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Nawal Mohamed Ibrahim Elnagar
(a) Chemistry Department, Faculty of Education, University of Kordofan. Sudan
(b) Chemistry Department, Faculty of Science, University of Hafr Albatin. KSA, Sudan

Barakat Mohammed Modawi
Chemistry Department, Faculty of Science, University of Khartoum. Sudan

Correspondence
Nawal Mohamed Ibrahim Elnagar
(a) Chemistry Department, Faculty of Education, University of Kordofan. Sudan
(b) Chemistry Department, Faculty of Science, University of Hafr Albatin. KSA, Sudan

Evaluation of the antimicrobial activities of root bark and leaves extracts of *Ziziphus spina Christi* grown in Sudan

Nawal Mohamed Ibrahim Elnagar, and Barakat Mohammed Modawi

Abstract
The antimicrobial activity of *Zizyphus Spina Christi* roots bark and leaves different extracts (petroleum ether, chloroform, ethanol and water) and sub fraction extracts of ethanol extract (petroleum ether, chloroform, ethyl acetate and n-butanol), were examined for their antimicrobial activity using cup-plate-agar diffusion method against four bacteria species, (staphylococcus aura, (S.a) Bacillus subtilis (B.s), Pseudomonas aerugionosa (Ps.a) and Esherichia coli (E.coli)), and two fungal species; (Candida albican (Ca.a) and Asparagellus Nigar (Asp.n)). Most extracts had considerable activity against tested organisms. The results obtained from the diameters of the growth inhibition zones of microorganisms (bacteria and fungi tested) provided good evidences that the ethanol crude extract of the root barks of zizyphus spina Christi, could be consider as promising source of antimicrobial ingredients.

Keywords: *Zizyphus Spina Christi*, root bark and leaves, antimicrobial activity

Introduction
Throughout human history, natural products have been widely used as remediesto cure and treat various illnesses. Humans are continuously learning more about the indispensible therapeutic properties of natural products as well as becoming conscious of the importance of a healthy life style-to gain life quality [1-5].

An impressive amount of natural substances has been high-lighted by the mediadue to their wide-ranging properties. Among these substances are the extracts of leaves and roots bark of *Zizyphus Spina Christi* [3, 6].

The use of natural products as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects. Infectious diseases account for about half of the death in tropical countries [7].

Natural products isolated from higher plants have been providing novel, clinically active drugs. The key to the success of discovering naturally occurring therapeutic agents rests on bioassay-guided fractionation and purification procedures [8].

Screening of both synthetic organic compounds and extracts of natural products has been an impressive history of identifying active agents [8].

The occurrence of fungal diseases is a serious problem of the present medicine because of the development of drug resistance against the antifungal activities in the pathogen. As compared to antibacterial antibiotics, there are only a few antibiotics which are used against fungal infection. Because of the serious problem of the development of resistance against the known antifungals, a great demand of some alternative chemotherapeutic agents was an argent necessatity. The possibility of getting certain active substances from plants is immense and earlier workers had isolated and characterized antifungal activity from various plants extracts and search of many substances continued to explore their potential [9].

Plants have always been considered a healthy source of life and the therapeutical properties of medicinal plants have been discovered and used since ancient times. Nowadays the therapeutic uses of medicinal plants for the treatment of various ailments represent the best medical solution [10].

Medicinal plants contain a variety of constituents which are known to be biologically active, eliciting a variety of pharmacological actions as evidence of interaction with physiological systems.

Among these pharmacological activities is the ability to interact with neural mechanisms which mediate behaviour in animals and man and to which these plants owe their medicinal potency [11].

The role of medicinal plants in the treatment of diverse disease, is well documented. Among these diseases where these plants have been used include mental and behavioural disorders [11].

Nature is and will still serve as the man's primary source for the cure of his ailments. However, the potential of higher plants as source for new druges is still largely unexplored [12].

Isoprenoids occurring in higher plants are known for their many biological activities. Triterpenoid saponins are widespread in medicinal plants and very often are responsible for their pharmacological effects. There are many plant-derived products containing significant amount of saponins, for example herbal infusions or tinctures [13].

Flavonoid aglycones (mainly methylated derivatives) are naturally accumulated in several medicinal plant species, mainly on their leaf and stem surfaces. Many intensive studies have indicated the increasing importance of flavonoid aglycones as biologically active natural products which emphasizes the importance of further studies on their distribution in medicinal plants and natural cures [14].

The physiological action of glycosides is intimately associated with and due to the aglycone and the role of sugar in the molecule is normally one of stabilization and solublization [15].

Plants are natures "Chemical Factories" providing the richest source of organic chemicals on earth and historically, plants have played an important role in medicine [16, 17].

