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Research Article

Larvicidal and Antibacterial Activities of Methanol Extract of *Acacia polyacantha Willd*

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Abstract

There is an immense need to develop an alternative antimicrobial source for treatment of several infectious diseases and restricting their vectors. So, evaluation of phytochemical constituents, antimicrobial and larvicidal activity of *Acacia polyacantha* bark methanol extract was undertaken in the present study. Proximate phytochemical analysis was performed according to standard methods using conventional protocols. Biological activates of the extract was conducted accordingly. The antibacterial screening was carried out against several bacterial pathogens. The larvicidal activity was evaluated by using *Culex quinquefasciatus* larva. The results revealed the presence of tannins, saponins, steroids, flavonoids, alkaloids and coumarins. The extract exhibited significantly higher diameter of inhibition zone against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* compared to amoxicillin, except with *Staphylococcus aureus*. The maximum diameter of inhibition was recorded as 19±0.05 mm against *E. coli*. The extract showed a significant dose dependent effect with mortality up to 100% at 500 µL. The LC_{50} obtained was 26.55 µL and 27.11 µL at 24 h and 48 h, respectively, while the LC_{90} was 318.5 µL and 328.1 µL at 24 h and 48 h, respectively. The results obtained in the present study suggest that the methanol extract of *A. polyacantha* revealed its potential anti-bacterial and larvicidal effects and clarifies its widely medicinal uses.

Keywords: *Acacia polycantha*, Biological activity, Larvicidal activity

Introduction

Medicinal plants are widely used to treat different diseases throughout the world. Medicinal plants, especially those with anti-infective properties are extensively used in Africa due to their availability and ease of access. Furthermore, lack of access to modern medicine in rural communities has made traditional medicinal practice as the only option in combating diseases. In fulfilling the immediate needs of Africa, such plants can be developed into phytomedicines as is the case in some third world countries. For example, traditional medicine has been extensively used in primary health care in China, Argentina and Papua New Guinea. Even in modern medicine, a considerable number of drugs used are derived from plants. It is therefore likely that plants will continue providing templates for the development of new drugs.[1]

As a part of Complementary and Alternative Medicine (CAM), a number of phytomedicines obtained from African plants, are in global markets.[2] It is therefore pertinent that African plants should be investigated systematically for better use in healthcare systems. On the other hand, the uses of medicinal plants have been traditionally transmitted

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through generations in several ways. However, traditional healers are secretive of their knowledge and hence there is a concern that the knowledge of traditional medicine in eastern and central Africa may be lost unless the information is documented; one such useful document compiled for researchers is to conduct modern phytochemical and pharmacological investigations.[3]

Many modern drugs have been reported to show resistance in bacterial and fungal infections. These modern drugs apart from showing resistance to bacterial and fungal infections, they are also more expensive.[4,5] At the same time most of the African population lives below poverty line and cannot afford expensive modern drugs. These challenges call for renewed strategies on treatment, especially in the development of antimicrobials. According to World Health Organization (WHO), medicinal plants can provide the best alternative source to obtain variety of drugs.[6]

Acacia polyacantha subsp. *campylacantha Willd*. (Leguminosae) known as hook thorn or white-stem thorn a tree with one stem bear branches commencing fairly high up, and a well-ordered flattish crown. The tree is spread through-out tropical Africa. In Sudan *A. polyacantha* (called Kakamut) different parts have been used for treatments of various illnesses; gum for general health tonic, antidote for snake bite, and cure for venereal diseases. The roots and the bark are used for medicinal treatments, like a general health tonic, antidote for snakebite, cure for venereal diseases and stomach disorders.[7] A preparation from the bark is used for stomach disorders, anti-healing and to bathe children who are restless at night.[7, 8] The aim of present work was to study the phytochemicals, antimicrobial and larvicidal activity of *A. polyacantha* bark extract. It is an attempt to document the biological activities of Sudanese Acacia in the light of the recent evidence.

