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Antimicrobial and preliminary phytochemical investigations of some traditional medicinal plants in iraqi kurdistan

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ABSTRACT

Many plants used in Kurdistan in folk medicine to treat a variety of illnesses, the following study incl uded antimicrobial evaluation of most commonly used traditional medicinal plants with their prelimin ary phytochemical screening. Antimicrobial activity of different extractsfrom nineteen traditional med icinal plants (Urtica dioica, Achillea mileforum, Viola odorata, Althea officinalis, Malva parviflora, T rigonella foenum-graecum, Glycyrrhiza glabra, Plantago major, Pegunm harmala, Pimpinellaanisum, Coriandrum sativum, Ammi vinaga, Nigella sativa, Hibiscus sabdarriffa, Foneucluim vulgari, Cichori um intybus, Melissa officinalis, Thymus vulgari, and Matricaria chamomilla) were evaluated against f our strains of gram negative bacteria and two strains of gram positive bacteria using agar well diffusio n method, and preliminary screening for main phytochemical natural product groups had been done us ing standard procedures. Eight plant species (Pegunm harmala, Hibiscus sabdarriffa, Achillea milefor um, Plantago major, Matricaria chamomilla, Nigella sativa, Thymus vulgari, and Althea officinalis) w ere showed activity against one or more of the tested bacterial strains. The highest antimicrobial activi ties were for ethanolic extracts of Pegunm harmala (MIC20mg/ml) against both Staphylococcus aureu s and Escherichia coli and for Hibiscus sabdarriffa (MIC 30 mg/ml) against Pseudomonas arigenosa. Alkaloids, flavonoids, saponins, condensed and hydrolysable tannins were detected in different studie d plants, flavonoids in Trigonella foenum-graecum and hydrolysable tannins in Ammi vinaga were re corded for the first time. Some of the studied plants are potentially good sources of antimicrobial agen ts and the results support the traditional medicinal uses of plants.

Keywords: **Antimicrobial; traditional medicinal plants; Kurdistan; phytochemicals**

1. Introduction

Medicinal plants are considerably useful and economicallyessential. They contain active cons tituents that are used in the treatment of many human diseases (Stary and Hans, 1998).Herbal remedies used in traditional folk medicineprovide an interesting and still largely unexplored s ource for the creation and development of potentially new drugs for chemotherapy which mig ht help to overcome the growing problem of drug resistance and also the toxicity of currently available commercial antibiotics (Al- wadh-Ali et al., 2001).In Kurdistan there are a number

of plants used in folk medicine to treat a variety of illnesses, although most of the herbs used were studied previously for their phytochemical constituents and bioactivities, but still more e fforts needed to find out other important chemical constituents and activities of those plants. The following study was decided to include antibacterial evaluation for a number of most co mmonly used plants by the traditional medicine in Kurdistan against a number of pathogenic bacteria, in addition to the preliminary screening for main phytochemical natural product gro ups of the studied plants.

2. Materials and Methods:

Plant material: The plants which were used in the study some are naturally found in Iraq espe cially in Kurdistan region and some are not native to Iraq. The plants were purchased from th e local herbalist markets and were authenticated by the department of pharmacognosy, colleg e of Pharmacy, Hawler medical university and department of biology, college of education, u niversity of Salahaddin, Erbil, Iraq.

2.1. Extraction: Plant materials were collected, the fresh ones were dried in air for seven days, and all plants were powdered with mechanical grinder. 50gm of each dried powdered plant material was extracted separately with 1000ml of chloroform using ultra sonic assisted extractor for one hour at 40 ^{0}C (Alupuluiet al., 2009). The extracts were obtained by filtration through Buckner funnel, and evaporated to dryness by rotary evaporator yielded chloroform extracts (CE). The residual plant materials were dried then re-extracted using 75% ethanol using ultra sonic assisted extractor for one hour at 40 ^{0}C (Alupuluiet al., 2009). The obtained extracts from filtration by Buckner funnel evaporated to dryness by rotary evaporator and yielded ethanol extracts (EE).

2.2. Antibacterial evaluation:

2.2.1 Plant extract preparation: The plants extracts were evaluated separately at two different concentrations 10mg and 100mg. the chloroform extracts and ethanol extracts for each plant were dissolved separately in 1ml of 20% tween 80 and 10% dimethyl sulfoxide (DMSO) respectively.

2.2.2. Tested microorganism: Bacteria which were used in the process of investigation are obtained from biology department, Science College, Salahaddin University. Bacterial strains include two gram positive bacteria (*Staphcoccusa ureus, Bacillus cerus*) and four gram negative bacteria (*Escherichia coli, Psedomonus arigenossa, Klepssila spp*and *Proteus spp*). The bacterial samples are frozen at -4c in cooled incubator, later reactivated before it's used.