The practice of herbal medicine has been growing more complex and chemists have been able to process natural substances into pills, tinctures and powders [15].

Some of the natural vegetations are useful for ornamental purposes, while many due to their odoriferous nature are useful in flavouring or as food additives and preservatives [16].

The results of investigation performed in the late 19th and 20th century and the advent of streptomycin and other antibiotics provide the ground for experimentation of a vast number of plants for antibiotic or antimicrobial activities that are useful to man [16].

Materials and Methods

The Plant Material

The plant Z. *spina Christi* was collected from Kordofan area (Sudan) and identified by the Botany department, Faculty of science University of Khartoum. The root bark and leaves were carefully peeled, shade-dried and finally powdered.

Chemicals

The following either analytical grade or carefully purified and perfectly dried chemicals were used. Petroleum ether (pet.ether) [60-80ºC], ethanol, methanol, acetone, dichloromethane (DCM), chloroform, ethyl acetate and n-butanol.

Bacterial Micro-organisms

The test organisms in this work were:

1. Bacillus subtilis (B.s) NCTC 8238
2. Staphylococcus aureus (S.a) ATCC 25923
3. Esherichia Coli (E.coli) ATCC 25922
4. Pseudomonas aeruginosa (Ps.ar) ATCC 27853

Fungal Micro-organisms

1. Candida albican (Ca.a) ATCC 7596
2. Asparagellus nigar (Asp.n) ATCC 9763

The standard organisms were obtained from the National Collection of Type Culture (NCTC), Colindale, England and American Type Culture Collection (ATCC), Rockville, Maryland, USA.

Preparation of the plant Materials

From each powdered plant extracts (leaves and roots bark) a 10% Solution was prepared in the following solvents.

Table 1

Extract	Solvent
Petroleum ether extract	Petroleum ether
Chloroform extract	Mixture of pet ether and methanol(1:2)
Ethanol extract	Methanol
Aqueous extract	Water

Preparation of the test organisms

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100ml of normal saline to produce a suspension containing about (10^8-10^9) colony forming units per ml. and the suspension was stored in the refrigerator at 4ºC until used.

The average number of viable organisms per 1ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938).

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and (0.02) ml volumes (one drop) of the appropriate dilutions were transferred by transfer pipette adjustable volume automatic microtitre pipette onto the surface of dried nutrient agar plates.

The plates were allowed to stand for 2 hours at room temp, for the drops to dry, and then incubated at 37ºC for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02ml) was multiplied by 50 and the dilution factor to give the viable count of the stock suspension expressed as the number of colony forming units. (C.F.U.)/per ml of suspension.

Each time a fresh stock suspension was prepared and all the above experimental conditions were maintained (constant) so that suspension with very close viable counts would be obtained.

Preparation of fungal suspensions

The fungal cultures were maintained on sabouraud dextrose agar, incubated at 25ºC for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

Preparation of the media

28.00g of Nutrient agar were dissolved in one liter of distilled water, sterilized by autoclave at 121 ºC, and 62.0g of Sabouroud dextrose agar dissolved in one liter of distilled water.

***In vitro* Testing of extracts for antimicrobial activity**

Testing for antibacterial activity

The cup-plate-agar diffusion method (Kavaragh 1972) was adopted with some minor modifications, to assess the antibacterial activity of the prepared extracts.

6.4ml of each of the four standardized bacterial stock suspensions (10^8-10^9 C.F.U./ml) were thoroughly mixed with 640 ml of sterile melted nutrient agar which was maintained at 45ºC.

20 ml of aliquots of the inocubated nutrient agar were distributed into sterile 32 petri-dishes. The agar was left to set and in each of these resulting plates, which were divided to two halves, two cups in each half (10 mm in diameter) were cut using a sterile cork borer (No.4). Each one of the 64 halves was designed for one of the extracts.

The agar disks were removed. Alternate cups were filled with 0.1 ml samples of each of the extracts using transfer pipette adjustable volume automatic microtitre pipette, and allowed to diffuse at room temperature for 2 hours.

The plates were then incubated in the upright position at 37 ºC for 24 hours.

Two replicates were carried out for each extract against each of the test organisms simultaneously; (positive) controls involving the addition of the respective solvents instead of the extracts were carried out separately. After incubation the diameter of the resultant growth inhibition zones were measured and mean values were tabulated.

Selection of Extract for further investigation

The results obtained from percentage yield extraction and diameters of the growth inhibition zoons of microorganisms, under investigation, (bacteria and fungi), provided good evidences that the ethanolic crude extract of root bark of zizyphus spina Christi, could be consider as promising source of antimicrobial ingredients. As a result the root bark of zizyhus spina Christi were selected for further studies.