Materials and Methods

Plant Material and Preparation of Extract

Bark of *A. polyacantha* subsp. *campylacantha* was collected in October 2016 from Abkarsholla city, South Kordofan, Sudan. The study was conducted during October 2016-April 2017 at College of Applied and Industrial Sciences, University of Bahri, Khartoum North, Sudan. The bark was firstly washed, dried in shades for 5 days, and then crunched using a hammer mill to obtain a coarse powder. The prepared bark material was stored in an air-tight container until use. For crude extraction, 30 g of the powdered bark were macerated in absolute methanol for 48 h. The extract was filtered with Whatman filter paper No. 41. The methanol was completely evaporated at room temperature (25-30°C). The process was repeated thrice and the extracts combined together. The dried extract was stored at 4°C until use.

Antimicrobial Activity

Bacterial strains of two gram +ve; *Bacillus subtilis*, and *Staphylococcus aureus*, and two gram -ve; *Escherichia coli*, *Pseudomonas argenosa* were used to study the antibacterial activity of *A. polyacantha* bark extract. The paper disc diffusion method was used for the antibacterial screen and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines.[9] Bacterial suspension was diluted with sterile physiological solution to 10^8 CFU/mL (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 min. Sterilized filter paper discs (Whatman No. 1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The principle of the agar plate dilution is the inhibition of growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into 4 segments. The organisms tested were growing in broth over night to contain 10^8 CFU/mL. Loop-full of diluted culture is spots with a standard loop that delivers 0.001 mL on the surface of segment. The endpoint (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results are reported as the MIC in mg/mL.

Phytochemical Analysis

Test for Alkaloids

The presence of alkaloids in extracts was tested by using Wagner reagent prepared.[10] Then, 2 mL of Wagner reagent was added to 2 mL of extracts. The formation of reddish-brown precipitate indicated the presence of alkaloids.

Test for Steroids

Test for steroids was done according to the method with modifications.[11] Hence, 1 mL of extract was taken in a test tube and dissolved with 10 mL chloroform, then equal volume of concentrated H_2SO_4 was added to the test tube by sides. The upper layer in the test tube appears red and sulphuric acid layer showed yellow with green fluorescence, which indicated the presence of steroids.

Test for Flavonoids

2 mL of extract was taken in a test tube and 2-3 drops of dilute NaOH was added.[11] An intense yellow color has appeared in the test tube. The solution became colorless when few drops of dilute H_2SO_4 was added confirming the presence of flavonoids.

Test for Saponins

Two grams of the powdered sample was boiled in 20 mL of

distilled water in a water bath and filtered.[12] To the filtered sample (10 mL), about 5 mL distilled water was added, shaken vigorously and observed for a stable persistent frothing for 25 min.

Test for Tannins

Test for tannins was done with some modifications.[12] From the dried powdered sample, 0.5 g was boiled in 20 mL water in a test tube and then filtered. One milliliter of 0.1% Ferric Chloride (0.01 Mol/dm^3) was added to 2 mL of extract. Brownish green colorations were indicated the presence of tannins.

Larvicidal Activity

The eggs of *Culex quinquefasciatus* Say were obtained from the Northern Kadrow area in Bahri, Khartoum, Sudan. The collected eggs were transferred into open petri dish contain distilled water with few powdered breads. The eggs were incubated for four days at room temperature (30ºC), until arrived to larval phase.

The larvicidal activity was carried against the third-instar larvae of *Culex*. For preparation of stock solution, 0.2 gram of extract was dissolved in 2 mL of distilled water, then 5 mL of Dimethyl sulfoxide (DMSO) was added. From the stock solution three aliquots viz. 5, 50, 500 µL were taken and applied each to a set of 30 larvae. The counts of dead larvae commenced after 24 h and 48 hours and larvae were considered dead when they fail to raise to the surface or settle motionless on the bottom of the dish.

Statistical Analysis

Every experiment was repeated three times. The obtained data were subjected to analysis of variance (ANOVA). The mean separation was analysed using Duncan multiple range test at p-value <0.05. All the values were expressed as mean ± SE.

