SCREENING FOR ANTIMICROBIAL ACTIVITY OF SOME PLANTS USED IN FOLKLORIC MEDICINE IN THE SUDAN

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المستخلص

أجريت هذه الدراسة لمعرفة مدى فعالية مستخلصات الإيثانول، الماء والبترولیوم إيثر لأربعة نباتات تستخدم في الطب الشعبي في السودان وهي ثمار شجرة السنط (القرض)، أوراق شجرة الجوافة، أوراق شجيرة الحناء والمجموع الخضري لشجيرة الطندب ضد خمسة انواع من البكتريا (العصوية الرقيقه، المكورة العنقودية الذهبية، الإشريشية القولونية، الزائفة الزنجارية والكلبسيلة) ونوعين من الفطريات (عفن الرشاشية السوداء وخميرة المبيضة البيضاء).

باستخدام طريقة الانتشار في الأجار المثقب فى طبق بتري تم اختبار فعالية المستخلصات النباتية بتراكيز5%, 10%, 15% و20%. قورنت فعالية هذه المستخلصات بفعالية بعض المضادات الحيوية المعروفة.

أظهرت مستخلصات القرض أعلى فعالية، تلتها مستخلصات أوراق الجوافة. أظهر المستخلص الإيثانولي للنباتات الأربعة المستخدمة أعلى فعالية، تلاه المستخلص المائي.

العصوية الرقيقة كانت اكثر حساسية للمستخلصات المستخدمة، تلتها المكورة العنقودية الذهبية ثم الإشريشية القولونية. من الفطرين المختبرين، كانت خميرة المبيضة البيضاء أكثر حساسية مقارنة بعفن الرشاشية السوداء والتي أظهرت حساسية فقط مع المستخلص الإيثانولي والمائي للقرض. أظهرت فعالية المستخلصات، بصورة عامة، تناسباً طردياً مع تركيزها.

وجدت فعالية المستخلصات النباتية ضد البكتريا، في معظم الحالات، أعلى من فعالية بعض المضادات البكتيرية شائعة الاستخدام، بينما كانت فعاليتها ضد الفطريات أقل من فعالية بعض المضادات الفطرية المعروفة.

Keywords: Acasia nilotica, Antimicrobial; Cup-plate agar diffusion, Medicinal plant extracts; Sudanese folkloric medicine.
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أوضحت هذه الدراسة أنّ المستخلص الايثانولي لثمار شجرة السنط (القرض) هو أقوي المستخلصات فعالية ضد البكتريا والفطريات ويرشح لدراسات متقدمة كمصدر لإيجاد مضادات بكتيرية وفطرية.

Abstract

The present study was carried out to evaluate the antibacterial and antifungal activity of ethanol, water and petroleum ether extracts of four plants used in Sudanese folkloric medicine. The plants tested were Acacia nilotica fruits, Psidum guajava leaves, Lawsonia inermis leaves and Capparis decidua shoot. The plant extracts were tested at four concentrations, 5%, 10%, 15% and 20%, and their antimicrobial activity was assessed using the cup-plate agar diffusion method against five bacteria (Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiela pneumoniae) and two fungi (Aspergillus niger and Candida albicans). Activity of the plant extracts was compared with that of known antibacterial and antifungal agents.

Acasia nilotica extracts showed the highest activity against both bacteria and fungi, followed by those of P. guajava. Ethanolic extract of the four plant species was the most active extract type against all organisms tested, followed by aqueous extract. B. subtilis was the most susceptible bacterium followed by S. aureus and then E. coli. Of the two fungal species, C. albicans was much more susceptible than A. niger, which showed susceptibility only to ethanolic and aqueous extracts of A. nilotica. The activity of extracts is generally increased with their concentrations. Antibacterial activity of the plant extracts was found, in most cases, superior to that of some known antibacterial agents, while their antifungal activity was inferior to that of some antifungal agents.

This study showed that ethanolic extract of Acasia nilotica (fruits) is an excellent candidate to be considered for further studies as source of both antibacterial and antifungal agents.

