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Antibacterial Activity and Preliminary Phytochemical Screening of Four Medicinal Plants

Nizar Sirag[1 *], Elhadi M M Ahmed[1], Anwar Mohammed[2] and Mohamed Abubakr [2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Sudan.

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Gezira, Sudan.

[*] Corresponding author. E-mail: nizarsirag@gmail.com

ABSTRACT

The present study was undertaken to investigate the antibacterial activity and to find the biologically active functional groups of four medicinal plants. The ethanolic extracts which were obtained by maceration were tested against *in vitro* five bacterial strains, using cup-plate agar diffusion method. Preliminary phytochemical screening techniques were also used to test for the presence and nature of the active constituents in these plant extracts.

Extracts of the plants; *Adansonia digitata*, *Eucalyptus globulus* and *Trigonella foenum graecum* showed a remarkable antibacterial activity against all tested bacterial strains. Meanwhile, the ethanolic extract of *Ficus sycomorous* exhibited a considerable antibacterial activity against *Proteus mirabilis*, *Pseudomonas aeuroginosa* and *Staphylococcus aureus.* The preliminary phytochemical screening revealed the presence of terpenes, tannins and saponins in all tested plant extracts, whereas alkaloids and cardiac glycosides were found in appreciable amounts in extracts *Trigonella foenum graecum* and *Adansonia digitata* respectively.

It can be concluded that the tested plants demonstrating broad spectra of activity which may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of health.

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Keywords: Antibacterial activity, Multi-drug-resistance, Medicinal plants.

INTRODUCTION

Many naturally occurring compounds found in medicinal plants, herbs, and spices have been shown to possess antimicrobial activities against many pathogens (Olaleye *et al*, 2007). Synthetic antibiotics provide the main basis for the therapy of bacterial infections. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug-resistant strains of several groups of microorganisms (Marjorie *et al*., 2011). The use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. The demand for plant based therapeutics is increasing in both developing and developed countries, because of growing recognition that they are natural products, non-narcotic, and easily biodegradable, producing minimum environmental hazards, having no adverse side effects, and being easily available at affordable prices (Marjorie *et al*., 2011). Thus, in light of the evidence of rapid global spread of resistant isolates, the need to find new antimicrobial agents is of paramount importance. Researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against resistant strains (Braga *et al.*, 2005). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Lewis and Ausubel, 2006). There have been several reports on the antimicrobial activity of different herbal extracts (Bonjar, 2004; De Boer *et al.*, 2005; Islam *et al.*, 2008).

In this study, the antibacterial activity and the presence of some active constituents of four medicinal plants used in Sudanese folk medicine were investigated.

MATERIALS AND METHODS

MATERIALS

Plants materials:

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Fresh leaves of *Eucalyptus globulus* and barks of *Ficus sycomorous* were collected from the garden of Faculty of Pharmacy, University of Gezira while *Trigonella foenum graecum* seeds and *Adansonia digitata* fruits were purchased from the local market in Wad Medani town .All plant samples were identified by the Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira (Table 1).

Table (1): Plants screened for their antibacterial activities and active constituents.

Botanical name/family	Common/vernacular name	Plant part tested
1. *Adansonia digitata* (Malvaceae)	Baobab (Tabaldi)	Fruits
2. *Eucalyptus globulus* (Myrtaceae)	Blue Gum (Ban)	Leaves
3. *Ficus sycomorous* (Moraceae)	Fig-mulberry (Gumez)	Barks
4. *Trigonella foenum graecum* (Fabaceae)	Fenugreek(Helba)	Seeds

METHODS

Extraction of plant materials:

Hundred grams of each powdered plant material were separately extracted by maceration using ethanol (70%) in a conical flask for 72 hours, filtered and evaporated by a rotary evaporator at 60 ^{o}C. The resulting solution was dried at room temperature and kept in a refrigerator until use.

