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Larvicidal, leishmanicidal, insecticidal and anthelmintic effects of Sterculia diversifolia stem bark and leaf

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Article Info	Abstract
Received:20 September 2019Accepted:10 February 2020Available Online:10 March 2020	The present study was conducted to evaluate the stem bark and leaf of <i>Sterculia diversifolia</i> for larvicidal, leishmanicidal, insecticidal and anthelmintic activities. In the larvicidal activity, dichloromethane and ethyl acetate
DOI: 10.3329/bjp.v15i1.43235	fractions of both stem bark and leaf showed significant results, while the ethyl acetate fraction showed mild activity (IC ₅₀ : 70.09 ± 0.09 µg/mL; 71.25 ± 0.01 µg/mL). In the insecticidal activity of the stem bark, <i>n</i> -hexane fraction showed mild activity (20-40% mortality) against all three subjects, while the insecticidal activity of leaf, methanolic extract and <i>n</i> -hexane fractions showed
Cite this article: Rabbi F, Zada A, Nisar A. Larvicidal, leishmanicidal, insecticidal and an- thelmintic effects of <i>Sterculia diversifo- lia</i> stem bark and leaf. Bangladesh J Pharmacol. 2020; 15: 32-38.	moderate activity (60% mortality) against <i>Rhyzopertha dominica</i> and <i>Calloso-bruchus analis</i> . In the anthelmintic activity of stem bark and leaf, ethyl acetate fraction exhibited activity in a dose-dependent manner giving time of paralysis and death shorter followed by dichloromethane fraction with 200 mg/mL concentration. Hence, it is clear that <i>S. diversifolia</i> stem bark and leaf possess larvicidal, leishmanicidal, insecticidal and anthelmintic agents.

Introduction

Extensive research on medicinal plants is highly desired to discovered convenient therapeutic moieties that are less toxic, highly potent and effective in case of resistant pathological conditions. Recently various plants reported larvicidal [Garcinia mangostana (Sasikumar and Ghosh, 2017), Zingiber officinale (Rahuman et al., 2008)], leishmanicidal [Tunisian terrestris (Bouabdallah et al., 2018)], insecticidal and anthelmintic [Coriandrum sativum (Hosseinzadeh et al., 2016), Viola betonicifolia (Rizwan et al., 2019)] effects.

Amongst medicinal plants, family Sterculiaceae is a source of bioactive compounds of different chemical classes such as alkaloids, glycosides, terpenoids, sterols, steroids, saponins, apigenins, flavonoids, polyphenols, essential oils, tannins, carbohydrates, proteins, triterpenes and reducing sugars (Khatiashvili et al., 2007; Ouédraogo et al., 2013). The genus Sterculia also possess

various types of biological activities such as S. vallosa bears anthelmintic and leishmanicidal activities while S. guttata and S. foetida bears larvicidal and insecticidal activities respectively (Das et al., 2017; Alam et al., 2012; Katade et al., 2006; Rani et al., 2010). S. diversifolia has antibacterial, antifungal and antioxidant activity. In earlier study, few fatty acid constituents were isolated from the S. diversifolia (Salem et al., 2014). The present study was designed to investigate the larvicidal, leishmanicidal, insecticidal and anthelmintic activity of methanolic extract of S. diversifolia stem bark and leaf and their fractions.

Materials and Methods

Plant material

Plant materials (stem bark and leaf) were collected from the botanical garden of Pakistan Forest Institute (34°



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00'50.6"N 71°29'03.0"E), University of Peshawar, Pakistan, in September, 2014. The identification of the plant was done a taxonomist Mr. Ghulam Jelani at the Department of Botany, University of Peshawar. Under the reference No: Bot.20098, a specimen was deposited in the above-mentioned institute herbarium.

Extraction and fractionation

S. diversifolia stem bark (17 kg) and leaf (13 kg) were dried completely in shade at room temperature. These parts were then crushed to powder and macerated with 90%hydro-methanolic solvent for two weeks. After maceration, the filtration was conducted with Whatman filter paper No. 1. The obtained crude extracts were concentrated under a reduced pressure at 40°C using a rotary evaporator (R-1001-V, Zhengzhou Great Wall Scientific Industrial and Trade Co., China) (Rabbi et al., 2017). The methanolic extract of stem bark and leaf was obtained in 950 g and 1.2 kg quantity respectively. The extract was mixed with distilled water (2.5 L) and soaked overnight. Fractionation was conducted with various organic solvents i.e. *n*-hexane $(3 \times 5 L)$, dichloromethane $(3 \times 5 L)$, ethyl acetate $(3 \times 5 L)$ and *n*-butanol (3 \times 5 L). The remaining was considered as watersoluble fraction i.e. aqueous fraction.

