Insecticidal and antimicrobial extracts from Leaves and Stem -Bark of Sudanese *Albizia anthelmintica*

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Research Article

Keywords: Albizia anthelmintica. Toxicity, Tribolium castaneum, Culex quinquefasciatus Antibacterial, Nutrients and Minerals

Posted Date: May 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-28167/v1

Abstract

The Purpose: of this study is to determine the toxicity of *Albizia anthelmintica* leaves and stem bark extracted compounds to some insects.

Methods: The phytochemical screening and physiochemical analysis for leaves and stem bark had been carried out by using Standard methods, to study the pharmacological activities of these materials.

Results: the findings indicated *Albizia anthelmintica* leaves powder had significant (p> 0.05) against *Tribolium castaneum*. Organic extracts of *Albizia anthelmintica* leaves had toxic effect on *Culex quinquefasciatus* larve and were effective in reducing the fecundity of *Tribolium castaneum* adults. The phytochemical screening demonstrated the presence of some secondary metabolites such as alkaloids, flavonoids, tannins, triterpenes, coumarin, cardia glycosides and saponins. The results indicated high nutrients like crude fibre levels 16.15% and 9.5% are found in leaves and stems-barks respectively; beside protein level 8.95%, Crude fat level 2.6%, Moisture content 2.5%, ash content 6.75% and Nitrogen free extract content 63.05%. Minerals like sodium 350 ppm, phosphous 245 ppm, calcium 215 ppm, potassium 1.65 ppm and magnesium 1.25 ppm were showed in two parts. The ethyl acetate of extract of *Albizia anthelmintica* leaves intermediate activity against some types of bacteria and fungi. The ethanol extract of leaves presented high activity against fungi *Candida Albicans*.

Conclusion: *Albizia anthelmintica* leaves powder and ethanolic extract had high efficiency on reducing number of pest and their ability of laying eggs.

Significance Statement

Albizia anthelmintica was chosen for this study due to its reputation in legend medicine as antimicrobial agent and utilization of its parts in curing diseases, crude extracts of its leaves, stem-bark were tested as insecticidal and antimicrobial.

Introduction

The genus Albizia (Mimosaceae) comprises about 150 species distributed in Africa, Asia, Central and South America. The *Albizia* members in Africa are used in folk medicine for the treatment of rheumatism, cough, diarrhea and injuries [1] and in Sudan for the stomach pain and vermifuge [2].

Albizia anthelmintica is a thorny/spiny, deciduous, multi-stemmed, medium canopied tree growing to about eight meters. Bark smooth, gray to brown. Young branchlets glabrous or sometimes shortly pubescent, twigs are often spine-tipped [3 - 5]. *A. anthelmintica* is effective in controlling infection with a variety of internal parasites in lambs. Furthermore, treatment of strongly type worms requires a biweekly dose of *A. anthelmintica* as an effective deworming protocol [6]. *A. anthelmintica* is a potent anthelmintic capable of slowly but surely eliminating the threat of Haemonchus and Trichuris worm burden in goats by making the eggs of these worms unviable [7]. Saponins of species of the genus *Albizia* showed many pharmacological properties as anticonvulsant, sedative, anti-inflammatory, antitumor, antifungal, antibacterial and anti- parasitic. It may be concluded that Albizia species shall be considered as a promising plant with various therapeutic properties and can be further explored pharmacologically against various ailments [8]. The leaf, root and stem bark ethanolic extracts of *Albizia anthelmintica* contains compounds with antibacterial and antioxidant properties and suggests that the plant could be a source of potential antibacterial and antioxidant agents [9]. *A. anthelmintica* twig extract inhibited *C. albicans* biofilm, and can thus be

useful as a toothbrush or chewing stick to remove this fungus from the mouth. The twig extract may also be effective against biofilm infections involving the strain S. aureus U3300, as it was able to remove some of the bacterium's pre-formed biofilm [10]. The aqueous ethanol extract of *A. anthelmintica* showed moderate anti-inflammatory activity and significant for both analgesic and antioxidant activities. Quercetin-3 - O - β - D -glucopyranoside, kaempferol – 3 – O – β – D -glucopyranosid, kaempferol-3-O-(6β-O -galloyl-β-D-glucopyranoside and quercetin-3-O-(6β-O-galloyl-β-D-glucopyranoside) exhibited potent antioxidant scavenging activity towards diphenyl-picrylhydrazine [11]. A systematic screening of plant extracts as a source of pharmacological compounds has been undertaken in different laboratories [12] and [13]. There is an urgent need to find new disposable and affordable remedies to face this problem [14].

The present work was aimed to test the insecticidal effect of *A. anthelmintica* leaves and stem- barks powder and its different organic extracts as well as investigation their antibacterial, antifungal and phytochemical responsible for this activities.

