Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 12 (7): 219-223
1st October, 2012
DOI: 10.3923/jms.2012.219.223

In vitro Antimicrobial Activity of Sudanese Medicinal Plants

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The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics has lead to the search for new antimicrobial agents mainly from plant extracts with the goal to discover new chemical structures. To overcome these disadvantages, the research was carried out to investigate the *in-vitro* antibacterial and antifungal activity of *Cadaba farinose* (Capparaceae), *Solanum nigrum* (Solanaceae), *Senna occidentalis* (Caesalpiniaceae) and *Maerua oblongifolia* (Capparaceae). Eighty percent Methanol and chloroform extracts of leaves of plants were screened for their antimicrobial activity against different pathogenic bacteria and fungi. These were *Staphylococcus aureus*, *Bacillus subtilus*, *Escherichia coli*, *Salmonella typhi* and *Aspergillus niger* and *Candida albicans* using the cup plate agar diffusion method. All methanol extracts exhibited inhibitory effects against all tested organisms with zones of inhibition ranging from 11-25 mm except the methanol extract of both parts of *Maerua oblongifolia* were active against *Escherichia coli* and *Aspergillus niger*. The results obtained from plants extracts were compared to some of the commercially used drugs. The Minimum Inhibitory Concentrations (MICs) of the most active extracts of these plants against standard bacteria and fungi were also determined and found that MICs a ranging between concentration 2.5-5 mg mL^{-1}. All of the plants extracts were phytochemically screened and triterpenes, saponins and tannins were present in all of the methanolic extracts. Coumarins, sterols and triterpenes were found in all choloroform extracts, this finding indicated that these extracts of such plants promising antimicrobial agents.

Key words: Antimicrobial activity, medicinal plants, phytochemical, Sudan

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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INTRODUCTION

Traditional medicine (TM) refers to the application, approach, knowledge and belief in incorporating plant or animal based properties in remedies, singularly or in combination, for the purpose of treating or preventing disease as well as to maintain the well-being of an individual (Joy *et al*., 2001). The types of biological that are commonly used as sources of biochemical or test resources for bio-discovery programs are microbial, marine organisms and plants. Insects and animals also have limited applications. Paradoxically, the plant genome is much larger than other natural sources (Bacteria: 1000 genes, Fungi: 10,000 genes, Plants: greater than 100,000 genes) and, therein offers a broader biochemical network for the generation of unique and novel chemical structures (Ecker and Crooke, 1995). Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds (Cragg and Newman, 2001) i.e., any part of the plant may contain active components like, alkaloids, flavanoids, glucosides, tannins, gums, resins, essential oils, fatty oils, carbon compounds, hydrogen, oxygen, nitrogen salts of some chemicals and others few of these chemicals are toxic with residual effects. Hence, preparation and administration of plants drugs should be done by experts only. Therefore, an extensive study is required to detect the medical properties of the plant. Several medicinal plants have been tried against pathogenic microorganisms (Haraguchi *et al*., 1999; Sashi *et al*., 2003). For this and various other reasons, drug discovery from plant and hence research in the area has got better appreciation and concern, nowadays. In Sudan a lot of medicinal plants have not yet been explored for their antimicrobial activity. Therefore, this study aims to screen the antibacterial and antifungal activity of four Sudanese plants using two solvents and comparing the result with Gentamicin, Ciprofloxacin and Nystatin as standard references drugs. Also aims to screen the phytochemical compound of these extracts.

MATERIALS AND METHODS

Collection and Identification of plants specimen: Four Sudanese medicinal plants were collected from February to May 2012 from Al-Gazira state. Identification was done by Dr. Haidar Abd Algadir, Plant Taxonomist, Herbarium Curator, Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum, Sudan. Identified as *Cadaba farinose* Forssk, *Solanum nigrum* L. *Senna occidentalis* (L.) Link and *Maerua oblongifolia* (Forssk).A.Rich.

