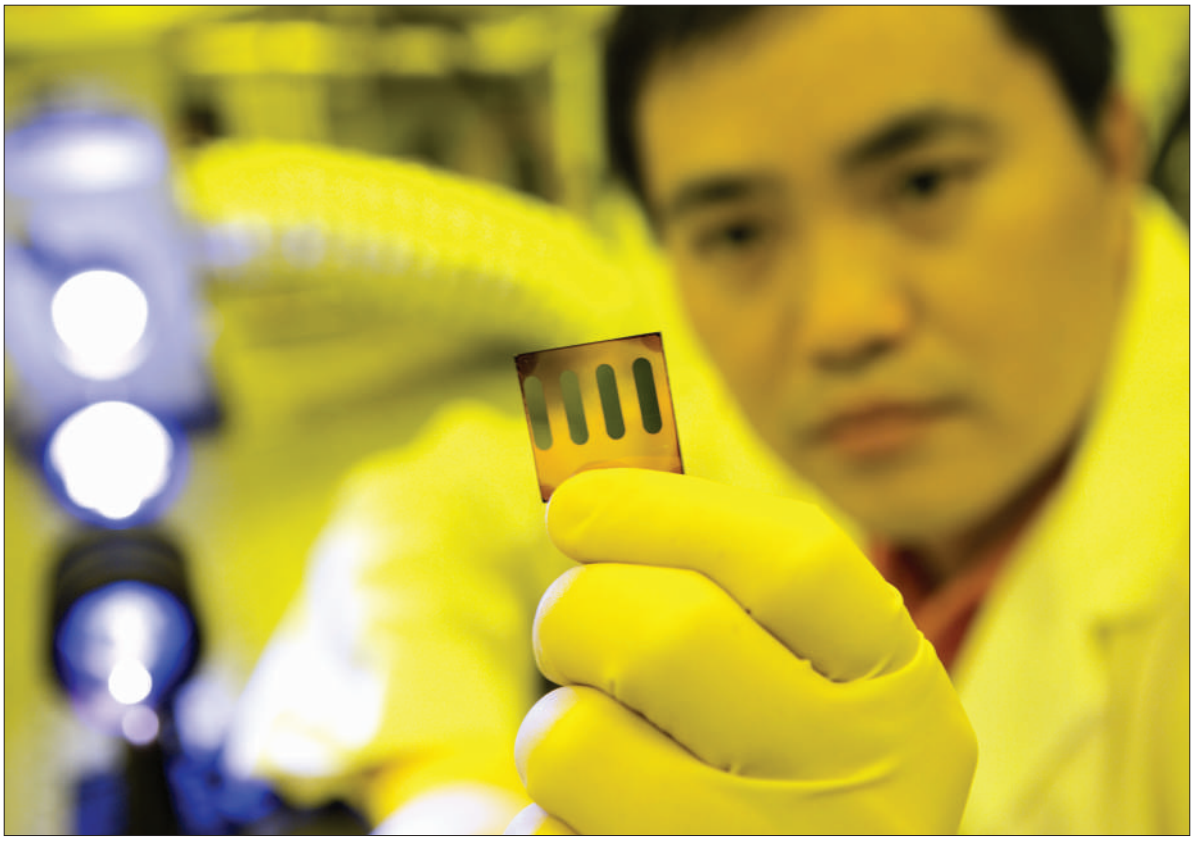

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Research Article
Insecticidal and Antimicrobial Extracts from Leaves and Stem-bark of Sudanese *Albizia anthelmintica*

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Abstract

Background and Objective: The different parts of *Albizia anthelmintica* tree were utilized historically in treating some human diseases and animals infections. The objective of this study was to determine the toxicity of *Albizia anthelmintica* leaves and stem bark extracted compounds to some insects. **Materials and 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 larvae 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, crude fat level, moisture content, ash content and nitrogen free were present. Minerals like sodium, phosphorus, calcium, potassium and magnesium were showed in two parts. The ethyl acetate extract of *Albizia anthelmintica* leaves exhibited 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.

Key words: *Albizia anthelmintica*, toxicity, *Tribolium castaneum*, *Culex quinquefasciatus*, antibacterial, nutrients and minerals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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]. In Sudan, it is used for the stomach pain and vermifuge[2].