Larvicidal activity

The larvicidal activity of the crude methanolic extract and its fractions of both stem bark and leaf were screened according to the protocol recommended by the WHO (1981). Various concentrations (25, 50, 100, 250, 500 ppm) of the test samples were used. All the extracts were dissolved in respective solvents. A glass beaker of 500 mL was used containing 250 mL tap water. Early third instar of *Aedes aegypti* (25) was introduced to each of the test solutions as well as the control. In the case of control, methanol was only used. Six replicates were maintained at a time for each experiment. The LC₅₀ was calculated using GraphPad prism 5 software (Bucker et

Box 1: Anthelmintic activity

Principle

The anthelmintic assay of crude methanolic extract and various fractions of both stem bark and leaf were evaluated on adult earthworms (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings

Requirements

Albendazole, Earthworm (length: 3-5 cm; width: 0.1-0.2 cm), Extract; Normal saline, Stopwatch

Procedure

Step 1: Earthworms were collected from the moist soil and then washed with normal saline for the removal of the adhering dirt

Step 2: Three earthworms were released in each glass beaker

al., 2013).

Leishmanicidal activity

The crude extract of S. diversifolia (stem bark and leaf), and its subsequent fractions were screened for antileishmanial activity using Leishmania major. NNN biphasic medium was used in which promastigotes of Leishmania were grown. This medium was modified earlier and normal physiological saline was used for this purpose. For culturing parasite, the RPMI medium (1640 Sigma) was used. Inactivated fetal bovine serum (10%) was also supplemented to the medium. Leishmania major parasites were harvested at log phase of growth and centrifuged for 10 min at 3,000 rpm. Parasites were washed three times with normal saline maintaining the same experimental conditions. With the addition of freshly prepared culture medium, final density (106 cells/mL) of parasites was achieved. About 20 µL test sample and 180 µL medium were added to each well of 96-well microplate and then serially diluted. 100 µL prepared culture of Leishmania was added to all wells. One row containing only media and Leishmania culture was served as negative control while the second row served as positive control con-taining pentamidine and amphotericin B. The number of living parasites was counted microscopically after incubation for 72 hours at 22°C using Neubauer counting chamber. Ezfit 5.03 software was used for the determination of IC₅₀ value (Ali et al., 2016).

Insecticidal activity

The crude methanolic extracts (stem bark and leaf) and their various fractions were studied for insecticidal activity against *Tribolium castaneum*, *Rhyzopertha dominica* and *Callosobruchus analis*. Insecticidal assay of crude methanolic extracts (stem bark, leaf) and their fractions were assessed by using the impregnated filter paper method (direct contact method). In this method, *T. castaneum*, *R. dominica* and *C. analis* were reared under

containing solution (50 mL) of reference drug albendazole and extract (50, 100 and 200 mg/mL each) in distilled water.

Step 3: Distilled water was served as negative control.

Step 4: The time taken to paralysis and death of earthworm was observed and noted.

Notes

The earthworms were authenticated by Mr. Abdur Rahim, an expert in animals taxonomy.

When the earthworms did not revive in the normal saline, it indicated water paralysis, while the earthworms lost their motility followed by fading away of their body color or even not moved when dipped in 50°C hot water concluded the death of animals.