The leaves were dried in the shade to prevent cells from sun light which destroy the cell for 1 week until a constant weight were obtained and ground to powder using mortar and pestle.

Extracts preparation: Samples were ground into fine powder then extraction was done using maceration procedures. In accordance with such method 50 g of the powdered leaves were macerated successively in chloroform and 80% methanol and kept for 5 days at room temperature with occasional shaking. Each mixture was then filtered and the filtrate was evaporated to dryness in an evaporating dish on a steam bath at a temperature of 70°C. The process was repeated four times with intervals of 5 days (Hagerman, 1987). These extracts were stored in screw-capped bottles and kept in the laboratory refrigerator.

Antimicrobial activity of plant extracts: A test stock concentration of 10 mg mL^{-1} for methanol/H_2O (80:20) extracts were prepared by dissolving 0.1 g of each extract in 10 mL of methanol in separate test tubes and choloroform extracts were dissolved in petroleum ether: methanol (1:2).The antimicrobial activities of each of the methanol/H_2O (80:20) and chloroform were tested against standard Gram positive bacteria (*Staphylococcus aureus* American Type Culture Collection ATCC 25923 and *Bacillus subtilus* National Culture Type Collection NCTC 8236), Gram negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella typhi* NCTC 0650) and fungi (*Aspergillus niger* ATCC 9763 and *Candida albicans* ATCC 7596) using agar well diffusion method (NCCLS, 2000) and the resultant inhibition zones were measured and tabulated as means. The zones were measured with a transparent ruler and the result recorded in millimeters. The screening was done in triplicates. Negative controls involving the addition methanol instead of the extracts were included.

Minimum inhibitory concentration (MIC): MICs were carried out according to the method described by Hirasawa *et al*. (1999). Different concentrations (2.5, 5, 10 and 20 mg mL^{-1}) were prepared using sterile distilled water as the diluents. Again, the agar well diffusion method was used. The test was carried out in duplicate and the mean recorded.

Antimicrobial activity of the standard reference drugs: Three antibiotics were used as standard reference drugs. They included two antibacterial drugs (Ciprofloxacin and Gentamicin) and Nystatin as antifungal drug. Antibacterial drugs were tested at different concentrations obtained by

taking 0.1 g of each powdered drug and dissolved in 100 mL sterile distilled water to give a concentration of 1000 μg mL^{-1} followed by serial dilutions to give concentrations of 5, 10, 20 and 40 μg mL^{-1}. There drugs were tested against standard bacteria i.e., *Staphylococcus aureus*, *Basillus subtilus*, *Escherichia coli* and *Salmonella typhi*. The antifungal drug was also tested at different concentrations obtained by taking 0.1 g of powdered drug and dissolved in 100 mL sterile distilled water to give a concentration of 1000 μg mL^{-1} followed by serial dilutions to give concentrations of 12.5, 25 and 50 μg mL^{-1} of Nystatin against standard fungi *Aspergillus niger* and *Candida albicans*. Clotrimazole was also tested.

Phytochemical screening: The dried extracts were reconstituted in the solvent used for their extraction and subjected to qualitative chemical screening to identify the presence of a variety of phytoconstituents. The methods used have been described by Harborne (1984) and Sukhdev *et al.* (2008), to identified the following chemical classes: alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, coumarins and anthraquenones.