Successive re- extraction of the root bark Ethanol extract

50g of root bark ethanol extract were dissolved in water and subjected to successive extraction in a separatory funnel with solvents in ascending polarity. Extraction was performed first with petroleum ether (60-80 ºC), chloroform, ethylacetate and finally n-butanol [scheme1]. The extracts were dried and carefully weighted (table) and investigated for their antimicrobial activity against bacteria and fungi (table…)

Investigation of the antimicrobial activity of subfractions from solvent solvent extraction

Petroleum ether, chloroform, ethyl-acetate and n-butanol subfractions obtained by solvent solvent re-extraction of the root bark ethanol extract were examined for their antimicrobial activity against bacteria and fungi and the results are in table (5) and (6).

Results

Investigation of the antimicrobial activity of the plant extracts

Leaves and roots bark of Zizyphus Spina Christi extracted in four solvents in ascending polarity, namely, petroleum ether, chloroform, ethanol and water. The extracts were investigated for their antimicrobial activity against four bacterial species, namely, staphylococcus aura, (S.a) Bacillus subtilis (B.s), Pseudomonas aerugionosa (Ps.a) and Esherichia coli (E.coli), and two fungal species; Candida albican (Ca.a) and Asparagellus Nigar (Asp.n). results reported in table 2 and 3.3.2 and figures 3.3.1and 3.3.2.

Table 2: Diameters of the resultant growth inhibition zones for bacterial species

Extraction solvent	Part of the plant used	E.coli (mm)	B.s (mm)	Ps.a (mm)	S.a (mm)
Ethanol	Leaves	30.0	19.5	14.5	17.5
	root barks	30.0	31.0	24.5	20.0
Water	Leaves	29.5	18.5	29.5	15.0
	Root barks	30.0	22.0	18.5	19.5
Petroleum ether (60-80 ºC)	Leaves	11.0	12.5	11.0	11.5
	Root barks	24.5	19.5	11.0	11.0
Chloroform	Leaves	12.0	19.5	12.5	-
	Root barks	11.5	11.5	11.0	11

The above result revealed that, both leaves and root barks ethanol extracts of zizyphus spina Christi possess the heights activities against E.coli, B.s and S.a also the ethanol extract of the roots bark show highest activity against ps.a. of all extracts of this part (24.5mm). water extract of the leaves show highest activity against Ps.a, of all tested organisms (29.5) and that of the two parts show activities about the same as that of ethanol extract. Petroleum ether extract of the root barks show considerable activity against E.coli and to some extent against B.s.

Table 3: Diameters of the resultant growth Inhibition Zones for fungal species

Extraction solvent	Part of the plant used	Ca-a	As-n
Ethanol	Leaves	12.0	12.0
	Root barks	20.5	12.5
Water	Leaves	15.5	11.5
	Root barks	19.5	12.0
Petroleum ether	Leaves	11.5	11.5
	Root barks	20.5	11.0
Chloroform	Leaves	11.5	-
	Root barks	11.0	-

Selection of plant part Extract for further investigation

The results obtained from percentage yield extraction and the diameters of the growth inhibition zones of microorganisms (bacteria and fungi tested) section 3.3, provided good evidences that the ethanol crude extract of the root barks of zizyphus spina Christi, could be consider as promising source

of antimicrobial ingredients. In addition to that the chemistry of root barks of Zizyphus.spina Christi has not been exhaustively studied yet. Thus the root barks of Zizyphus spina Christi were selected for further studies, fractionation and Isolation of pure compounds.

Solvent solvent extraction of roots bark ethanol extract

50g of root bark ethanol extract was subjected to solvent solvent extraction (aqueous-organic) using solvents in descending polarity: petroleum ether, chloroform, ethyl acetate and n-butanol-in a separatory funnel. Extraction repeated with each solvents till colourless solvents were obtained (sec 2.6). Results contained in table 4.