References

Panda et al., 2015

Table I								
Larvicidal activity of methanol extract and its fractions								
Test sample	%Mortality ± S.D							
-	25 ppm	25 ppm 50 ppm 100 ppm 250 ppm			500 ppm			
Stem bark								
Methanol	22.2 ± 1.0	26.5 ± 1.2	38.2 ± 0.8	42.4 ± 1.0	52.5 ± 0.4	66.0		
<i>n</i> -Hexane	8.4 ± 1.8	10.8 ± 1.0	16.0 ± 1.6	18.3 ± 0.4	22.1 ± 0.8	326.5		
Dichloromethane	45.6 ± 1.2	48.8 ± 0.4	56.9 ± 0.2	70.2 ± 0.2	82.6 ± 1.8	28.7		
Ethyl acetate	42.9 ± 0.8	52.5 ± 0.6	60.5 ± 1.6	70.2 ± 0.2	81.8 ± 1.4	31.5		
<i>n</i> -Butanol	06.0 ± 0.2	09.2 ± 1.8	12.6 ± 1.0	15.8 ± 0.6	19.3 ± 1.2	353.2		
Aqueous	06.0 ± 0.8	06.9 ± 0.2	07.3 ± 1.8	09.4 ± 0.8	12.2 ± 1.4			
Control					2.2 ± 0.8			
Leaf								
Methanol	28.4 ± 0.2	32.8 ± 0.8	39.5 ± 0.0	46.2 ± 0.4	54.2 ± 0.6	64.2		
<i>n</i> -Hexane	6.2 ± 1.2	9.0 ± 0.0	9.8 ± 0.8	11.3 ± 0.6	14.0 ± 1.8	399.2		
Dichloromethane	48.6 ± 0.8	54.9 ± 0.8	59.1 ± 0.4	72.1 ± 1.8	88.1 ± 1.4	12.0		
Ethyl acetate	46.3 ± 1.2	53.6 ± 1.6	58.2 ± 0.6	70.0 ± 1.2	87.4 ± 1.6	14.4		
<i>n</i> -Butanol	07.9 ± 0.4	08.2 ± 1.0	12.0 ± 1.2	14.8 ± 0.2	17.2 ± 0.8	371.8		
Aqueous	04.2 ± 0.6	05.2 ± 0.4	07.2 ± 1.4	09.5 ± 1.0	12.9 ± 0.2			
Control					2.2 ± 0.8			

controlled and specific conditions of humidity and temperature in the laboratory. Those insects were selected which are uniform in size and age. The test sample (200 mg) was dissolved in acetone (3 mL) to prepare the stock solution. In petri dish, filter paper (90 mm) was placed and then it was loaded with the test sample (1019.1 μ g/cm²). The volatile organic solvent was evaporated after 24 hours. Ten active insects were transferred to the next day to each petri dish with the help of a brush and incubated for 24 hours ($27 \pm 1^{\circ}$ C) with 50% humidity in the growth chamber. Acetone and permethrin (239.50 μ g/cm²) were used as negative and positive control respectively. The percent mortality was determined by comparing the test sample with the positive control (Saeed et al., 2010). It was determined by counting the number of survived insects as below:

Number of insects alive in test

%Mortality= 100 -

×100 Number of insects alive in control

Results

Larvicidal activity

The maximum activity was shown by dichloromethane and ethyl acetate fractions of stem bark (Table I). The dichloromethane fraction exhibited 45.6, 48.8, 56.9, 70.2 and 82.6% mortality at the test doses of 25, 50, 100, 250 and 500 ppm respectively. Ethyl acetate fraction showed %mortality of 42.9, 52.5, 60.5, 70.2 and 81.8% at the same concentrations. The methanolic extract showed mortality of 22.2, 26.5, 38.2, 42.4 and 52.5% at the same concentrations respectively. Other fractions did not show significant activity. The maximum activity of stem bark was observed against dichloromethane fraction followed by ethyl acetate, methanolic extract, *n*-hexane, *n*-butanol and aqueous fractions with LC_{50} values 28.7, 31.5, 66.0, 326.5 and 353.2 µg/mL respecttively.

Dichloromethane and ethyl acetate fractions of leaf showed the maximum activity. Dichloromethane fraction exhibited 48.6, 54.9, 59.1, 72.1 and 88.1% mortality at the test doses of 25, 50, 100, 250 and 500 ppm respectively. Ethyl acetate fraction showed mortality of

Table II						
Leishmanicidal activity of methanol extract and its fractions						
Test sample	$\begin{array}{llllllllllllllllllllllllllllllllllll$					
Methanol	>100	>100				
<i>n</i> -Hexane	77.3 ± 0.3	> 100				
Dichloromethane	79.3 ± 0.0	79.3 ± 0.1				
Ethyl acetate	70.1 ± 0.1	71.3 ± 0.0				
<i>n</i> -Butanol	>100	>100				
Aqueous	>100	>100				
Amphotericin-B	0.3 ± 0.1	0.3 ± 0.1				
Pentamidine	5.1 ± 0.1	5.1 ± 0.1				