Materials And Methods

Collection area of Albizia anthelmintica

Albizia anthelmintica locally in Sudan known as "Umm –takirni " its leaves and stem- barks were collected from Algoz area (Figure 1) in September 2015. Algoz area is situated in the northern part of South Kordofan state, and its borders by Northern Kordofan state from the north and northeast, West Kordofan state from the northwest, Dellang locality from the south and Habella locality from the southeast direction [15]. It is located between latitudes 12°–12° 30 N and longitudes 29° 48–300 E and 622 m above sea level, with a total area of 35,000 km^2. Short grass and short scattered trees prevail. The White Nile which is the main tributary of the River Nile bounds the hydrologic system to the east. Khor Abu Habil is a major seasonal wadi that crosses the area and flows from the west to the east [15]. *Albizia anthelmintica* plant was identified and authenticated by authorities of herbarium of Institute of Medicinal and Aromatic Plants-National Centre for Research, Khartoum, Sudan.

Figure 1. Morphological appearance of Albizia *anthelmintica* in its nature "Algoz area"

Plant materials

The leaves and stem- barks of *Albizia anthelmintica* were removed from their plant, and then were air dried for ten days. The samples were crushed into Crouse powder using hammer mill and stored in cloth bags for further use.

Preparation of crude extracts

Two hundred grams of each dried leaves and stem bark were weighed, then extracted with n-hexane firstly by placing in conical flask, by using magnetic stir device for four hours at room temperature, then filtered and air dried. The same procedure was repeated for extraction with ethyl acetate for 18 hours and ethanol for the same period. Each extract was filtered through Whatman No.1 filter paper and concentrated. The crude extracts were kept at 20^0C in sterile universal bottles.

Phytochemical analysis

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was carried out on the extracts with few modifications as described by Evans [16] Sofowora, [17], Harborne [18] , Harborne, [19] Gibbs, [20] and Harbone [21].

Test for Akaloids

Three ml of extract was poured on petri dish and dried in water path, then dissolved in ten ml of HCL 2% OR NH_4OH 10% and transferred in three test-tubes each one contain one ml, few drops of the following reagent were added into each tube (Dragedorff's gave orange precipitation, Wagner's gave reddish precipitation and Hager's gave yellow precipitation which indicated the present of alkaloids Evans [16] and Sofowora [17].

Test for Flavonoids

Two ml of extract evaporated on petri dishes and then ten ml of ethanol were added, then transferred into four test tubes, the one added 1ml of 1%NaOH that give yellow color, the second test tube was poured a few powder of magnesium turnate piece followed by adding concentrated HCL, the formation of a pink, crimson red which indicate the present of Flavonoid, the third test tube was treated with 1 ml of 10% $ALCL_3$ solution, the formation of creamy color indicated the present of Flavonoid, the fourth test tube was treated with Ammonium solution, the formation of yellow/orange color indicated the present of flavonoid [17].

Test for Tannins

Two ml of extracts was stirred with 1 ml of distilled water, filtered and few drops of ferric chloride were added to the filtrate. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins [16 & 21].

Test for Saponins

Two ml of extracts was concentrated in water bath, then was shaken with 5 ml of distilled water in a test tube. Frothing which persists on warming was taken as evidence for the presence of saponins [18 & 19].

Test for Triterpenes and Sterols

Two ml of extract was dried in water path and dissolved in 6ml of chloroform, a few drops of concentrated sulfuric acid were added along the side of the test tube two layers was formed, the upper green color indicated the presence of sterol and the middle red brown ring indicated the presence of triterpenes [19 & 20].

Test of Cardia Glycosides

About 0.1 g of plant powder was dissolved in one ml glacial acetic acid containing one drop of ferric chloride solution, 1ml the sulfuric acid was added under layer, a brown ring obtained was indicated the presence of cardenolides [22].

Test of Anthraquionone Glycosides

About 0.1g of plant powder was dissolved in one ml water and five ml of chloroform was added and shake for five min , after shaking two layer were formed , the chloroform layer was separated. One ml of ammonia solution

(10%) was added into 1ml of chloroform, the appearance of pink or red or violet color indicated the presence of anthraquionone [23].

Bioassay phytotoxicity effect of Tribolium castaneum experiments

Tribolium castaneum culture

Adults of *T. castaneum* were sieved from stored wheat seeds, stored at a house in Algabal area. These adults were kept in a conical flask containing crushed wheat seeds in laboratory. The adults were placed in the conical flask for three days to obtain eggs and then removed covered with muslin cloth to allow for aeration. At intervals of about three weeks, the wheat was replaced by fresh wheat crush to avoid contamination of the culture by fungi and bacteria which could develop on skin and fecal matter of cultured insects (Figure 2 a). The emerged larvae were left to give new adults for further experiments.