Table 4: weights, and percentages of the extraction yield for solvent solvent extraction of roots bark ethanol extraction

Solvent	Weight (g)	% yield
Petroleum ether	0.06	0.12
Chloroform	0.12	0.24
Ethyl acetate	1.20	2.40
n-butanol	1.01	2.02

Investigation of the antimicrobial activity of sub fractions

Petroleum ether, chloroform, ethyl acetate and n-butanol above sub fractions were examined for their antimicrobial activity (sec 2.7), the results contained in tables 5 and 6

Table 5: Diameters of the resultant growth inhibition zones for bacterial species using sub fractions from solvent solvent extraction

Sub fraction	Diameters of inhibition zones (mm)			
	E.coli	B.s	Ps.a	S.a
Pet. ether sub fraction	-	-	11.0	-
Chloroform sub faction	-	-	-	-
Ethyl acetate sub fraction	12.5	12.0	24.5	12.5
N-butanol sub fraction	17.5	15.0	25.0	16.5
Aqueous sub fraction	13.5	15.0	17.5	20.0

Table 6: Diameters of the resultant growth inhibition zones for fungal species using sub fractions of solvent solvent extraction

Sub fraction	Diameters of Inhibition zones (mm)	
	ca-a	As - n
Pet. ether sub fraction	-	-
Chloroform sub fraction	-	-
Ethyl acetate sub fraction	17.5	11
n-butanol sub fraction	25.5	12.5
Aqueous sub fraction	35.0	15.0

Disucssion

Zizyphus Spina Christi roots bark and leaves different extracts (petroleum ether, chloroform, ethanol and water) and sub fraction extracts of ethanol extract (petroleum ether, chloroform, ethyl acetate and n-butanol). were examined for their antimicrobial activity using cup-plate-agar diffusion method against four bacteria species, (staphylococcus aura, (S.a) Bacillus subtilis (B.s), Pseudomonas aerugionosa (Ps.a) and Esherichia coli (E.coli)), and two fungal species; (Candida albican (Ca.a) and Asparagellus Nigar (Asp.n)). Most extracts had considerable activity against tested organisms.

The result obtained revealed that both leaves and root barks ethanol extracts of zizyphus spina Christi possess the heights activities against E.coli, B.s and S.a also the ethanol extract of the roots bark show highest activity against ps.a. of all extracts of this part (24.5mm). water extract of the leaves show highest activity against Ps.a, of all tested organisms (29.5) and that of the two parts show activities about the same as that of ethanol extract. Petroleum ether extract of the root barks show considerable activity against E.coli and to some extent against B.s.

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References

1. Harbone JB. phytochemical methods, third edition, Chapman & Hall, an imprint of Thomson Science,2-6 Boundary Row, London ESI 8HN, UK, 1998.
2. Peter B, Kaufman Leland J. Seke Sara Warber James A. Duke Harry L Brielmann, Natural Products from plants, 1995.
3. Richard JP. Cannell/ Natural Products isolation, Glaxo welcome Research and Development Stevenage, Herts UK, Human press Totwa, New Jersey, 1998.
4. New man DJ, Cragg GM. Natural Products as source of new Drugs over the last 25 years, Journal of Natural Products. 2007; 70:461-477.
5. WWW/Wikipedia Forever/ from Wikipedia, the free encyclopedia, 2009.
6. Mentzer C. Comparative Phytochemistry, (ed. Swain) Academic press, 1966, 21.
7. Elizabeth M. Willcamson, Phytotherapy Research, 1987, Jon wiley and Sons. from Wilkipedia, 2009.
8. Acharya, Deepak andShrivastava Anshu, Indogenous Herbal Medicines: Trial formulations and Traditional Herbal Practices, Aavishkar publishers Distributor, Jaipur- India, 1975, 440.
9. Edgar J DaSilva, Elias Baydoun, Adnan Badran. Biotechnology and the developing world. Electronic Journal of Biotechnology. 2002; 5(1)fulltext-1.
10. Sofowora, Medicinal plants and Traditional Medicine in Africa, John Wiley and Son ltd, 1982.
11. Gani A. Chemical constituents and uses of Medicinal Plants of Bangladesh, 1998.
12. Ezike AC, Akah PA, Okoli Charles O. Pharmacology Sciences, Ethnopharmacology. 2008; 82:143-177.
13. Elvin Lewis M. Journal of Ethnopharmacology. 2001; 75(2-3):141-146.
14. Awadh Ali, *et al*. Afr. J Trad. CAM. 2006; 3(1):1-10.
15. OKE JM, Hamburger MO. African Journal of Biomedical Research. 2002; 5:77-79.
16. Anna Szakiel, Max Henry. Acta Biochemica Polonica. 2007; 54(4):853-856.
17. Milena Nikolova, Reneta Gevrenova, Stephanka Iuancheva, Acta Pharm. 2003; 53:145-149.
18. Hazem A, Kadry, Dr. Makboul A. Makboul Chemistry of Natural Products; Part 1; Dar Al-Hamed, 1998.
19. Egwaikhide PA, Gimba CE. Middle-East Journal of Scientific Research. 2007; 2(3-4):135-138.
20. Green Medicine, Plant Conservation Alliance – Medicinal Plants Working group. Last updated, 2009.
21. Ayurvedic Medicinal Plants, 2008, 12.