Table III							
Insecticidal activity of methanolic extract and its fractions (% Mortality)							
Test sample	Tribolium castaneum		Rhyzopertha dominica		Callosobruchus analis		
-	Stem bark	Leaf	Stem bark	Leaf	Stem bark	Leaf	
Methanol	20	-	-	60	-	-	
<i>n</i> -Hexane	40	-	20	-	30	60	
Dichloromethane	-	-	-	-	-	10	
Ethyl acetate	-	-	-	-	-	10	
<i>n</i> -Butanol	-	-	-	-	-	20	
Aqueous	-	-	-	-	-	10	
Positive control (permethrin)	100	100	100	100	100	100	
Negative control	-	-	-	-	-	-	

46.3, 53.6, 58.2, 70.0 and 87.4% at the same concentrations. The methanolic extract also showed mortality of 28.4, 32.8, 39.5, 46.2 and 54.2% at the same concentrations. The maximum activity of leaf was observed against dichloromethane fraction followed by ethyl acetate, methanolic extract, *n*-butanol and *n*-hexane with LC₅₀ values 12.0, 14.4, 64.2, 371.8 and 399.2 μ g/mL (Table I).

Leishmanicidal activity

The leishmanicidal activity of the crude methanolic extract and its fractions of stem bark and leaf were evaluated against promastigotes of *Leishmania major* (Table II). In the leishmanicidal activity of stem bark, ethyl acetate fraction showed mild activity (IC_{50} : 70.1 µg/mL) followed by *n*-hexane fraction (IC_{50} : 77.3 µg/mL). In the leishmanicidal activity of leaf, ethyl acetate fraction showed mild activity (IC_{50} : 71.3 µg/mL) followed by dichloromethane fraction (IC_{50} : 79.3 µg/mL).

Insecticidal activity

The insecticidal activity of crude methanolic extract and its fractions were studied against R. dominica, T. castaneum and C. analis. The results revealed that the crude methanolic extract stem bark showed low insecticidal activity (20%) against T. castaneum and inactive against rest of the insects (Table III). The n-hexane fraction showed good activity, 40% against R. dominica, 20% against T. castaneum and 30% against C. analis. Other fractions; dichloromethane, ethyl acetate, n-butanol and aqueous were found inactive against test insects. In case of leaf, crude methanolic extract showed good insecticidal activity (60%) against R. dominica and inactive against the rest of the insects. Other fractions; *n*-hexane, dichloromethane, ethyl acetate, n-butanol and aqueous showed 60, 10, 10, 20, 10% insecticidal activity respectively against C. analis, while were found inactive against R. dominica, T. castaneum.

Anthelmintic activity

In the anthelmintic activity, extract of S. diversifolia

(stem bark and leaf) and its fractions demonstrated worms paralysis as well as death as presented in Table IV. The extracts in higher concentrations produced much earlier paralytic effect and shorten the time to death for all worms. Ethyl acetate fraction exhibited anthelmintic activity in dose-dependent manner giving time of paralysis and death shorter followed by dichloromethane fraction with 200 mg/mL concentration. The crude methanolic extract and aqueous fraction exhibited moderate anthelmintic activity, paralyzed worm after 57.5 and 65.9 min, while killed after 72.7 and 78.3 min respectively at the tested dose of 200 mg/ mL. The *n*-hexane and *n*-butanol fractions showed mild activity and paralyzed the worm after 62.4 and 80.6 min while killed after 82.6 and 91.6 min respectively at the test dose of 200 mg/mL. The ethyl acetate and dichloromethane fractions of leaf showed good activity and paralyzed the worm after 23.1 and 28.3 min while killed after 39.7 and 54.9 min respectively at the tested dose of 200 mg/mL. crude methanolic extract and n-hexane fraction of leaf exhibit moderate anthelmintic activity paralyzed worm after 59.4 and 60.5 min, while killed after 75.2 and 77.6 min respectively at the tested dose of 200 mg/mL. Aqueous and n-butanol fractions of leaf exhibit mild activity and paralyzed worm after 70.5 and 72.3 min while killed after 87.4 and 89.5 min respectively at the dose of 200 mg/mL. The value of paralysis and death for albendazole (standard) was 0.8 and 4.2 min respectively.