Figure 2. a/The morphology of *Tribolium castaneum* larva and adult,

b/ Morphological Larvicidal 3rd instar larvae of *Culex quinquefasciatus* specimen

Test one

The effect of powder and three extract of *Albizia anthelmintica* leaves and stem- barks on mortality of *Tribolium castaneum* adults under laboratory conditions at college of Applied and Industrial Sciences University of Bahri at Alkadro area. These experiments were designed for the purpose of assessing the effect of *A. anthelmintica* leaves and stem bark powder, ethanolic extract, ethyl acetate extract and hexane extract in comparison with neem leaves powder on mortality of *T. castaneum* adults for each of these treatments, three disposable (replicates) each containing 25 gm wheat crush treated with one gm *A. anthelmintica* leaves powder (4%), *Azadirachta indica* (Neem) powder (4) % were prepared. Twenty *T. castaneum* adults were selected at random and introduced into each Petri dish. The Petri dish were covered with muslin cloth and arranged in a *completely* random design. Counts were made after three and seven days from the treatment to determine the effect of each extracts and powder.

Test two

The effect of powder and methanolic extract of *A. anthelmintica* leaves and stem bark on fecundity of *T. castenum.* Treatments described for experiment 1 were repeated to follow the effect on fecundity of *T. castaneum* for each treatment, three disposable Petri dishes, each containing 25 gm wheat crush and twenty adults of *T. castaneum* selected at random were released. Petri dishes were covered with muslin cloth and arranged in completely randomized design. After one week adults were removed from petri dishes after laying eggs. The crushed wheat containing eggs were left for another week for hatching and counts were made for larvae of *T. castaneum.* These numbers of larvae stand for the number of eggs.

Test three

The effect of different organic extract, in different concentrations of *A. anthelmintica* leaves and stem bark on mortality of *Culex quinquefasciatus* 3rd instar larvae, it was conducted to compare between the effects of different

organic extracts namely hexane, ethyl acetate, and methanolic extract against 3rd instar larvae *Culex quinquefasciatus.* These tests were executed in similar test tubes, each containing 2 ml of distilled water, replicated three times. Thirty 3rd instar larvae were placed in each test tube and mortality count after 24 hrs and 72 hrs, larvae were considered dead when they fail to rise to the surface or settled on the bottom.

Larvicidal activity of Culex quinquefasciatus experiments

Collection of Culex quinquefasciatus eggs

Culex quinquefasciatus egg rafts were collected from various natural breeding sites at Alkadaro north area.

Culex quinquefasciatus eggs Hatching

The egg rafts were kept in dishes 10.6 inches wide and 1.6 inches deep containing distilled water till hatching. Larvae were fed with fine powdered bread. Dead larvae were continuously removed to avoid contamination of cultures with pathogens. For experiments 3rd instar larvae were used (Figure 2 b), [24] & [25].

Statistical Analysis

Results were expressed as mean ± standard error of mean. The data was statistically analyzed using analysis of variance (ANOVA) with Duncan Multiple Range Test (DMRT) comparisons versus control groups. The values of $p<0.05$ were considered as significant [26].

Collection of standard organisms for antimicrobial activity according to ATCC [27]

Name of standard organism	Type of organism	ATCC code
Candida albicans	Fungi	7596
Escherichia coli	Gram negative bacteria	25922
Pseudomonas aeruginosa	gram negative bacteria	27853
Bacillus subtilis	gram positive bacteria	8236
Staphylococcus aureus	gram positive bacteria	25923

Preparation of Fungal Suspensions

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

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 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U ml^{-1}. The suspension was stored in the

refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique [28]. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained [28 &29].

Results And Discussion

The phytochemical screening of *Albizia anthelmintica* leaves and stem bark was presented in Table 1 showed that very high amount of the alkaloids were found in ethyl acetate, followed by ethanol extracts and moderate in n-hexane extract. Flavonoids were found to be very high amount in ethanol extract and moderate amount in both of the ethyl acetate and n-hexane extracts. References to saponins were present in trace amounts in all extracts. Whereas tannins were very high in ethanol extract and totally absent in ethyl acetate and n-hexane extracts. *Albizia anthelmintica* leaves had high amount of sterols in ethyl acetate when compared with ethanol and n-hexane extracts. Triterpenes presented in moderate amount in ethanol extract and low amount in both of ethyl acetate and n-hexane extracts. The coumarins were found in trace amounts in all extracts.