Results

The results on qualitative phytochemical analysis for the methanol extract of *Acacia polyacantha* bark were presented in Table (1). It's clear that alkaloids, tannins, saponins, coumarins, steroids, and flavonoids were existed in high concentrations.

The methanol extract of *A. polyacantha* (bark) showed strong inhibition against four types of bacteria (Table 2). The significant (P<0.05) highest activity was 19±0.05 mm recorded against *E. coli* by the higher concentration (100 mg/L). While the significantly (P<0.05) lowest activity 10.3±0.1 mm was recorded against *B. subtilis* by the lower concentration (12.5 mg/L). In the present study, no activity was noted against *S. aureus* except at high concentration (100 mg/L) with 13.6±0.02 mm (Table 2). Significant (P<0.05) activity of *A. polyacantha* bark methanol extract was found against *E. coli* followed by *P. aeruginosa*, *B. subtillus* and *S. aureus* at all used concentrations. On the other hand, the bark extract of *A. polycantha* at 100 mg/L showed larger zone of inhibition with *E. coli*, *P. aeruginosa*, and *B. subtillus* than the standard antibiotic used, amoxicillin (Table 2).

Table 3 showed the experiment preliminary results on *Culex quinquefasciatus which* sensitive to *A. polyacantha* extract as concentrations (5, 50 and 500) µL. The application of 500 µL results in 100% mortality within 24 and 48 hours. The concentration 500 µl produced significantly (P <0.05) high mortality compared to 50 and 5 µL concentrations. The effect was dose dependent and significantly different from the control. Increasing the exposure period resulted in significantly (P <0.05) a progressive increase in mortality.

Table 1.Phytochemical constituents identified in *A. polyacantha* bark extract

Phytochemical	Index
Tannins	+++
Flavonoids	+++
Saponins	+++
Steroids	++
Alkaloids	+++
Coumarins	+++

Index key: ++; high, +++; very high.

Table 2.Antibacterial activity of *Acacia polyacantha* bark extracts against standard bacteria

Antibiotic (mg/mL)		Diameter of zone of inhibition (mm)			
		Bs	Pa	Ec	Sa
Control		0.0±0.0^e	0.0±0.0^f	0.0±0.0^f	0.0±0.0^d
A. polyacantha bark extract	12.5	10.3±0.1^d	11.7±0.021^e	12.7±0.02^e	0.0±0.0^d
	25	14±0.07^c	14.7±0.02^d	15.3±0.04^d	0.0±0.0^d
	50	16.6±0.08^b	16.3±0.04^c	17±0.05^c	0.0±0.0^d
	100	18±0.00^b	18.33±0.03^b	19±0.05^b	13.6±0.02^c
Ciprofloxacin	100	30.1±0.1^a	26.1 ±0.1^a	31.2±0.1^a	28.4±0.2^a
Amoxicillin	100	17±0.2^b	16±0.2^c	18 ±0.2^{bc}	17±0.01^b

Bs: *Bacillus subtilis*, Pa: *Pseudomonas aeruginosa*, Ec: *Escherichia coli*, Sa: *Staphylococcus aureus.* Values represent mean± standard errors. *Different* letters indicated statistically significant *differences* between means according to Duncan's multiple range test (P<0.05).

Table 3.Larvicidal effect of *Acacia polyacantha* extract on *Culex quinquefasciatus* larvae after 24 h and 48 h of incubation

Treatment	Concentration (µL)	No. of larvae	Mortality (%)	
			24 h	48 h
Control	0	30	0.0±0.0^{d}	0.0±0.0^{d}
Extract	5	30	21.1±1.11^{c}	31.1±0.22^{c}
	50	30	56.67±0.19^{b}	68.89±0.4^{b}
	500	30	100±0.0^{a}	100±0.0^{a}

Table 4.Lethal dose of *Acacia polyacantha* extract on *Culex quinquefasciatus* larvae after 24 h and 48 h of incubation

Exposure period	LC_{50} µL (LC_{50} Log)	LC_{90} µL (LC_{90} Log)	Regression equation	r^2
24 h	27.11 (1.433)	328.1 (2.516)	y = 36.937x + 2.9337	0.9945
48 h	26.55 (1.424)	318.46 (2.503)	y = 37.072x - 2.7933	0.9946

LC_{50}: lethal concentration that kills 50% of the exposed larvae, LC_{90}: lethal concentration that kills 90% of the exposed larvae, r^2: regression coefficient.