RESULTS

The results showed that methanol was the best solvent for extracting antimicrobial substances from the tested plants (Table 1). This finding was based on the number of pathogenic microorganisms inhibited and the diameter of inhibitory zones produced. It was also observed that, *Bacillus subtilus* was the most sensitive microorganism inhibited by all extracts followed by *Salmonella typhi* and *Escherichia coli* were inhibited by seven extracts (87.5%) and *Staphylococcus aureus* and *Aspergillus niger* inhibited by six extracts (75%). While *Candida albicans* was the most resistant organism inhibited by four extracts (50%) (Table 1). Futhermore, all of the methanol extracts exhibited inhibitory activity against the entire tested organism with zones of inhibition ranging from 11-25 mm except the methanol extracts of *Cadaba farinose* not active against *Candida albicans* and *Maerua oblongifolia* was effective against *Bacillus subtilus, Escherichia coli* and *Aspergillus niger*. Methanol leave extracts of *Solanum nigrum* was superior and active against all of the tested microorganisms with high inhibition zone of *Salmonella typhi* 25 mm. Chloroform extracts of all plants samples were found less active against all of the tested microorganisms as compared to methanol extracts. From Table 2, 3, the results obtained indicate that these plants were effective against pathogenic bacteria and fungi in comparing with reference drugs.

The Minimum Inhibitory Concentrations (MICs) of the most active extracts were determined against reference organisms (*Staphylococcus aureus*, *Bacillus subtilus*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans* and *Aspergillus niger*) it was found that MICs a ranging between concentration 2.5-5 mg mL^{-1}.

The result of phytochemical screening, showed that flavonoids, alkaloids, saponins, tannins, sterols, triterpenes and coumarins were present in some of these plants with different in concentration (Table 4).

DISCUSSION

The result obtained in this study agree with Prescott *et al.* (2008) whom reported that, the antimicrobial activity of medicinal plants and drugs varies in their inhibitory effect, depending on the concentration in crude extracts or synthetic drug, size of inoculums, temperature, nature of organism and rate of diffusion. Medicinal plants produce slow recovery; the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms (Seyyednejad and Motamedi, 2010). The inhibitory activities exhibited by the extracts tend to agree with the reports of Jayaveera *et al.* (2010) and

Table 1: Antimicrobial activity of plant extracts against certain reference microorganisms

			Test organisms used MDIZ (MM)*						
			Bacteria					Fungi	
Name of the Plants	Family	Parts used	Solvents used	*S.a*	*B.s*	*E.c*	*S.t*	*As.n*	*Ca.a*
Solanum nigrum L.		Leaves	Chloroform	-	15	20	14	15	15
	Solanaceae		Methanol 80%	15	15	15	25	19	15
Senna occidentalis (L.)		Leaves	Chloroform	11	14	-	12	-	15
	Caesalipiniaceae		Methanol 80%	15	15	16	15	17	15
Cadaba farinose		Leaves	Chloroform	14	13	20	15	-	-
	Capparaceae		Methanol 80%	14	13	13	15	12	-
Maerua oblongifolia		Leaves	Chloroform	13	13	15	14	15	-
	Capparaceae		Methanol 80%	-	15	15	-	15	-

S.a: Staphylococcus aureus, *B.s*: *Bacillus subtilus*, *E.c*: *Escherichia coli*, *S.ty*: *Salmonella typhi*, *Ca.a: Candida albicans* and *As.n: Aspergillus niger*,
**M.D.I.Z: Mean Diameter of Inhibition Zones (mm) MDIZ >18 Sensitive; 14-18 Intermediate; <14 = Resistant (-) No activity