Discussion

Larvicidal, leishmanicidal, insecticidal and anthelmintic activities are reported for the first time in this work. Keeping in view the importance of the larvicidal activity of stem bark and leaf, maximum activity was shown by dichloromethane (28.7 and 12.0 ppm) followed by ethyl acetate (31.5 and 14.4 ppm) fractions. Results indicated the potency of leaf fractions over stem bark fractions. In the leishmanicidal activity, maximum

Table IV							
Anthelmintic activity of methanolic extract and its fractions							
Test sample		Stem bark			Leaf		
	Conc. (mg/mL)	Time taken for paral- ysis (min)	Time taken for death (min)	Time taken for paralysis (min)	Time taken for death (min)		
Methanol	50	66.3 ± 0.8	84.2 ± 1.3	70.2 ± 1.0	87.3 ± 0.4		
	100	62.5 ± 1.1	78.7 ± 1.0	65.9 ± 2.4	81.5 ± 3.1		
	200	57.5 ± 2.2	72.7 ± 3.1	59.4 ± 1.9	75.2 ± 0.3		
<i>n</i> -Hexane	50	70.9 ± 1.4	93.8 ± 0.8	68.3 ± 2.9	87.2 ± 2.2		
	100	68.1 ± 1.9	88.6 ± 2.9	64.0 ± 0.6	80.3 ± 1.1		
	200	62.4 ± 3.1	82.6 ± 2.7	60.5 ± 0.3	77.6 ± 2.3		
Dichloromethane	50	50.3 ± 1.0	89.6 ± 2.7	48.3 ± 3.0	82.4 ± 0.4		
	100	40.4 ± 2.5	68.3 ± 0.6	38.1 ± 1.6	62.8 ± 1.5		
	200	31.2 ± 2.0	60.0 ± 0.9	28.3 ± 2.2	54.9 ± 2.7		
Ethyl acetate	50	45.2 ± 0.5	70.6 ± 3.0	42.7 ± 1.2	67.3 ± 1.3		
	100	32.7 ± 1.8	59.8 ± 2.7	31.1 ± 2.1	50.6 ± 0.7		
	200	24.8 ± 2.7	41.8 ± 0.2	23.1 ± 1.1	39.7 ± 2.9		
<i>n</i> -Butanol	50	102.4 ± 0.5	123.7 ± 1.5	100.8 ± 3.0	121.0 ± 0.8		
	100	88.8 ± 3.2	102.3 ± 2.2	81.8 ± 0.7	99.5 ± 1.5		
	200	80.6 ± 0.7	91.6 ± 1.2	72.3 ± 1.5	89.5 ± 2.7		
Aqueous	50	91.4 ± 1.2	112.7 ± 0.5	93.7 ± 1.6	117.5 ± 0.5		
	100	80.4 ± 3.2	98.4 ± 2.5	85.0 ± 2.7	101.7 ± 2.9		
	200	65.9 ± 1.7	78.3 ± 1.0	70.5 ± 1.4	87.4 ± 1.0		
Negative control (1% carboxymethyl cellulose in normal saline)							
Positive control (albendazole)	50	1.8 ± 0.5	7.3 ± 2.0	1.8 ± 0.5	7.3 ± 2.0		
	100	1.2 ± 1.7	5.2 ± 1.0	1.2 ± 1.7	5.2 ± 1.0		
	200	0.8 ± 0.2	4.2 ± 0.2	0.8 ± 0.2	4.2 ± 0.2		
All values represent mean ± S.E.M; p<0.05							

activity was observed for ethyl acetate fraction (IC50 70.1 μ g/mL and 71.3 μ g/mL respectively) amongst test samples of Sterculia diversifolia stem bark and leaf showing almost similar results. The results of insecticidal activity reveal that methanolic extract of stem bark, n-hexane fraction showed mild to moderate activity (percent mortality) against all three insects followed by crude methanolic extract, while the insecticidal activity of leaf, none of the fraction showed insecticidal activity against all three insects, however, the methanolic extract showed 60% mortality against Rhyzopertha dominica while n-hexane fraction showed 60% activity against Callosobruchus analis. In anthelmintic activity of stem bark and leaf, ethyl acetate fraction exhibited activity in dose-dependent manner giving time of paralysis and death shorter followed by dichloromethane fraction with the highest concentration (200 mg/mL), showing almost comparable outcomes.