Values represented mean± standard errors. Different letters indicated statistically significant differences between means according to Duncan's multiple range test (P<0.05).

Discussion

All the studied secondary metabolites viz.: alkaloids, tannins, saponins, coumarins, steroids, and flavonoids were previously reported in extracts of *A. polyacantha* bark. [13, 14] Other phytochemical analysis on *A. polyacantha* revealed the presence of alkaloids, tannins, saponins, coumarins, sterols and terpenes.[15] Moreover, only alkaloids, tannins, and saponins was identified in *A. polyacantha* extract.[16] Therefore, in contrary to the present results, both those previous studies indicated that flavonoids were not detected in *A. polyacantha* extracts. The change in metabolites noticed in our sample from previous work might be related to the time of harvest, to the soil or climate factors.[17] Diversity of secondary metabolites of *A. polyacantha* plant could explain its uses in the treatment of inflammatory diseases, diarrhea, parasitic, Salmonellosis and gastrointestinal diseases.

The methanol extract of *A. polyacantha* bark revealed variable inhibition degrees against studied bacteria with higher inhibition value on *E. coli*. Similarly, methanolic extracts of *A. polyacantha* bark recorded the maximum zone of inhibition 25±7 mm against *E. coli* compared to leaf extracts tested.[18] Also, hydro-ethanolic extract of *A. polyacantha* bark inhibited *E. coli* and *Salmonella typhi* growth.[15] In contrary to these studies, ethanolic extract of *A. polyacantha* bark showed no activity against the tested strains of *Streptococcus pneumonia*.[19] Likewise, *S. aureus* revealed resistant behavior to the aqueous extract of *A. polyacantha* bark.[15]

The results on larvicidal activity confirmed the sensitivity of *C. quinquefasciatus* to *A. polyacantha* extract and can be used as a natural insecticide. However, in contrast, Azokou et al.[20] considered *A. polyacantha* bark extract to be inactive against the fourth instar larvae of *Cx. quinquefasciatus* and exhibited LC_{50} value up to 370 ppm.

The antibacterial and larvicidal activities herein can be attributed to the ability of methanol to extract a wide range of chemical constituents from *A. polyacantha* bark. Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, and saponins, are secondary metabolites of plants that serve a defense mechanism against many microorganisms and insects. The presence of tannins and saponins in *A. polyacantha* would justify its uses in the treatment of diarrheal diseases and gastrointestinal diseases of animals.[21] It reported that tannins and alkaloids are natural products that have medicinal properties.[22] These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Tannins bind to proline rich proteins and interfere with the protein synthesis.[23] It stop infections on the skin surface, internally tannins continue to heal the wound. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and they have been found to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls.[24] Coumarins are known to act against gram positive bacteria and it is produced in carrots in response to fungal infection which could be attributed to its antimicrobial activity.[24] Saponins are heterosides of plant origin with insecticidal activities including anti-feeding, disturbance of the moult, growth regulation, and mortality. The insecticidal activity of saponins is due to their interaction with cholesterol, causing a disturbance of the synthesis of ecdysteroids.[25] Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cells. Steroid have been reported to have antibacterial

properties, due to the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes.[26] *A. polyacantha* root emits chemical compounds with its strong odor that repel animals including crocodiles, snakes and rats.[27]

Conclusion

The methanolic extract of *A. polyacantha* bark possesses both antibacterial and larvicidal activities, which encourage its use as an effective phytomedicine with its rich secondary metabolites.

Conflict of Interest: None

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