Albizia anthelmintica is a thorny/spiny, deciduous, multi-stemmed and medium canopied tree growing to about 8 m. Bark smooth, gray to brown. Young branchlets glabrous or sometimes shortly pubescent, twigs are often spine-tipped[3-5]. *Albizia anthelmintica* is effective in controlling infection with a variety of internal parasites in lambs. Furthermore, treatment of strongly type worms requires a bi-weekly dose of *A. anthelmintica* as an effective deworming protocol[6]. *Albizia 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 is important to consider *Albizia* species as natural source for medicines to treat various diseases[8]. The leaf, root and stem bark ethanolic extracts of *Albizia anthelmintica* contains compounds with antibacterial and antioxidant properties while suggested that the plant could be a source of potential antibacterial and antioxidant agents[9]. *Albizia 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]. Some antioxidant and analgesic properties exhibited when *A. anthelmintica* was extracted with ethanol as Quercetin-3-O-β-D-glucopyranoside, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-(6β-O-galloyl-β-D-glucopyranoside and quercetin-3-O-(6β-O-galloyl-β-D-glucopyranoside)[11]. A systematic screening of plant extracts as a source of pharmacological compounds has been undertaken in different laboratories[12,13]. 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

Study area: The study was carried out at Microbiology and Biochemistry Labs, College of Applied and Industrial Sciences, University of Bahri, Sudan, during period from January, 2017-April, 2017.

Collection area of *Albizia anthelmintica*: *Albizia anthelmintica* locally in Sudan known as "Umm-takirni" its leaves and stem-barks were collected from Algoz area[15] as presented in Fig. 1 in September, 2015. *Albizia anthelmintica* plant was identified and authenticated by authorities of herbarium of Institute of Medicinal and Aromatic Plants, National Centre for Research, Khartoum, Sudan.

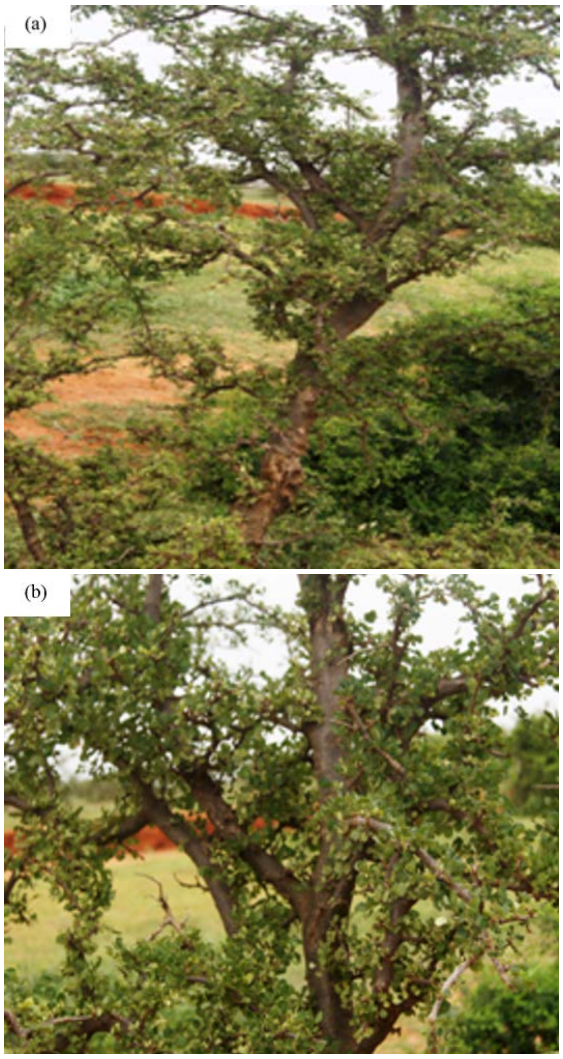

Fig. 1(a-b): Morphological appearance of *Albizia anthelmintica* in its nature
Source: Issa *et al.*[15]

Plant materials: The leaves and stem-barks of *Albizia anthelmintica* were air dried for 10 days. The samples were crushed and stored in cloth bags for further use.

Preparation of crude extracts: Two hundred grams of each dried leaves and stem bark were extracted with n-hexane for 4 h, ethyl acetate for 18 h and ethanol for 18 h at room temperature and then filtered followed by drying. Each extract was filtered with Whatman No.1. The crude extracts were kept at 20°C 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[16-21].

Test for alkaloids: Three milliliter of extract was treated with 10 mL of HCl 2%, NH_4OH 10% and transferred in three test-tubes each one contain 1 mL, few drops of Dragendorff's gave orange precipitation, Wagner's gave reddish precipitation and Hager's gave yellow precipitation were added indicated the presence of alkaloids[16,17].