Introduction

The importance of alternative medical therapy is increasing due to increase in emergence of multi-drug resistant infectious organisms, high

cost of synthetic compounds as well as undesirable side effects of certain drugs. Herbal medicine, some-times referred to as herbalism or botanical medicine is the use of herbs for their therapeutic value (Lai, 2004). Herbal plants produce and contain a variety of chemical substances that act upon the human body. A number of higher plants have been used for centuries as remedies for diseases (Lewington, 1993). This has encouraged the scientists to screen higher plants for various biological activities including antimicrobial effects. Many studies indicated that some plants contain many substances such as peptides, aldehydes, alkaloids, some essential oils, phenols and some other compounds which are with potentially significant therapeutic application against human pathogens, including bacteria, fungi or viruses (Jantan et al., 2003; Holetz et al., 2002; Horvath et al., 2002; Khan et al.**,** 2003; Perez, 2003).

Traditional medicine in the Sudan has roots in cultures of all tribes. People in many areas of the country depend on herbal medicines, which are integral part of the health care system and there is a wide experience with the use of herbs in medical treatment. The Medicinal and Aromatic Plants Research Institute was founded 25 years ago and has trained a considerable number of specialists in different fields required for research in medicinal plants. Sudan Atlas of medicinal plants record the scientific name of more than 2000 medicinal herbs collected from different parts. All of these herbs are in current use in traditional medicine (WHO, 2001). This study was carried out to evaluate the antimicrobial activity of aqueous, ethanolic and petroleum ether extracts of four Sudanese folkloric medicinal plants (Acacia nilotica [fruits], Lawsonia inermis [leaves], Capparis decidua [shoot] and Psidum guajava [leaves]) against some bacteria and fungi.

Materials and Methods

2.1. Plant materials

Four of the local Sudanese medicinal plants were selected to be tested for their antimicrobial activity. Their names and parts used were: Acacia nilotica (fruits), Lawsonia inermis (leaves), Capparis decidua (shoot) and Psidum guajava (leaves). The plants were collected from Khartoum State in January 2008, then identified and authenticated in the Department of Botany, Medicinal and Aromatic Plant Research Institute, National Center

for Research, Sudan. They were air-dried at room temperature and then coarsely powdered and kept at room temperature until their use.

2.2. Preparation of the petroleum ether and ethanolic crude extracts
By using balance type H6T (Mettler, England), 50 g of A. nilotica, 40 g of L. inermis, 50 g of C. decidua and 60 g of P. guajava powders were weighed. Then each powder was put into Soxhlet apparatus (Grant Instrument Ltd, England) and exhaustively extracted for about 4 h with petroleum ether (boiling point 40-60 °C, Merck, Germany). The petroleum ether extract was filtered off through Whatman's grade No. 1 filter paper and evaporated under reduced pressure using rota-vapor (Grant Instrument Ltd, England). Then the plant material in the Soxhlet was taken out to evaporate the remaining petroleum ether and then put back and exhaustively extracted with ethanol (BDH, England) for about 12 h. The ethanolic extract was then filtered and evaporated under reduced pressure using rota-vapor.

2.3. Preparation of the aqueous extracts
One liter of distilled water was added to 100 g of each plant powder in a conical flask and left for about 4 h at room temperature. Extract was then filtered using Whatman grade No. 1 filter paper and stored in a deep freezer till its use. Before use, the extract was freeze-dried using freeze-dryer apparatus (Virsta, USA) till all of the ice was removed out and a powdered extract was obtained.

For each of the three extracts of each of the four plants, yield percentage was calculated as follows:

$$\frac{\text{Weight of dry extract (g)}}{\text{Weight of plant powder used (g)}} \times 100\%$$

2.4. Preparation of culture suspensions of test organisms
Reference strains and methods of preparation of culture suspensions of test organisms were obtained from the Medicinal and Aromatic Plant Research Institute, National Center for Research, Sudan.