Table 1. Phytochemical screening of *Albizia anthelmintica* leaves and stem bark

Secondary metabolites	Tests	Successive extraction of leaves with			Successive extraction of stem bark with		
		n-hexane	ethyl acetate	ethanol	n-hexane	ethyl acetate	ethanol
Alkaloids	Drogndroff's	++	++++	+++	+++	++	++
	Hager's	+	+++	+++	+++	++	+
	Wagner's	+	+++	+++	+++	++	+
Flavonoids	1%Na OH	++	++	++++	+++	+	++
	NH_4OH	++	++	+++	+++	++	+
	10%$ALCL_3$	++	++	+++	+	+++	+++
	Mg/HcL	-	-	-	++	+++	+
Saponins	Foam	-	+	+	-	++	+++
Tannins	$FeCl_3$	-	-	++++	++	+	++
	10%Gelatin salt	-	-	+++	+++	++	++
Sterols/Triterpene	Liebermman's	++/+	++++/+	+++/++	+++/	+/	++/
	Salkowski	+++/+	++++/+	+++/+	+++/	+++/	+++/
Coumarin	KOH/UV	+	+	+	- -	-	-
Glycosides	Anthraquinone	-	-	-	-	+	+
	Cardic	-	+	+	-	+	+

++++ ≡ very high concentration

+++ ≡ high concentration

++ ≡ moderate concentration

+ ≡ trace amount

- ≡ absent

Table 1 presented the results of phytochemical screening of *Albizia anthelmintica*, stem bark. The high amount of alkaloids showed in n-hexane extract associated with a moderate amount in ethyl acetate extract and low amount in ethanol extract. The results of *Albizia anthelmintica*, stem bark extract showed high amount of flavonoid in ethyl acetate and a moderate amounts in n-hexane and ethanol extracts. Tannins were high in ethanol, followed by moderate in ethanol and absent in n-hexane extracts. The high amount of sterol/ triterpene in n-hexane and ethanol extracts associated with moderate amount in ethyl acetate extract. The coumarins and Anthraquinone glycosides were totally absent in all extracts with low amount of cardic glycoside in ethyl acetate and ethanol extracts. In general the ethanol extract showed high amount of alkaloids, flavonoids, tannins and sterol, trace amount of saponins, triterpenes, coumarin and cardia glycosides with absence of anthraquionone. Ethyl acetate extract showed high amount of alkaloids and sterol, moderate amount of flavonoids, trace amount of saponins, triterpenes, coumarin and cardia glycoside and absence of tannin and athraquinone. Hexane extract had high amount of alkaloids, moderate amount of flavonoids, sterol and trace amount of saponins, cardia glycoside and absence of tannins and anthraquinone. *Albizia anthelmintica* leaves different extracts showed minor differences from *A. anthelmintica* Stem-barks extract in their relation to secondary metabolite contents. The results of the proximate analysis of the *Albizia anthelmintica* leaves and Stem bark showed that had higher ash content 6.75% compared with that of stem bark 3.1%, as well as the crude protein of leaves was 8.95% whereas that of stem bark was 6.2%, the crude fibers of leaves and stem bark were 16.2 and 9.5 respectively. The crude fat with (ether extract) for both leaves and stem bark were more or less similar (2.6%) however the nitrogen free extract (carbohydrates by difference) was 63.1 % and 82.5 % for stem bark. The results of the mineral analysis of *A. anthelmintica* leaves and Stem-bark were given in Table 2.

Table 2. Mineral Composition (ppm) of *Albizia anthelmintica* of leaves and stem bark

Leaves		Stem- bark	
minerals Analyzed	Concentrations (ppm)	minerals Analyzed	Concentrations (ppm)
Na	350	Na	340
K	1.65	K	285
P	245	P	555
Ca	215	Ca	170
Mg	1.25	Mg	1.55

Values are means of duplicate determinations.

The leaf was rich with calcium when compared to Stem-bark, whereas Stem-bark, was rich with Phosphate, however, a little amount of magnesium were observed in both parts Table 2.

The results in Table 3 indicated that the *Azadirachta indica* leaves powder 4% after three days was significantly ($p < 0.05$) had best effect on mortality of *Tribolium castaneum* adults, followed by *Albizia anthelmintica* leaves powder 4% then extract with ethanol.

Table 3 .The mortality of *Tribolium castaneum* (Red flour beetle) adults after three days with application of *Albizia anthelmintica* leaves powder, extracts of leaves with ethanol

Treatments	Number of adults	Mortality Replicates			Total	Mean ±0.707	Duncan test F Pr.<0.05
		R1	R2	R3			
Control	20	0	0	0	0	0	a
Azadirachta indica Leaves powder 4%	20	4	2	5	11	3.67	c
Albizia anthelmintica Leaves powder 4%	20	1	2	2	5	1.67	b
Albizia anthelmintica Leaves ethanol extract 4%	20	1	0	1	2	0.66	b

The results after seven days from treatments Table 4 showed that *Azadirachta indica* leaves powder 4% was still the best treatment in relation to mortality of *Tribolium castaneum* and the difference between it and all other treatments was significant ($P<0.05$) and the powder of *Albizia anthelmintica* leaves and ethanol extract were significantly ($P>0.05$) effective against *T. castaneum* adults and the difference between them was not significant.