2.2.3. Method of antibacterial evaluation: The antibacterial activity of the two types of plant extracts were tested against six strains of bacteria using agar- well diffusion method (Turkoglu*et al*., 2007; Park *et al*., 1997).From the frozen bacteria inoculation was done into nutrient agar media, and incubated at 37^{o}C for 24hr.The grown bacteria were suspended in a normal saline solution (0.85% sodium chloride w/v) to a turbidity of 0.5 McFarland standards (108 cfu/ml).The prepared bacterial suspension was used to inoculate into Muller-Hinton agar plate with a sterile non-toxic cotton swab on a wooden

applicator. Four wells were done by a sterile cork borer of 5mm in diameter in each plate.100 µl of dilution of plant extracts in 10% DMSO for ethanol extract and 20% tween 80 for chloroform extract to give final concentration of 1mg and 10mg of each plant extract was added in each well, 10% DMSO and 20% tween 80 were used as negative control and streptomycin antibiotic used as positive control in concentration of (10µg/ml) added in a well in each plate. Plates are incubated at 37 C^0 for 24 hr.

2.3. Determination of minimum inhibitory concentration (MIC): MIC values for biologically active extracts against *Staphylococcus aureus, Bacillus cerus, Escherichia coli, Pseudomonas arigenossa, Klepssila* spp., and *Proteus* spp. were determined by agar well diffusion method (Park *et al.*, 1997; Turkoglu*et al.*, 2007).

2.4. Phytochemical screening: Fifty g of dried powdered plant materials were separately extracted using 75% ethanol for the phytochemical investigation using ultra sonic assisted extractor for 1hr at 40 ^{0}C (Alupului*et al.*, 2009) .

2.4.1. Alkaloids test: Hydroalcoholic extracts of plants were separately treated with dilute sodium hydroxide (5% NaOH) solution, extraction is then carried out with organic solvent (chloroform). The concentrated organic liquid is then shaken with aqueous acid (5% HCl) and allowed to separate; the aqueous extract was used for detection of alkaloidal compounds (Evans, 2000). Few drops of reagent were added to 1ml of extract. The appearance of a reddish-brown to orange precipitate indicates the presence of alkaloids (Harborne, 1984; Evans, 2000; Seema, 2008).

2.4.2. Flavonoids test: 2 ml of hydroalcoholic extracts of plant materials were tested separately for the presence of flavonoid glycoside by the addition of few drops of NaOH solution, intense yellow color is formed which turn to colorless on addition of few drops of dilute acid solution indicate the presence of flavonoids (Ashutosh, 2003).

2.4.3. Anthraquinone glycosides test: The tested plant extract was boiled with 1ml of dilute acid in a test tube over a pre-heated water bath for 5 minute. The contents were cooled and extracted with chloroform, the chloroform layer was separated and ammonia solution was added. The appearance of a rose-pink color in ammonia layer is indicating the presence of anthraquinone glycosides (Ashutosh, 2003).

2.4.4. Cardioactive glycosides test: Dried plant extracts were dissolvedin chloroform and evaporated to dryness. The residues were dissolved in (0.4ml) glacial acetic acid with few drops of ferric chloride (FeCl3), concentrated sulphuric acid (H2SO4) was added along the side of the test tube to settle at the bottom. The reddish brown color changing to bluish green color appears at the junction of the two reagents within 2-5 min. spreading slowly into the acetic acid layer indicated the presence of cardioactive glycosides (Ashutosh, 2003).

2.4.5. Saponin glycosides test: A volume of 2ml of each hydroalcoholic plant extracts were shaken with 1ml of water. The formation of semi-permanent foam indicates the presence of saponin natural products (Banso, 1984).

2.4.6. Tannins test: A few drops of 1% ferric chloride reagent were added to 1ml of each plant extracts. The appearance of blue color indicated the presence of hydrolysable tannins and appearance of green color indicated the presence of condensed tannins (Banso, 1984; Evans, 2000).

3. Results

Among the evaluated plants, eight plant species showed antimicrobial activities against o ne or more of the tested bacterial strains, table 1.Different natural product groups were det ected from the phytochemical screening studyin the evaluated plants, table 2.