2.5 Bacterial culture suspensions

One millilitre aliquots of 24 h broth culture of test organisms (Bacillus subtilis (NCTC 8236), Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 35657), Pseudomonas aeruginosa (ATCC 27853) and Staphylococcus aureus (ATCC 25923) were aseptically added to Nutrient agar slopes and incubated (Griffin and George Ltd, England) at 37 °C for 24 h. The bacterial growth was harvested and washed off by addition of sterile normal saline. The harvest was suspended in a suitable volume of normal saline to produce a suspension containing about 10^8-10^9 CFU/ml. The suspension was stored in the refrigerator at 4 °C till its use. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938).

2.6. Preparation of fungal culture suspensions

Cultures of Aspergillus niger (ATCC 9763) and Candida albicans (ATCC 7596) were grown on Sabouraud Dextrose agar, and incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and then suspended in 100 ml of sterile normal saline and the suspension was stored in refrigerator till its use.

2.7 Preparation of concentrations of extracts

Four different concentrations in 10ml-volumes (5%, 10%, 15% and 20%) were made from each dry extract with the respective solvent except the ethanol was replaced by methanol (BDH, England). The different concentrations were then kept in refrigerator till their use.

2.8 Testing of plant extracts for antibacterial activity

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted to assess the antibacterial activity of the prepared extracts. Two-millilitres of each bacterial stock suspension were mixed with 200 ml of sterile molten Nutrient agar. Twenty millilitres aliquots of the inoculated Nutrient agar were distributed in sterile Petri dishes. The agar was left to set and four cups (10 mm in diameter) were cut using sterile cork porer (No. 4) in each plate and the agar discs were removed using a sterile wire loop. Each cup was filled with 100 µl of one of the four extract concentrations using microtiter pipette and the extract was allowed to diffuse at room temperature for 2 h. Two replicates were carried out for each extract against each of the bacterial organisms. The plates were then incubated at

37 °C for 18 h. simultaneously, in separate Petri dishes; cups were made for each solvent to see its effect on growth of each organism. After incubation, the diameters of the growth inhibition zones were measured, and the average values were tabulated.

2.9. Testing of plant extracts for antifungal activity

The same method as for bacteria was adopted. Instead of Nutrient agar, Saboraud Dextrose agar was used. The molten medium was inoculated with the specific organism and the medium was incubated at 25 °C for 48 h for C. albicans and three days for A. niger.

2.10. Sensitivity testing of test organisms with some common antibiotics

Four concentrations (40, 20, 10 and 5 µg/ml) from Ampicillin, Gentamicin, Cloxacillin and Benzyl penicillin as antibacterial agents and three concentrations (50, 25 and 12.5 µg/ml) from Clotrimazole and (20, 10 and 5 µg/ml) from Nystatin as antifungal agents were prepared in sterile distilled water. The same method which was used to determine the antimicrobial activity of plant extracts was adopted. An average of diameter inhibition zones of each concentration of each antibiotic was calculated and then compared with that of plant extracts.

Results

3.1. Antimicrobial activity of plant extracts

Results of inhibition zones of plant extracts on both bacteria and fungi tested (Table 1) indicated that the ethanolic extract of each plant was, in general, the most active extract type followed by the water extract. The activity of extracts is generally increased with their concentrations. Extracts of Acacia nilotica fruits were the most powerful and those of Capparis decidua are the least powerful. Ethanolic extract of A. nilotica showed high to very high activity against all bacteria even at lowest concentrations. B. subtilis was the most susceptible bacterium to extracts followed by E. coli and the least susceptible organism was K. pneumoniae.

The antifungal activity of the plant extracts was far less than their antibacterial activity, especially against A. niger, which showed only low to moderate susceptiblity to only A. nilotica (ethanolic and water extract).

C. albicans was most susceptible to A. nilotica ethanolic and water extracts, followed by ethanolic extract of P. guajava.

3.2. Comparison between the antimicrobial activity of plant extracts and antibiotics

Results of the antibiotic sensitivity of bacteria and fungi tested (Tables 2 and 3) displayed that the plant extracts were, in most cases, of higher antibacterial activity than antibiotics. However, the antifungal activity of the plant extracts was lesser than that of antifungal agents.

Yield percentage of plant extracts varied between plant species and solvent type. The highest yield percentage was obtained from ethanolic extract of each plant and the lowest was from petroleum ether (Table 4).