Testing for antibacterial activity:

The cup-plate agar diffusion method was conducted as describe by El-kamali and Moneer, (2006) to assess the antibacterial activity of the prepared ethanolic extracts (500 mg /ml). Microorganisms were sub-cultured from slope agar into nutrient agar, incubated at 37 ^{o}C for 18 hours in an incubator. Wells were made at the centre of each of the four different sectors of the plate, three of them were filled with 100 µl of plant extract and the

other was filled with 100µl of the control (Cefuroxime 100 mg /ml). Each extract was tested against the micro organisms in four replicates. Plates were left for 2 hours at room temperature and then incubated at 37 ºC for 18 hours. The inhibition zones were observed and the results were interpreted by measuring the diameter of clear zones of inhibition recorded as means± standard deviation.

Phytochemical screening:

The major secondary metabolite classes such as alkaloids, tannins, saponins, cardiac glycosides, terpenes and flavonoids were screened according to the common phytochemical methods described by Harborne, (1998) that include:

(a)Test for alkaloids:

To 2 ml ethanolic extract, 1 ml of 1 % hydrochloric acid was added in a test tube, and heated in a water bath for 10 minutes. 1 ml from the acidified solution was taken and 6 drops of Dragendorff's reagent / Wagner's reagent / Mayer's reagent were added and mixed separately. Orange precipitate / brownish-red precipitate / creamish precipitate, respectively, indicate the presence of alkaloids.

(b) Test for tannins:

To 2 ml ethanolic extract, 2 ml of 10% ferric chloride solution wrer added in a test tube. Blue-black precipitate indicates the presence of tannins.

(c) Test for saponins (Frothing test):

To 0.5 ml ethanolic extract, 5 ml distilled water was added in a test tube and vigorously shaken. Persistent froth volume produced, checked each 10 minutes for 30 minutes, indicates the presence of saponins.

(d)Test for cardiac glycosides (keller –kiliani test):

To 2 ml ethanolic extract, 1 ml glacial acetic acid, 6 drops of 10% ferric chloride solution and 6 drops of concentrated sulphuric acid were added in a test tube. Green-blue colour, indicates the presence of cardiac glycosides.

(e)Test for terpenes (liebermman –Burchard reaction):

To 2 ml ethanolic extract, 2 ml acetic anhydride and few drops concentrated Sulphuric acid were added in test tube. Pink- purple ring, indicates the presence of terpenes.

(f) Test for flavonoids:

Test for flavonoids was done by three methods:

- To 2 ml ethanol extract, 0.5 ml concentrated hydrochloric acid and few of magnesium turnings were added in a test tube. Pink-tomato red colour, indicates the presence of flavonoids.
- To 2 ml ethanol extract, 1 ml of 1% potassium hydroxide solution was added in a test tube. Dark yellow colour, indicates the presence of flavonoids.
- To 2 ml ethanol extract, 0.5 ml concentrated Hydrochloric acid and few drops of amyl alcohol were added in a test tube and shaken. Red colour, indicates the presence of flavanoidal glycosides.

RESULTS AND DISCUSSION

Antibacterial activity:

The *in vitro* antibacterial activity of the plant ethanolic extracts (500 mg/ ml) against five bacterial strains is shown in Table 2. Results were compared to Cefuroxime in a concentration of 100 mg/ml being a broad spectrum antibiotic .

As shown in Table 2, *Adansonia digitata*, *Eucalyptus globulus* and *Trigonella foenum graecum* extracts showed a remarkable antibacterial activity against all the tested bacterial strains. Meanwhile, the ethanolic extract of *Ficus sycomorous* exhibited a considerable antibacterial activity against *Proteus mirabilis*, *Pseudomonas aeuroginosa* and *Staphylococcus aureus.* Hwoever it is now effective against *E.cole klebsilla pneanoniae*.

Table (2): *In vitro* antibacterial activity of the four tested plant extracts.

