolic content of the dried green parts powder.

Tested microorganisms:

The bacterial species (*E. coli, Klebsiella pneulnoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*) used in this study were isolated from clinical cases. Standered cultural and biochemical tests were used to identify all bacterial isolates[17].

Study of the effect of *A. maurorum* phenolic extract on the growth of bacteria:

The Muellar- Hinton agar (MHA) well diffusion method was used to determine the antibacterial effect of the extract. Gentamicin (10 µg/disc, Oxoid) was used as positive control.

Determination of minimal inhibitory concentration of phenolic extract on bacterial growth:

Two ml of each phenolic extract was used (2.0, 1.0, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015, 0.007, 0.003 mg/ml) with l8ml of Muellar-Hinton agar. The mixture was poured in a Petri dish to obtain the final concentration. One Petri dish without extract was used as a control. Bacterial inoculum was prepared; 0.l ml of the inoculum was cultured as small spot on MHA medium mixing with plant extract. The plates were incubated at 37C for 24 hrs and the results were recorded[18].

III. RESULTS

Table 1 showed that the effect of the phenolic extract in a concentration of 32-128 mg/ml was similar to that of gentamicin in a concentration of 10 mg/disc against *Escherichia coli*, while the extract in a contration of 0.25- 4 mg/ml possessed the same effect exerted by gentamicin in a concentratioln of l0 µg/disc against *Klebsiella pneumoniae*. However, the effect of phenolic extract in a concentration of 0.25-8 mg/ml was similar to that of gentamicin in a concentratioln of l0 µg /disc against *Pseudomonas aeruginosa*, However when the concentration of 32-l28 mg/ml was used, it's effect became significantly better than gentamicin in the above concentration($p< 0.0l$). The effect of the extract in a concentration of 0.25-1 mg/ml was similar to that of gentamicin in a concentration of 10 µg/disc against *Staphylococcus aureus*. On the other hand, the effects of all concentrations of the phenolic extract of *A. maurorum* were better than negative control ($p< 0.000l$) against all speices of examined bacteria. As mentioned in table (2), the least (MIC) was recorded against *S. aureus* (3.1 mg/ml),while the highest (MIC) was recorded against *E.coli* (12.5 mg/ml).

Table 1: The diameter of the zone of growth inhibition of phenolic extract of *Alhagi maurorum* against bacterial isolates

Concentration mg/ml	Inhibition zone diameter (mm) (mean±SD)			
	E. coli	*Klebsiella pneulnoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
128	11.0 ± 0.5 [a]	15.6 ±0.5 [a]	16.0 ±1.0 [a]	18.0±0.5[a]
64	10.3 ± 0.5 [ab]	15.3 ±0.5 [a]	15.0 ± 1.1 [ab]	16.3±0.5[ab]
32	9.6 ±1 .1 [abc]	14.6 ± 0.5 [ab]	14.3 ± 0.5[abc]	15.6±1.0[b]

16	8.6± 0.5 ^{bcd}	14.3 ±0.5 ^{ab}	13.3±0.5^{bcd}	14.0±1.0^{bc}
8	7.6 ± 0.5 ^{cd}	14.0 ± 1.0 ^{ab}	12.0±1.0 ^{cdef}	12.3±0.5^{cd}
4	7.3 ± 1.0 ^{cd}	13.6 ± 1.1 ^{abc}	11.6±0.5 ^{cdef}	11.6±1.1^{cd}
2	6.6 ±1.0 ^{d}	12.0± 1.1 ^{bc}	11.3±0.5 ^{cdef}	11.0±0.5^{cde}
1	6.3 ± 0.5 ^{d}	11.6 ± 0.5 ^{c}	10.6±1.0 ^{ef}	10.0±0.5 ^{cef}
0.5	5.6 ±0.5 ^{d}	11.3 ± 0.5 ^{c}	9.6±1.1 ^{ef}	8.6±1.0 ^{ef}
0.25	13.0 ± 0.5 ^{d}	10.0 ±1.0 ^{c}	8.3±0.5 ^{f}	7.0±0.5 ^{f}
Gentamycin 10 µg/disc	13.0 ± 0.5 ^{a}	13.0 ± 0.5 ^{abc}	10.0±1.0 ^{def}	10.0±0.5 ^{cef}
Negative control (DMSO)	0.0 ± 0.0			

Vertically: similar letter means not significant

Table 2: MIC (µg/ml) for phenolic extract of *Alhagi maurorum*

Bacterial species	MIC (µg/ml) for phenolic extract of *Alhagi maurorum*
E. coli	12.5
Klebsiella pneulnoniae	6.2
Pseudomonas aeruginosa	6.2
Staphylococcus aureus	3.1