Table 4. The mortality of *Tribolium castaneum* (Red flour beetle) adults after seven days with application of *Albizia anthelmintica* leaves powder, extracts of leaves with ethanol

Treatments	Number of adults	Mortality Replicates			Total	Mean ±0.745	Duncan test F Pr.<0.05
		R1	R2	R3			
Control	20	0	0	0	0	0	a
Azadirachta indica Leaves powder 4%	20	5	4	6	15	5	c
Albizia anthelmintica Leaves powder 4%	20	1	3	4	8	2.67	b
Albizia anthelmintica Leaves ethanol extract 4%	20	1	1	1	3	1	b

The powder of *Albizia anthelmintica* leaves and the extract with ethanol were also tested for their effectiveness in reducing the fecundity (number of eggs) of *T. castaneum* adults (Figure 2 a), all treatments showed significant (P< 0.001) reduction of the number of eggs produced within one week when compared with control. As mentioned in test one results shown in Tables 3 and 4, the effects of powder and ethanolic extract of *A. anthelmintica* leaves and stem bark on mortality of *T. castaneum*, all products of *A. anthelmintica* (leaves powder, stem bark powder, ethanolic extracts (4%), were came after neem leaves powder (4%) in term of reducing the number of *T. castaneum* adults but both were affected significantly (P > 0.05) and (P> 0.007) respectively. There was no significant difference when compared with control. The same results were obtained after seven days of treatments (Table 4).

Results presented in Tables 5 and 6 indicated that all organic extracts with concentration 500 µg ml^{-1} were highly significant (p< 0.001) on mortality of *Culex quinquefasciatus* 3^{rd} instar larvae after 24 hours The mortality of larvae compared to other treatments after 48hours, n-heane extract at 500 µg ml^{-1} ranked 1^{st} followed by ethanol (500 µg ml^{-1}), n-hexane (50 µg ml^{-1}), ethyl acetate (500 µg ml^{-1}) ethanol (50 µg ml^{-1}) ethyl acetate (50 µg ml^{-1}) ethanol (5 µg ml^{-1}) n-hexane (5 µg ml^{-1}) and ethyl acetate (5 µg ml^{-1}) respectively. The ethyl acetate extract (50 µg ml^{-1}) ethanol extract (5 µg ml^{-1}) ethyl acetate (5 µg ml^{-1}) and n-hexane (5 µg ml^{-1}) were not significantly reduced the number of larvae. The highest concentration of n-hexane (500 µg ml^{-1}) ethanol extract (500 µg ml^{-1}) and ethyl acetate (500 µg ml^{-1}) respectively the best treatments and were significant (p<0.001). After 48 hours (Table 6) showed that n-hexane extract (500 µg ml^{-1}) was still the best treatment in relation to mortality of the *Culex quinquefasciatus* 3^{rd} instar larvae followed by ethanol extract (500 µg ml^{-1}). In general the effect of organic extracts of *Albizia anthelmintica* leaves on mortality of *Culex quinquefasciatus* 3^{rd} instar larvae increase with increase of concentration of the extract. This result that *Albizia anthelmintica* leaves may be a good source of insecticide against insects. The high Alkaloids, flavonoids and tannins constituents also show that it could be a potent insecticide.

Table 5. The effect of *Albizia anthelmintica* leaves organic extracts on mortality of *Culex quinquefasciatus* instar 3rd larva after 24 hours.

Treatment	No of larva	Concentration µg ml^{-1}	Mortality Replicates R1	R2	R3	Total	Mean± 0.530	Duncan test F Pr.<0.001
Control	30	0	0	0	0	0	0	a
Albizia anthelmintica Leaves ethanol extract	30	500	13	8	26	47	15.7	c
	30	50	6	4	2	12	4	b
	30	5	4	2	0	6	2	ab
Albizia anthelmintica Leaves ethyl acetate extract	30	500	5	2	7	14	4.7	b
	30	50	4	2	3	9	3	ab
	30	5	4	2	0	6	2	ab
Albizia anthelmintica Leaves n-hexane extract	30	500	22	30	30	82	27.3	d
	30	50	18	6	13	37	12.7	c
	30	5	0	0	2	2	0.66	Ab

Table 6. The effect of *Albizia anthelmintica* leaves organic extracts on mortality of *Culex quinquefasciatus* instar 3rd larva after 48 hours.