Test for flavonoids: Two milliliter of extract dried, 10 mL of ethanol were added, then transferred into four test tubes, the 1 test tube added 1 mL 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 presence of flavonoid, the fourth test tube was treated with ammonium solution the formation of yellow/orange color indicated the presence of flavonoid[17].

Test for tannins: Two milliliters of extracts were mixed with few drops of ferric chloride. A blue-black color indicating the presence of tannins was obtained[16,21].

Test for saponins: Two milliliters of extracts was concentrated in water bath and 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: About 2 mL of extract dissolved in 6 mL of chloroform, a few drops of concentrated sulfuric acid were added 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 1 mL glacial acetic acid containing one drop of ferric chloride solution, 1 mL the sulfuric acid was added under layer, a brown ring obtained was indicated the presence of cardenolides[22].

Test of anthraquinone glycosides: Five milliliter of chloroform were added to 0.1 g of plant powder two layers were formed. Ammonia was added and pink, red, 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, kept in a conical flask for 3 days to obtain eggs and covered with muslin cloth to allow for aeration (At) intervals of about 3 weeks, the emerged larvae were left to give new adults for further experiments (Fig. 2a).

Test one: The effect of powder and three extract of *Albizia anthelmintica* leaves and stem-barks on mortality of *Tribolium castaneum* adults. 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 g wheat crush treated with 1 g *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.

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 one were repeated to follow the effect on fecundity of *T. castaneum* for each treatment, three disposable Petri dishes, each containing 25 g wheat crush and 20 adults of *T. castaneum* selected at random were released. 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

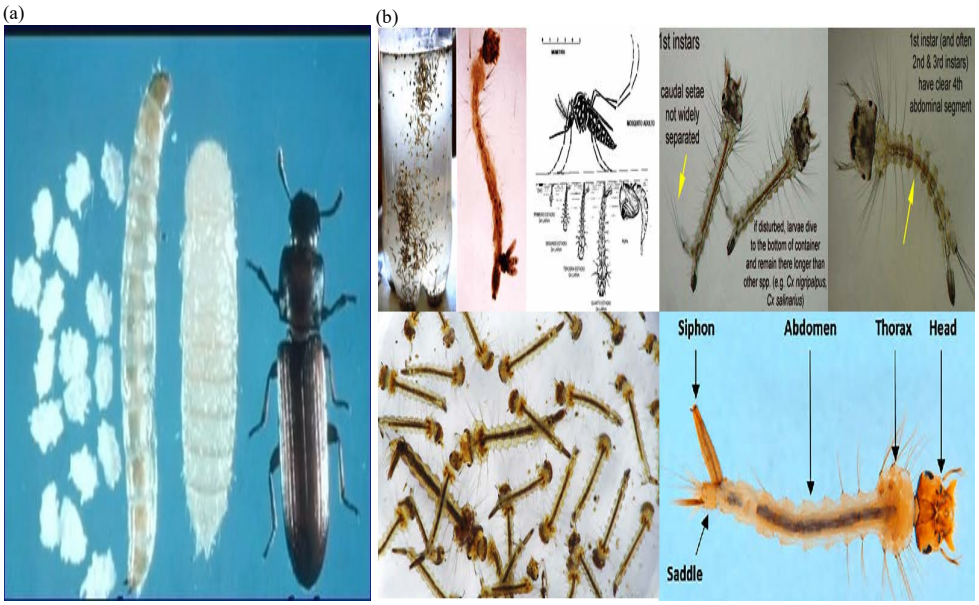

Fig. 2(a-b): (a) Morphology of *Tribolium castaneum* larva and adult and (b) Morphological Larvicidal 3rd instar larvae of *Culex quinquefasciatus* specimen
Source: Mathews *et al.*[24] and Darsie and Morrise[25]

of distilled water, replicated 3 times. About 30, 3rd instar larvae were placed in each test tube and mortality count after 24 h and 72 h, 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'' wide and 1.6'' deep containing distilled water till hatching. Larvae were fed with fine powdered bread. For experiments 3rd instar larvae were used (Fig. 2b).

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

Antimicrobial activity: Table 1 shows the collection of standard organisms for antimicrobial activity.

Preparation of fungal suspensions: The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days.