IV. DISCUSSION

The previous pharmacological studies revealed that medicinal plants possessed wide range of antibacterial activities[19-24]. These plants included: *Achillea santolina*[25], *Adiantum capillus-veneris*[26], *Agrimonia eupatoria*[27], *Agropyron repens*[28], *Ailanthus altissima*[29], *Allium* species[30], *Alpinia galangal*[31], *Althaea officinalis*[32], *Althaea rosea*[33], *Ammannia baccifera*[34], *Ammi visnaga*[35], *Anchusa strigosa*[36], *Anethum graveolens*[37], *Anthemis nobelis*[38], *Antirrhinum majus*[39], *Apium graveolens*[40], *Arachis hypogaea*[41], *Arctium lappa*[42], *Artemisia campestris*[43], *Arundo donax*[44], *Asclepias curassavica*[45], *Asparagus officinalis*[46], *Avena sativa*[47], *Bacopa monniera*[48], *Ballota nigra*[49], *Bauhinia variegate*[50], *Bellis perenni*[51], *Benincasa hispida*[52], *Betula alba*[53], *Bidens tripartite*[54], *Brassica rapa*[55], *Bryophyllum calycinum*[56], *Caesalpinia crista*[57], *Calendula officinalis*[58], *Calotropis procera*[59], *Capparis spinosa*[60], *Capsella bursa-pastoris*[61], *Capsicum annuum* and *Capsicum frutescens*[63-62], *Carthamus tinctorius*[64], *Carum carvi*[65], *Cassia occidentalis*[66], *Casuarina equisetifolia*[67], *Celosia cristata*[68], *Centaurea cyanus*[69], *Chenopodium album*[70], *Chrozophora tinctoria*[71], *Chrysanthemum cinerariaefolium*[72], *Cicer arietinum*[73], *Cichorium intybus*[74], *Cistanche tubulosa*[75], *Citrullus colocynthis*[76], *Citrus* species[77], *Clerodendrum inerme*[78], *Clitoria ternatea*[79], *Colchicum balansae*[80], *Convolvulus arvensis*[81], *Corchorus aestuans*[82], *Corchorus capsularis*[83], *Cordia myxa*[84], *Coriandrum sativum*[85], *Coronilla varia*[86], *Cotoneaster racemiflora*[87], *Cressa cretica*[88], *Crotalaria juncea*[89], *Cyminum cuminum*[90], *Cupressus sempervirens*[91], *Cuscuta planiflora*[92], *Cydonia oblonga*[93], *Cymbopogon schoenanthus*[94], *Cynodon dactylon*[95], *Cyperus rotuntdus*[96], *Dactyloctenium aegyptium*[97], *Dalbergia sissoo*[98], *Daphne mucronata*[99], *Datisca cannabina*[100], *Datura metel* and *Datura stramonium*[101], *Daucus carota*[102], *Delphinium brunonianum*[103], *Desmostachya bipinnata*[104], *Dianthus caryophyllus*[105], *Dodonaea viscose*[106], *Dolichos lablab*[107], *Echinochloa crus-galli*[108], *Echium italicum*[109], *Ephedra alata* and *Ephedra foliate*[110], *Equisetum arvense*[111], *Erigeron Canadensis*[112], *Erodium cicutarium*[113], *Eryngium creticum*[114], *Eucalyptus* species[115], *Eupatorium cannabium*[116], *Euphorbia hirta*[117], *Euphorbia tinctoria*[118], *Fagopyrum esculentum*[119], *Ficus carica*[120], *Ficus religiosa*[121], *Fritillaria imperialis*[122], *Foeniculum vulgare*[123], *Fraxinus ornus*[124], *Fumaria officinalis*[125], *Fumaria parviflora*[126], *Galium aparine*[127], *Galium verum*[128], *Geum urbanum*[129], *Glaucium corniculatum*[130], *Glossostemon bruguieri*[131], *Glycyrrhiza glabra*[132], *Gnaphalium luteoalbum*[133], *Gossypium* species[134], *Haplophyllum* species[135], *Hedera helix*[136], *Helianthus annuus*[137], *Helicophyllum rauwolffii*[138], *Heliotropium* species[139], *Herniaria glabra*[140], *Herniaria hirsute*[141], *Hibiscus cannabinus*[142], *Hibiscus rosa-sinensis*[143], *Hibiscus sabdariffa*[144], *Hyoscyamus* species[145], *Hypericum triquetrifolium*[146], *Inula graveolens*[147], *Jasminum officinale*[148], *Jasminum sambac*[149], *Juglans regia*[150], *Juncus maritimus*[151], *Juniperus communis*[152], *Juniperus oxycedrus*[153], *Jussiaea repens*[154], *Kochia scoparia*[155], *Lagerstroemia indica*[156], *Lallemantia iberica*[157], *Lallemantia royleana*[158], *Lathyrus sativus*[159], *Lemna minor*[160], *Leontice leontopetalum*[161], *Lippia nodiflora*[162], *Lithospermum officinale*[163], *Lycopus europaeus*[164], *Lythrum salicaria*[165], *Malva neglecta*[166].

The phenolic extract of *Alhagi maurorum* contained many phenolic compounds 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[6-8]. Phenols can exert antibacterial activities through multiple mechanisms, included disruption of cytoplasmic membrane, inhibition of nucleic acid synthesis, inhibition of energy metabolism, inhibition of folic acid synthesis, inhibition of cell membrane synthesis and function in addition to anti-virulence mechanisms[167]. Therefore the antibacterial effects of phenolic extract in this study could be attributed to all these mechanisms.

V. CONCLUSION

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

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