Discussion

Plants are important source of potentially useful chemical compounds for the development of new therapeutic agents. The first step towards this goal is the in vitro antimicrobial activity assay. This study was designed to evaluate the antimicrobial activity of ethanolic, aqueous and petroleum ether extracts from four Sudanese folkloric medicinal plants against five bacterial and two fungal species.

In general, crude extracts from the four plants showed different degrees of activity. This could obviously be attributed to the differences in the chemical constituents of these plants. The finding that A. nilotica extracts had the highest activity against both bacterial and fungal species was also reported by Abdel Nabi et al. (1992) and Ali et al. (2001). Duke (1983) and El Ghazali et al. (1994) mentioned that A. nilotica extracts were used for the treatment of diarrhoea, which is commonly caused by E. coli (Jawetz et al., 2001). In this study E. coli was found susceptible especially to A. nilotica. Aspergillus niger showed no response to all extracts except ethanolic and aqueous extracts of A. nilotica, Such antifungal activity of A. nilotica was also reported by Umalker et al. (1976), who mentioned that A. nilotica inhibited at least four species of pathogenic fungi. Resistance of A. niger could be attributed to its possible high tolerance to osmotic pressure, acidic environments (pH as low as 5.0) of extracts, structural factors or the extracts may not contain potential compounds against it. The broad antimicrobial action of the ethanolic and aqueous extracts of A. nilotica fruits could be ascribed to presence of tannins in the

fruits of A. nilotica (Elkhalifa et al., 2005). Sotohy et al. (1997) reported that the number of total bacteria in the rumen of goats decreased significantly when the animals were fed tannin-rich plant (Acacia nilotica). Tannins are most effective component of this plant against bacteria (McSweeney et al., 1999). The activity of A. nilotica might also be due to presence of other components such as gallic acid and m-digallic acid (El Ghazaly et al., 1994).

Ethanolic extract of P. guajava leaves had also showed a broad antimicrobial action. This high activity could be attributed to presence of alkaloids, tannins, saponins, cardenolides with steroided rings (steroidal nucleus) and cardenolides with deoxy sugar (cardiac glycosides) (Elekwa et al., 2009). P. guajava extracts also contain tannin as well as essential oil (Morton, 1987). Taylor (2005) and Morton (1987) found that P. guajava was effective against a number of diseases; at least some of them are caused by organisms used in this study.
Petroleum ether extracts showed lower action as antimicrobial agents. This may be due to little diffusion properties of these extracts in the agar or because fresh plants contain active substances which may be affected or disappeared by the steps of extraction methods or the active components of these plants may be polar compounds.

The result of antimicrobial activity of Henna (Lawsonia inermis) was similarly reported by Revil et al. (1988). Activity of Henna against C. albicans was also noted by Bosoglu et al. (1998). The effect of Henna may be due to action of one or more of the following constituents: lawsone, various phenolic glycosides, coumarins, and tannin-gallic acid (Amelio, 1999).

Caparis decidua has the lowest antimicrobial action amongst the plants tested. It showed moderate activity against E. coli and B. subtilis. However, it was found to be useful for treatment of some diarrheal causes (Von Maydell, 1986). E. coli is a common cause of diarrhoea; this may support its moderate susceptibility to effect of this plant. This low activity might be due to the low concentration of active components in the extracts or components have little or no antimicrobial activity against test organisms.

The higher antibacterial action of some plant extracts compared to that of antibiotics was clearly indicated that some potential antibacterial chemical compounds are present in these extracts. Also, it may indicate that these bacterial species became resistant or less susceptible to the antibiotics used. This finding is especially important in case of difficult to treat bacteria such as P. aeruginosa. This and other results should encourage researchers to conduct further studies to determine the active constituents and to study their safety in vivo so as to be exploited to manufacture, at the end, new antibiotic agents.

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Table I. Inhibition zone of some plant extracts at various concentrations on some microorganisms.