Table 1: Antibacterial activity for chloroform and hydroalcoholic extracts (100 mg/ml):

		Inhibition zone diameter in millimeter (MIC)[1]				
Plants[2]	Plant part[3]	S. aureus	E. coli	P.arigenosa	B. cerus	Proteus spp.
Achilleamileforum(C)	F	10 ±0.10 (80)	---	---	---	10±0.3 (80)
Achilleamileforum(E)	F	10±0.71 (90)	---	---	---	---
Althea officinalis (C)	F, L	10±0.173 (70)	---	---	---	---
Hibiscus sabdarriffa (C)	F	10±0.10 (80)	---	---	---	---
Hibiscus sabdarriffa (E)	F	10±0.81 4±0.17 1 0±0.20 (80)	(70)	(30)		20±0.10 (40)
Matricariachamomilla (C)	F	---	---	---	---	14±0.30 (90)
Nigella sativa (C)	S	14±0.264 (80)	---	---	---	---
Pegunmharmala (C)	S	4±0.20 (80)	4±0.17 3 (90)	6±0.36 (80)	---	---
Pegunmharmala (E)	S	20±0.265 (20)	10±0.1 0 (20)		20±0.30 (30)	10±0.173 (50)
Plantago major (C)	L	---	---	---	---	10±0.1(80)
Thymus vulgari(C)	L,S	14±0.33 (70)	---	---	---	---
Thymus vulgari(E)	L,S					10±0.26 (70)

[1]Mean of triplicates ± SD values.

[2] C, chloroform extract; E, hydroalcoholic extract

[3] F, flowers; L, leaves; S, seeds

Table 2: Phytochemical screening results for the studied plants:

Plants	Alkaloid	Flavonoid	Saponin	Hydrolysable Tannin	Condensed Tannin
Achilleamileforum	---	+	+	---	+
Althea officinalis	---	+	---	---	+
Ammivinaga	---	+	---	+	---
Cichoriumintybus	+	+	---	---	+
Coriandrumsativum	---	+	---	---	+
Foneucluimvulgari	---	+	---	---	---
Glycyrrhizaglabra	---	+	+	+	---
Hibiscus sabdarriffa	+	+	---	---	---
Malvaparviflora	---	+	---	---	---
Matricariachamomilla	---	+	---	---	+
Melissa officinalis	---	+	---	---	+
Nigella sativa	+	---	---	---	+
Pegunmharmala	+	---	---	+	---
Pimpinellaanisum	+	+	---	---	+
Plantago major	---	+	---	---	+
Salvia officinalis	---	+	+	+	+
Thymus vulgari	---	+	---	---	+
Trigonellafoenum-graecum	+	+	+	---	---
Urticadioica	---	+	---	---	
Viola odorata	---	+	---	---	+

(+) Positive result; (---) Negative result

4. Discussion

Many published reports show the effectiveness of traditional herbs against microorganisms, a s a result, plants are one of the bedrocks for modern medicine to attain new principles (Shahi di, 2004). Scientific analysis of plant components follows a logical pathway. Twenty one extr acts of the thirty eight extracts exhibited antimicrobial activity against selected strains of bact eria, most of them were chloroform extract even though that in some plants hydroalcoholic ex tract showed a greater activity. Among the plant studied, Peganum harmala showed the stron gest antibacterial activity. It gives activity against five types of tested bacterial strains. The E extract of the plant showed a greater biological activity than C extract, from literature review

it was found that the pharmacological active constituents in the seeds of the Peganum harmal a are alkaloids (Mahmodianet al, 2002) which can be extracted by ethanol (Ivanovska et al, 1 996), the antibacterial activity is related to the alkaloidal content of the plant (Prashanthet al, 1999). Thymus vulgaris has been shown to be effective against Gram-positive and Gram-neg ative bacteria, fungi, and yeasts (Greives, 1996), the two main secondary metabolite groups r esponsible for the antibacterial activity are tannins and essential oils (Marjorie, 1999). Antiba cterial activity of Hibiscus sabdariffa was carried out and results revealed that the activity ma y be due to polyphenolic nature of the flavonoids (Mahadevanet al, 2008). The results reveale d that most of the evaluatedplants were found to contain flavonoidal compounds and the same results can be observed for condensed tannins, while none of the plants were found to contai n cardioactive and anthraquinone glycosides, flavonoids in Trigonella foenum-graecum and h ydrolysable tannins in Ammi vinaga were recorded for the first time. Many factors responsibl e for the variation in the results with the literature reviewed. The chemical constituents of pla nts vary depending on the species, variety and part of the plant, with conditions of growth (so il, water and temperature), and with the age of the plant. The phytochemistry also varies acco rding to the geographical regions, season and time of collection and different climatic conditi ons (Chaudhury, 1999; Dean and David, 2008).

Conclusion

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