Treatment	No of larva	Concentration µg ml^{-1}	Mortality Replicates R1 R2 R3			Total	Mean± 0.530	Duncan test F Pr.<0.001
Control	30	0	0	0	0	0	0	A
Albizia anthelmintica	30	500	16	16	26	58	19.33	Ef
Leaves ethanol extract	30	50	6	6	4	16	5.33	C
	30	5	4	4	2	10	3.33	Bc
Albizia anthelmintica	30	500	10	12	14	36	12	De
Leaves ethyl acetate extract	30	50	12	8	17	37	12.33	De
	30	5	11	12	12	35	11.66	Cd
Albizia anthelmintica	30	500	23	30	30	83	27.66	F
Leaves n-hexane extract	30	50	18	8	17	58	19.33	De
	30	5	1	1	2	4	1.3	Abk

Antimicrobial activity of the crude extracts of *A. anthelmintica* leaves at different concentrations expressed in diameter of zone of inhibition, mm against standard organisms was presented in Figure 3. The n-heane extract (100 mg ml^{-1}) had showed intermediate activities against *Bacillus subtilis* (17 mm), *Escherichia coli* (16 mm) and *Staphylococcus aureus* (16 mm), there were no activities against *Pseudomonas aeruginosa* and *Candida Albicans*. The extract with ethanol (100 mg ml^{-1}) was also showed intermediate activity against *C. Albicans* (17 mm) and low activity against two gram positive bacteria *Bacillus subtilis* (13 mm) and *Staphylococcus aureus* (12 mm). With no inhabitation zone against two gram negative bacteria *Escherichia coli* and Pseudomonas *aeruginosa*. Ethyl acetate extract (100 mg ml^{-1}) showed intermediate activities against fungi *C. Albicans* (14 mm) and the four bacteria microorganisms *Bacillus subtilis* (16 mm) *Staphylococcus aureus* (16 mm) *Pseudomonas aeruginosa* (16 mm) and *Escherichia coli* (15 mm). These intermediate microbial activity of *A. anthelmintica* leaves may due to presence of high amount of flavonoids. Meanwhile the antimicrobial activity of the crude extracts of *A. anthelmintica* stem-barks at different concentrations against standard organisms was presented in Figure 4 the ethyl acetate extract (100 mg ml^{-1}) of *A. anthelmintica* stem bark was found sensitive to *E. coli* (20 mm), *P. aeruginosa* (20 mm), *B. subtilis* (19 mm), and fungi *C. albicans*, but face inactive against *Staphylococcus aureus* (13 mm). The ethanol extract (100 mg ml^{-1}) had showed sensitive effect to *B. subtilis* (18 mm), intermediate effect to *E. coli* (16 mm), and fungi *B. subtilis* (16 mm). The hexane extract (100 mg ml^{-1}) showed sensitive effects to *P. aeruginosa* (20 mm), intermediate to fungi *Bacillus subtilis* and resistance to fungi *B. subtilis* (12 mm).The test reflected that ethyl acetate extract (100 mg ml^{-1}) had higher growth inhibition zone against three bacteria, *P. aeruginosa*, *E. coli, B. subtilis.* Ethanol extract (100 mg ml^{-1}) had higher growth inhibition zone against *B. subtilis* whereas n-hexane extract had higher inhibition zone against *P. aeruginosa.* The mentioned results disagree with (Maitera, [30]), may be because they used aqueous extracts.

Figure 3. Antimicrobial activity of *Albizia anthelmintica* leaves different extracts at concentrations 25, 50 and 100 mg ml^{-1}against standard organisms

Figure 4. Antimicrobial activity of *Albizia anthelmintica* stem- barks different extracts at concentrations 25, 50 and 100 mg ml^{-1}against standard organisms

Where: *E. c≡ Escherichia coli; P. a ≡ Pseudomonas aeruginosa; S. a≡ Staphylococcus aureus; B. s≡ Bacillus subtilis and C. a≡ Candida albicans*

Conclusion

Albizia anthelmintica was chosen for this study due to its reputation in legend medicine as antimicrobial agent and utilization of its different parts in curing diseases. *Albizia anthelmintica* leaves powder and ethanolic extract had high efficiency on reducing number of pest and their ability of laying eggs. The effect of extracts increased with increase of their concentrations. The antimicrobial examination indicated that the ethanol extract for leaves has a high effectiveness against the fungus; the ethyl acetate extract has medium effect against all kinds of bacteria used. A high quantity of tannins was found in ethanol extract but not present in ethyl acetate, as well as presence a medium amount of alkaloids and trace amount of saponins. Some active ingredients may responsible for the antimicrobial activities of this plant leaves. The antimicrobial examinations indicated that extract for leaves and stem- bark had a high effectiveness against the fungus, while ethyl acetate extract for leaves had medium effect against all kinds of bacteria applied whereas stem bark had high effect against three bacteria *E. coli*, *P. aeruginosa* and *B. subtilis*. The hexane extract of leaves showed intermediate activity against *E. coli* and *B. subtilis*.