Extracts		**Inhibition zone (mm)**						
Plant species	**Conc.**	**Bacteria**					**Fungi**	
Ethanolic extracts		S. aureus	B. subtilis	E. coli	P. aeruginosa	K. pneumoniae	C. albicans	A. niger
A. nilotica	20%	25	32	40	35	31	28	13
P. guajava		29	28	28	28	28	28	-
L. inermis		30	32	25	25	23	15	-
C. decidua		20	15	18	25	17	12	-
A. nilotica	15%	32	31	30	30	30	25	13
P. guajava		26	25	25	25	25	20	-
L. inermis		25	30	25	20	23	15	-
C. decidua		18	16	16	20	17	11	-
A. nilotica	10%	30	30	30	30	28	21	11
P. guajava		25	25	25	25	25	18	-
L. inermis		25	28	25	18	20	13	-
C. decidua		14	17	18	18	18	11	-
A. nilotica	5%	30	31	25	24	23	19	11
P. guajava		16	20	23	18	18	15	-
L. inermis		11	25	25	13	20	15	-
C. decidua		-	15	-	13	11	-	-
Aqueous extracts								
A. nilotica	20%	31	27	30	30	27	29	13
P. guajava		18	19	23	19	22	-	-
L. inermis		26	23	23	27	24	19	-
C. decidua		15	16	22	15	15	11	-
A. nilotica	15%	28	25	25	26	27	22	15
P. guajava		16	19	20	20	24	-	-
L. inermis		25	21	21	25	22	19	-
C. decidua		-	18	18	-	13	-	-
A. nilotica	10%	26	25	25	25	25	21	15
P. guajava		16	19	20	20	24	-	-
L. inermis		20	21	20	25	20	18	-
C. decidua		-	-	12	-	-	-	-
A. nilotica	5%	25	21	20	21	21	20	14
P. guajava		15	14	11	15	16	-	-
L. inermis		18	20	16	20	15	16	-
C. decidua		-	-	-	-	-	-	-

Table 1. (cont.)

Petroleum ether extracts								
A. nilotica	20%	-	14	-	-	-	-	-
P. guajava		14	23	-	-	-	-	-
L. inermis		-	15	-	-	-	13	-
C. decidua		-	16	-	-	-	-	-
A. nilotica	15%	-	12	-	-	-	-	-
P. guajava		11	22	-	-	-	11	-
L. inermis		-	13	-	-	-	-	-
C. decidua		-	13	-	-	-	-	-
A. nilotica	10%	-	11	-	-	-	-	-
P. guajava		-	20	-	-	-	-	-
L. inermis		-	-	-	-	-	-	-
C. decidua		-	11	-	-	-	-	-
A. nilotica	5%	-	-	-	-	-	-	-
P. guajava		-	-	-	-	-	-	-
L. inermis		-	-	-	-	-	-	-
C. decidua		-	-	-	-	-	-	-

Table 2. Inhibition zone of some antibacterial agents on some bacterial species.

Drug		**Inhibition zone (mm)**				
Name	Conc. (µg□ml)	B. subtilis	S. aureus	E. coli	K. pneumoniae	P. aeruginosa
Ampicillin	40	16	25	-	-	-
	20	14	20	-	-	-
	10	13	18	-	-	-
	5	12	15	-	-	-
Benzyl penicillin	40	-	38	-	-	-
	20	-	33	-	-	-
	10	-	28	-	-	-
	5	-	24	-	-	-
Cloxacillin	40	-	29	-	-	-
	20	-	27	-	-	-
	10	-	22	-	-	-
	5	-	18	-	-	-
Gentamicin	40	30	20	-	-	-
	20	20	16	18	13	16
	10	16	14	15	-	12
	5	16	12	11	-	-

Table 3. Inhibition zone of two antifungal agents on two fungal species

Drug		**Inhibition zone (mm)**	
Name	Conc. (µg□ml)	Aspergillus niger	Candida albicans
Clotrimazole	20	24	43
	10	19	33
	5	16	30
Nystatin	50	17	28
	25	14	28
	12.5	-	23

Table 4. Yield percentage of plant extracts

Plant species	**Yield percentage**		
	Ethanol	Water	Petroleum ether
Acacia nilotica	13.4	8.5	3.5
Psidum guajava	26.8	2.6	2.0
Lawsonia inermis	19.1	2.7	4.4
Caparis decidua	11.2	4.4	1.1