Table 1: Collection of standard organisms for antimicrobial activity

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

Source: Pfaller *et al.*[27]

Preparation of fungal suspensions: The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days.

Preparation of bacterial suspensions: One milliliter aliquots of a 24 h broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 h. Production of suspension containing about 10^8-10^9 CFU Ml^{-1} was prepared. The average number of viable organisms per milliliter 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 2 h at room temperature for the drops to dry and then incubated at 37°C for 24 h. After incubation, the number of developed colonies in each drop was counted[28,29].

RESULTS

The phytochemical screening of *Albizia anthelmintica* leaves and stem bark was presented in Table 2, it showed that very high amount of the alkaloids were found in ethyl acetate. Flavonoids were found to be very high amount in ethanol extract. Saponins were present in trace amounts in all extracts. Tannins were very high in ethanol extract *Albizia anthelmintica* leaves had high amount of sterols in ethyl acetate when compared with ethanol and n-hexane extracts (Table 2).

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%. 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 3.

The results in Table 4 indicated that the *Azadirachta indica* leaves powder 4% after 3 days was significantly ($p<0.05$) had best effect on mortality of *Tribolium castaneum* adults, followed by *Albizia anthelmintica* leaves powder (4%) then with ethanol. The results after 7 days from treatments, Table 5 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. 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, 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 Table 4 and 5, 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 at $p>0.05$ 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 5).

Table 2: Phytochemical screening of *Albizia anthelmintica* leave and stem-bark

		Successive extraction of leaves with			Successive extraction of stem bark with		
Secondary metabolites	Tests	n-hexane	Ethyl acetate	Ethanol	n-hexane	Ethyl acetate	Ethanol
Alkaloids	Dragendorff'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 3: Mineral composition (ppm) of *Albizia anthelmintica* of leaves and stem bark

Leaves		Stem-bark	
Minerals analyzed	Concentrations (ppm)	Minerals analyzed	Concentration (ppm)
Na	350.00	Na	340.00
K	1.65	K	285.00
P	245.00	P	555.00
Ca	215.00	Ca	170.00
Mg	1.25	Mg	1.55

Values are means of duplicate determinations

Table 4: 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 R1	R2	R3	Total	Mean±0.707	Duncan test F (p<0.05)
Control	20	0	0	0	0	0.00	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

a: p≤0.05 significant difference compared with cytotoxic control, b: p≤ 0.05 significant difference compared with normal control, c: p≤0.05 significant difference compared with standard, Duncan test (p≤0.001) a, b and c adjustment for multiple comparisons

Table 5: 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 R1	R2	R3	Total	Mean±0.745	Duncan test F (p<0.05)
Control	20	0	0	0	0	0.00	a
Azadirachta indica leaves powder (4%)	20	5	4	6	15	5.00	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	1.00	b

a: p≤0.05 significant difference compared with cytotoxic control, b: p≤0.05 significant difference compared with normal control, c: p≤0.05 significant difference compared with standard, Duncan test (p≤0.001) a, b and c adjustment for multiple comparisons

Table 6: Effect of *Albizia anthelmintica* leaves organic extracts on mortality of *Culex quinquefasciatus* instar 3rd larva after 24 h

Treatments	No. of larva	Concentration (µg mL^{-1})	Mortality replicates R1	R2	R3	Total	Mean±0.530	Duncan test (p<0.001)
Control	30	0	0	0	0	0	0.00	a
Albizia anthelmintica leaves ethanol extract	30	500	13	8	26	47	15.70	c
	30	50	6	4	2	12	4.00	b
	30	5	4	2	0	6	2.00	ab
Albizia anthelmintica leaves ethyl acetate extract	30	500	5	2	7	14	4.70	b
	30	50	4	2	3	9	3.00	ab
	30	5	4	2	0	6	2.00	ab
Albizia anthelmintica leaves n-hexane extract	30	500	22	30	30	82	27.30	b
	30	50	18	6	13	37	12.70	c
	30	5	0	0	2	2	0.66	ab

a: p≤0.05 significant difference compared with cytotoxic control, b: p≤0.05 significant difference compared with normal control, c: p≤0.05 significant difference compared with standard, Duncan test (p≤0.001) a, b and c adjustment for multiple comparisons

Table 7: Effect of *Albizia anthelmintica* leaves organic extracts on mortality of *Culex quinquefasciatus* instar 3rd larva after 48 h