More research work are needed on *A. anthelmintica* specifically on purification of plant extracts to isolate the bioactive metabolites and their structure must be elucidate and specify the active components that can effect in *T. castaneum* adults and *Culex quinquefasciatus* larvaes.

Declarations

Acknowledgements

We would like to thank, students Islam Mohammed Shaeib and Ahmed Ibrahim for their technical support and University of Bahri for funding and using the labs.

Ethics approval

Not applicable

The present study is purely based on chemical analysis in lab, not in human or animal trails were carried out.

Consent for publication

Not applicable

Availability of data and materials

We have already included all data in the manuscript, the lab and data, phytochemical screening, cytotoxicity, antioxidant and antimicrobial activities.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was financed by the University of Bahri, Sudan, with Code No: U of B-1-201.

Authors' contributions

S. A. was carried out and supervised the Insecticidal and antimicrobial experiments. T. O. I. was carried out and supervised the phytochemical and physiochemical experiments and Y. S. M. was collected the raw materials and identification of the plant. A. M. M had provided technical and financial support and helped in the rewrite-up and revision and T. O. K and S.Y. wrote the draft manuscript, designed the study and supervised the project. All authors read and approved the final manuscript.

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Figures

Figure 1

Morphological appearance of Albizia anthelmintica in its nature "Algoz area"

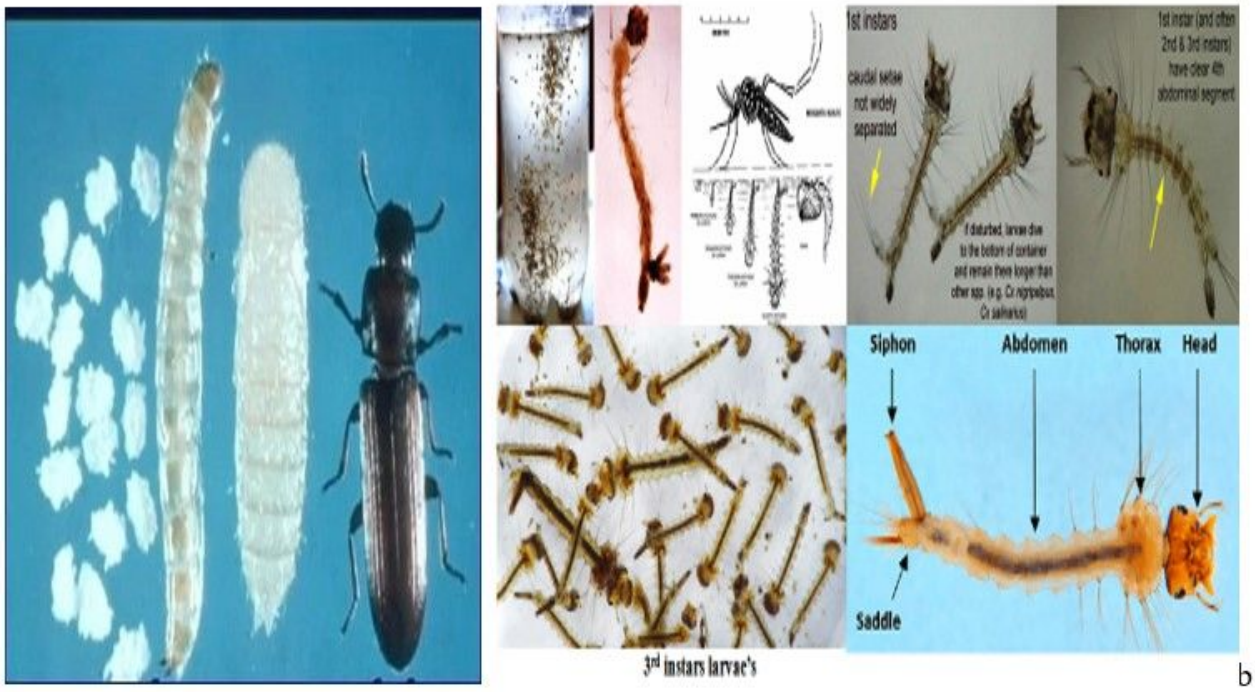

Figure 2

a/The morphology of Tribolium castaneum larva and adult, b/ Morphological Larvicidal 3rd instar larvae of Culex quinquefasciatus specimen