El-Mahmood *et al.* (2008) all of whom linked antimicrobial properties of plants to the presence of bioactive secondary metabolites. For example, Sadiq *et al.* (2012) investigated the antibacterial activity of leaf extracts of *Cassia occidentalis* (L.) and reported that the bioactive compounds found in the plant inhibited the growth of *S. typhi*, *E.coli*, *P. aeruginosa*, *Staphylococcus aureus* and *Shigella* spp and that the ethanol extract was most active. The earlier report of Doughari and Okafor (2007) revealed that methanol extract of *Senna alata* leaf control the growth of *S. typhi* moderately (Zone of inhibition 8 mm). So these results are reconfirming the use of multiple solvents for the extraction of chemical compounds from the natural materials may help to derive of one or more active principles from the crude extracts. The activity of the plant extracts against bacteria is an indication of the presence of broad or narrow spectrum antibiotic compounds or simply metabolic toxins in the plant (Parekh and Chanda, 2007). The activity of extracts also varied based on the mode of preparation of the extracts either simple soaking the plant powder in respective solvents at room temperature or extracted by using Soxhlets at solvent dependent temperature. Antibacterial activities shown by plants samples (Table 1), is due to the presence of bioactive substances in these plants (Table 4) such as alkaloids, flavonoids, saponins, sterols, triterpens, tannins, coumarins and the solubility of those active compounds in the solvent used. It could also be seen from Table 1 that, plants used in this study different extracts were different in their antimicrobial efficacy depending on the extractive solvent used. This result agrees favourably with the suggestion of Oloke and Kolawole (1998) that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvents used. The result supports the traditional use of *Cadaba farinose*, *Solanum nigrum*, *Senna occidentalis* and *Maerua oblongifolia* for the treatment of various infectious diseases in different regions of the Sudan. The study also showed that these plants may be good as an antibacterial and antifungal recipe. More work is needed to isolate the bioactive components of these plants.

Table 2: Antimicrobial activity of reference drugs against standard pathogenic bacteria

		Standard bacteria **MDIZ (mm)			
Drugs used	Concentration (μg mL^{-1})	*S.a*	*B.s*	*S.ty*	E.c
Gentamicin	40	20	19	29	22
	20	18	17	28	18
	10	16	15	27	15
	5	15	14	25	11
Ciprofloxacin	40	26	26	25	30
	20	21	25	20	25
	10	18	24	20	23
	5	16	22	14	21

S.a: *Staphylococcus aureus*, *B.s*: *Bacillus subtilus*, *E.c*: *Escherichia coli*, *S.ty*: *Salmonella typhi*, *Ca.a*: *Candida albicans* and *As.n*: *Aspergillus niger*, **M.D.I.Z: Mean Diameter of Inhibition Zones (mm) MDIZ >18 Sensitive; 14-18 Intermediate; <14 = Resistant (-) No activity

Table 3: Antifungal activity of certain drugs used against the reference pathogenic fungi

		Standard fungi **MDIZ (mm)	
Drugs used	Concentration (μg mL^{-1})	*Candidia albicans*	*Aspergilus niger*
Clotrimazole	20	43	24
	10	33	19
	0	30	16
Nystatin	50	28	17
	25	28	14
	12.5	23	-

M.D.I.Z.(mm): Mean diameter of inhibition zone (mm), M.D.I.Z.>18 Sensitive M.I.Z.D = 14-18 mm Intermediate <14 = Resistant -: No Inhibition

Table 4: Phytochemical screening of plant extracts

Name of the plants and family	Parts used	Sovent used	Fla	Alk	Sap	Tan	An	Cou	Tri	Ste
Solanum nigrum L.	Leaves	Chloroform	-	-	-	-	-	+	+++	+++
Solanaceae		Methanol 80%	-	+	+++	+	-	-	+	-
Senna occidentalis (L.)	Leaves	Chloroform	-	-	-	-	-	+	+++	+++
Caesalipiniaceae		Methanol 80%	-	+	+++	+++	-	-	+	-
Cadaba farinose	Leaves	Chloroform	-	-	-	-	-	+	+++	+++
Capparaceae		Methanol 80%	+	+++	+	++	-	-	+	-
Maerua oblongifolia	Leaves	Chloroform	-	-	-	-	-	+	+++	+++
Capparaceae		Methanol 80%	+++	+++	+	+++	-	+	+	++

Functional group: Fla: Flavonoids, Alk: Alkaloids, Sap: Saponins, Tan: Tannins, Ste: Sterols, Cou: Coumarins, Tri: Triterpens and An: Anththraquenones, +: positive reaction trace concentration, ++: moderate concentration, +++: High concentration and -: negative reaction

ACKNOWLEDGMENT

The Corresponding Author would like to express her appreciation to traditional healer Suraj Ibrahim Suliman, who collected the plants.

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