Crude extracts are a complex mixture of active compounds (Anees, 2008). Various extracts possess larvicidal activity against mosquitoes have the advantage in reducing resistance issue and also environmentfriendly. The disease can be easily controlled by controlling the vectors (e.g. parasitic vectors). The insect population can be reduced by controlling the mosquito larvae which is a result reduce disease burden (Ghosh et al., 2012). Throughout the world and especially in tropical countries, dengue fever is a measure of health problem. Dengue fever could be controlled by reducing *Aedes aegypti* production (Raveen et al., 2014). It is evident from the result that various concentrations of *Sterculia diversifolia* stem bark and leaf were the main cause of mortality in *A. aegypti* larvae as reported from plants *Citrus grandis and Tinosphora rhumpii* (Gutierrez et al., 2014)¹.

Leishmaniasis, a common disease in the subtropical and tropical regions of the world, is an infection of the protozoal parasite of genus *Leishmania* (Monzote et al., 2014). Currently leishmaniasis is considered as a serious disease due to lack of availability of specific treatment; still some semi synthetic and synthetic drugs are implicated to treat leishmaniasis (Mears et al., 2015; Tiuman et al., 2011; Zucca et al., 2013). Drugs such as amphotericin B and pentamidine are also used in the management of leishmaniasis, lacking the desired efficacy (Aronson et al., 2016; Cunha et al., 2015; Sadeghi-Nejad et al., 2011). The extracts *Achillea biebersteinii* showed promising activity with potent leishmanicidal activities as compared to *Sterculia diversifolia* stem bark and leaf, which showed mild activity (Al -Sokari et al., 2015).

Insects control relies heavily on the use of synthetic insecticides; however, their widespread use has led to various problems such as the development of insect strains resistant to insecticides (Zia-Ul-Hag et al., 2012). The plant produces various secondary metabolites for defense purposes. These secondary metabolites protect as well as repel the harmful insects to control pests Pavela, 2016; Dubey et al., 2008). Monoterpenes, sesquiterpenes lactones and triterpenes are some of the secondary metabolites of various plants that possess insecticidal activity (Barney et al., 2005; Cespedes et al., 2015; Sosa and Tonn, 2008). Crude extracts of Lepidium sativum and Ipomoea hederacea showed the highest insecticidal activity against all three insects as compare to Sterculia diversifolia (Zia-Ul-Haq et al., 2012). Sterculia diversifolia stem bark, n-hexane fraction showed mild to moderate activity against all insects, while the MESD and n-hexane fraction of leaf showed highest activity against Rhyzopertha dominica and Callosobruchus analis respectively.

The anthelmintic potential of tested extract may be due to the presence of similar phytoconstitutes, which was evident. Studies have shown that phenolic and tannin possess plants to produce anthelmintic activities because tannins chemically belong to polyphenolic compounds. Phenolic and tannins are known to interfere with the generation of energy in helminth parasites by uncoup-ling the oxidative phosphorylation and also bind to free proteins in GIT of host animal or glycoprotein on the parasite cuticle, leading to death (Mali and Wadekar, 2008). It is possible that phenolic compounds and tannins also possessing in Sterculia diversifolia stem bark and leaf extracts may be responsible for the anthelmintic activity. Sterculia diversifolia stem bark and leaf shown moderate anthelmintic activity comparable to other medicinal plants e.g. Blumea lacera (Pattewar et al., 2012).

S. diversifolia stem bark and leaf possess various types of phytochemical constituents such as alkaloids, saponins, flavonoids, steroids, tannins etc. These constituents may carry larvicidal, leishmanicidal, insecticidal and anthelmintic potential, although further phytochemical screening is needed. Crude extracts require preliminary screening because it is a complex mixture of bioactive compounds. Hence, these plant extracts are an

economical, safe, effective and environment-friendly alternative.

Conclusion

S. diversifolia stem bark and leaf possess larvicidal, leishmanicidal, insecticidal and anthelmintic agents.

Conflict of Interest

The authors declare that there is no conflict of interest.

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