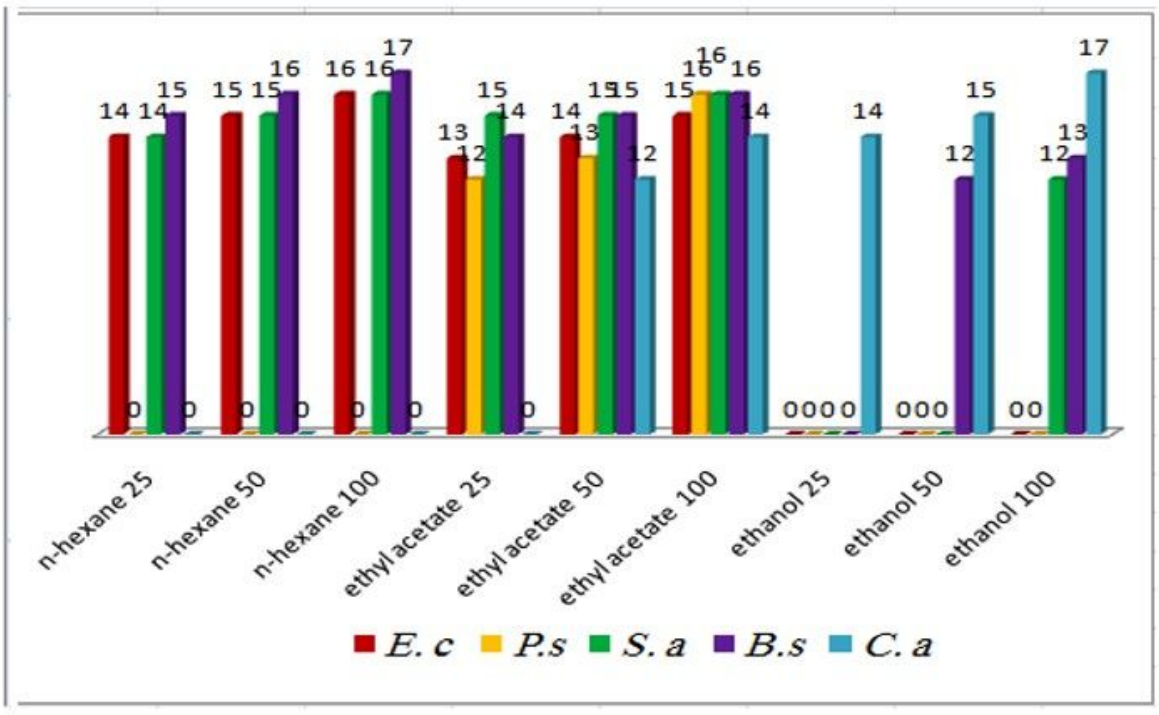

Figure 3

Antimicrobial activity of Albizia anthelmintica leaves different extracts at concentrations 25, 50 and 100 mg ml-1against standard organisms

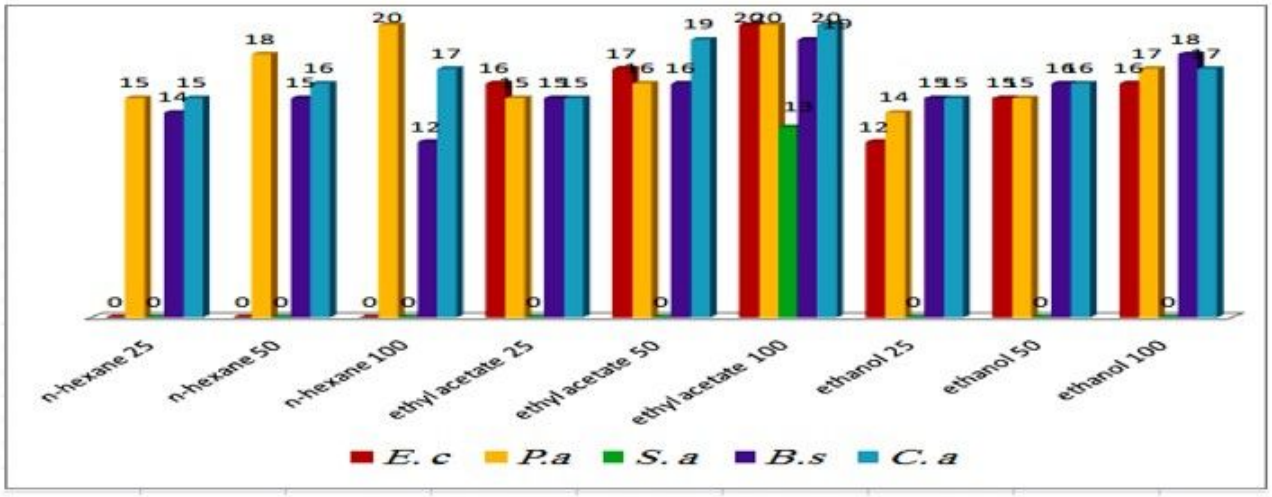

Figure 4

Antimicrobial activity of Albizia anthelmintica stem- barks different extracts at concentrations 25, 50 and 100 mg ml-1against standard organisms Where: E. c≡ Escherichia coli; P. a ≡ Pseudomonas aeruginosa; S. a≡ Staphylococcus aureus; B. s≡ Bacillus subtilis and C. a≡ Candida albicans

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