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Preparation	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeuroginosa*	*Staphylococcus aureus*
	Diameter of zone of inhibition in millimeters				
Adansonia digitata fruit (500 mg/ ml)	33	35.7	27	34.3	34.7
Ficus sycomorus (500 mg/ ml)	-	-	18	15.3	44.3
Eucalyptus globulus (500 mg/ ml)	30.3	30.3	28.3	30.7	23
Trigonella foenum grecum (500 mg/ ml)	31.7	31	24.3	27	30.7
Control: Cefuroxime (100 mg/ml)	20	25	46	45	45

The antibacterial activity produced by these plants was similar to those reported by other researchers (Takahashi *et al*., 2004; Adenshina *et al*, 2010; Donatien *et al*., 2011; Premanath *et al*., 2011).

The results of the antibacterial testing of the plant extracts provide valuable information and highlight the potentiality of these plants in drug development as candidate sources of antibacterial agents being safe and edible. More investigation is required to ascertain that the *in vitro* results are attainable *in vivo*.

Preliminary phytochemical screening:

The presence of phytoconstituents in the four plant extracts is illustrated in Table 3. Terpenes, tannins and saponins were detected in all tested plant extracts whereas alkaloids were found in appreciable amounts in *Trigonella foenum grecum* extract and in trace amounts in *Ficus sycomorus* extract. Meanwhile, flavonoids were only found in trace amounts in *Eucalyptus globulus* extract and cardiac glycosides were detected only in *Adansonia digitata* extract.

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The detected phytoconstituents may be responsible for the antibacterial activities of these plant extracts. For instance, saponins, flavonoids and tannins have been reported to inhibit the growth of bacteria (Sodipo *et al*., 1991; Chung *et al*., 1998; Cushine and Lamb, 2005; Lewis and Ausubel, 2006).

Table (3): Preliminary photochemical screening of the investigated plants.

Plant extract	Phytoconstituents					
	Alkaloids	Flavonoids	Terpenes	Cardiac glycosides	Saponins	Tannins
Adansonia digitata	-	-	+	+	+	+
Eucalyptus globulus	-	±	+	-	+	+
Ficus sycomorus	±	-	+	-	+	+
Trigonella foenum graecum	+	-	+	-	+	+

Key: (+) present in appreciable amounts, (±) present in trace amounts, (-) absent.

It can be concluded that the tested plants demonstrating broad spectra of activity which may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of health.

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النشاط المضاد للبكتيريا والمسح النباتي الكيميائي الاولي لاربع نباتات طبية

نزار سراج[1], الهادي محمد محمد احمد[1], انور محمد[2] و محمد ابوبكر[2]

[1] قسم العقاقير الطبية–كلية الصيدلة– جامعة الجزيرة.

[2] قسم الكيمياء الصيدلانية –كلية الصيدلة– جامعة الجزيرة.

الملخص

اجريت هذه الدراسة لاستقصاء النشاط المضاد للبكتريا و المجموعات الوظيفية النشطة احيائياً لاربعة نباتات طبية.اختبر المستخلص الايثانولي المتحصل عليه عن طريق عملية النقع علي اربعة انواع من البكتيريا باستخدام طريقة انتشار الاجار بواسطة الكاس. تم استخدام طرق المسح النباتي الكيميائي الاولية لمعرفة وجود وطبيعة المكونات الفعالة في تلك المستخلصات النباتية.

أظهرت مستخلصات التبلدي, البان والحلبة نشاطاً ملحوظاً ضد كل الانواع البكتيرية المختبرة. أبدي المستخلص الايثانولي نشاطاً معتبراً ضد المتقلبة الرائعة , الزائفة الزنجبارية والمكورات العنقودية الذهبية. أوضح المسح النباتي الكيميائي وجود مركبات تيربينات, التانينات و صابونينات في كل المستخلصات النباتية بينما وجدت القلويدات والجليكوسيدات القلبية بكميات مقدرة في مستخلصي الحلبة والتبلدي علي التوالي.

يمكن أن نستنتج أن النباتات المختبرة تمتلك نشاطاً واسعاً مما يساعد علي اكتشاف مجموعات كيميائية جديدة من المضادات الحيوية تستخدم في المحافظة علي الصحة.

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