Treatments	No. of larva	Concentration (µg mL^{-1})	Mortality replicates R1	R2	R3	Total	Mean±0.530	Duncan test (p<0.001)
Control	30	0	0	0	0	0	0.00	a
Albizia anthelmintica leaves ethanol extract	30	500	16	16	26	58	19.33	c
	30	50	6	6	4	16	5.33	b
	30	5	4	4	2	10	3.33	ab
Albizia anthelmintica leaves ethyl acetate extract	30	500	10	12	14	36	12.00	b
	30	50	12	8	17	37	12.33	ab
	30	5	11	12	12	35	11.66	ab
Albizia anthelmintica leaves n-hexane extract	30	500	23	30	30	83	27.66	b
	30	50	18	8	17	58	19.33	c
	30	5	1	1	2	4	1.30	ab

a: p≤0.05 significant difference compared with cytotoxic control, b: p≤0.05 significant difference compared with normal control, c: p≤0.05 significant difference compared with standard, Duncan test (p≤0.001) a, b and c adjustment for multiple comparisons

Results presented in Table 6 and 7 indicated that all organic extracts with concentration 500 µg mL^{-1} were highly significant (p<0.001) on mortality of *Culex quinquefasciatus* 3rd instar larvae after 24 h. In general the effect of organic extracts of *Albizia anthelmintica* leaves on mortality of *Culex quinquefasciatus* 3rd 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.

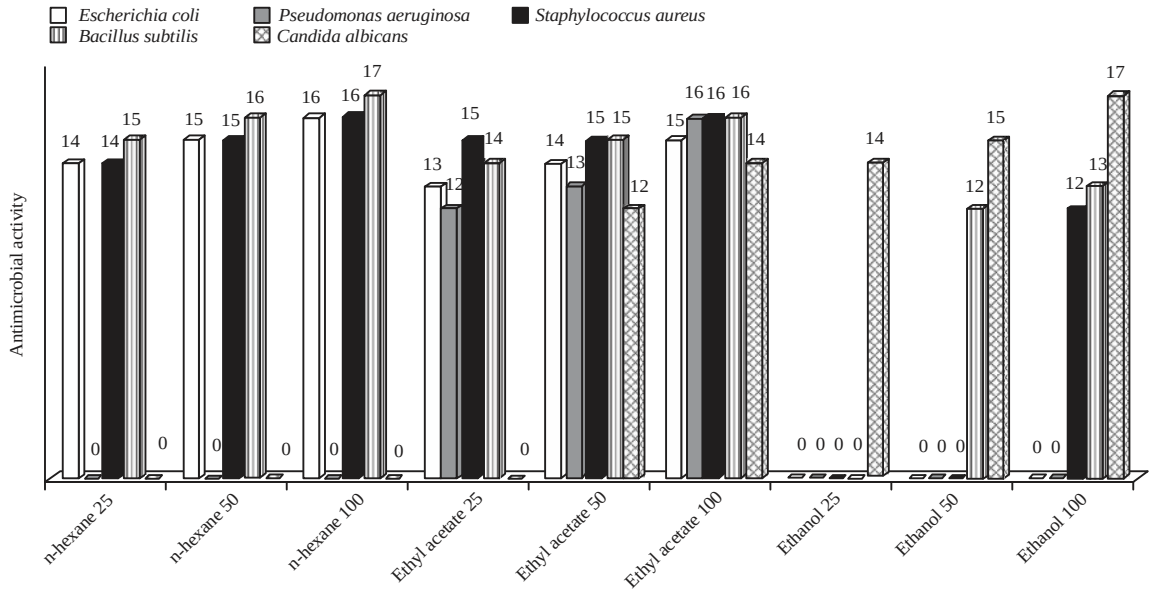

Fig. 3: Antimicrobial activity of *Albizia anthelmintica* leaves different extracts at concentrations 25, 50 and 100 mg mL^{-1} against standard organisms

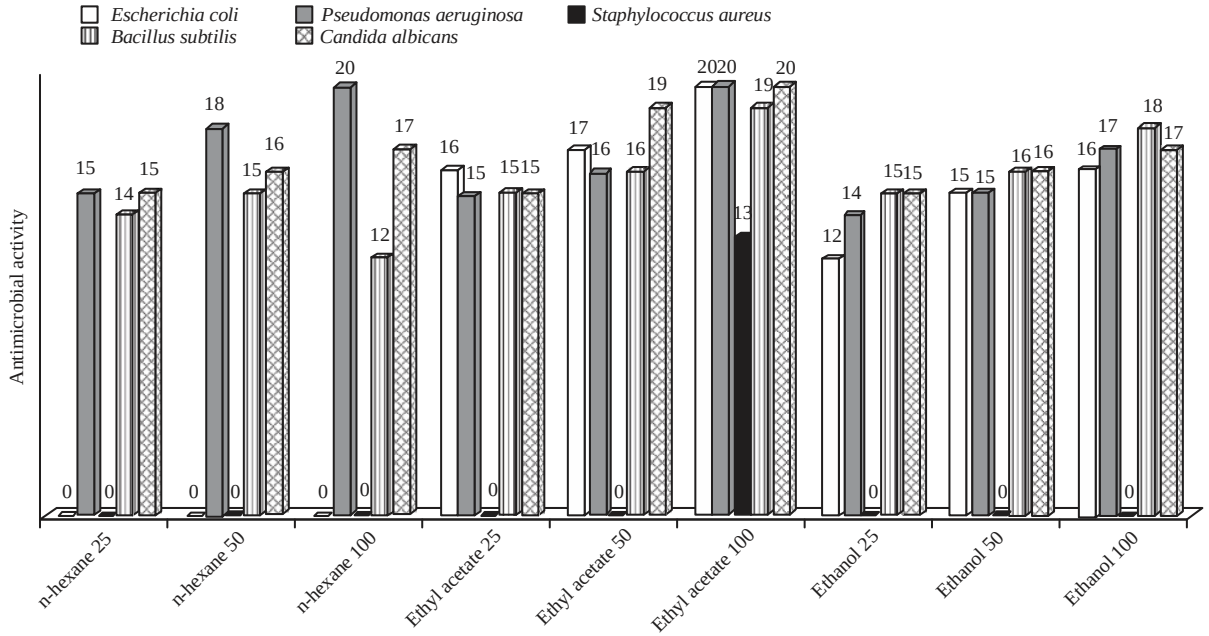

Fig. 4: Antimicrobial activity of *Albizia anthelmintica* stem-barks different extracts at concentrations 25, 50 and 100 mg mL^{-1} against standard organisms

Antimicrobial activity of the crude extracts of *A. anthelmintica* leaves at different concentrations expressed in diameter of zone of inhibition, millimeter against standard organisms was presented in Fig. 3. The n-hexane extract 100 mg mL^{-1} had showed intermediate activities against *Bacillus subtilis* 17 mm, *Escherichia coli* 16 mm and *Staphylococcus aureus* 16 mm. The extract with ethanol 100 mg mL^{-1} was also showed intermediate activity against *C. Albicans* 17 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 Fig. 4.

DISCUSSION

The presence of high amount of alkaloids, flavonoids positively correlated with findings of Kokila *et al.*[30], although Wale *et al.*[31] found only terpenoids when extracted other parts of the tree (bark and root). Tannins and sterols especially in ethyl acetate and ethanol in both leaves and stem of *Albizia anthelmintica*, indicated their suitability to be applied as antimicrobial and insecticidal agents. The chemical analysis of leaves and stem exhibited high ash and nitrogen contents.

Albizia anthelmintica leaves 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 effect of all organic extracts of *Albizia anthelmintica* leaves showed good morality rate on the adults of *Tribolium castaneum*, larvae of *Culex quinquefasciatus* and reduced the number of egg within limited time, but Azadirachta indica leaves powder exhibited higher mortality rate compared with *Albizia anthelmintica* leaves. However the organic extracts of *Albizia anthelmintica* leaves could be considered promising source for insecticides. The antimicrobial examination indicated that the ethanol extract for leaves has high effectiveness against the fungus; the ethyl acetate extract has medium effect against all kinds of bacteria used. Kilonzo *et al.*[32] concluded that extracts from different plants including *Albizia anthelmintica* gave promising results on 7 g positive bacteria supported by findings of Mutembei *et al.*[33]. A high quantity of tannins was found in ethanol extract but not present in ethyl acetate, this is may be due to presence of large amount of flavonoids especially in *A. anthelmintica* leaves. However the evidences of active antimicrobial have been mentioned in previous research[34-38].

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*) larvae.

Highly purified extract can increase the novelty of the work, however the absence of specialized analyzers as High Performance Liquid Chromatography, Ultraviolet-visible Spectroscopy and Ultraviolet-visible Spectrophotometry in the labs resulted in limited findings.

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. 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*.

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.

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