Journal of Genetics and Cell Biology
JGCB, 2(1): 36-43
www.scitcentral.com

ISSN: 2639-3360

Original Research Article: Open Access

Antimicrobial Activity and Phytochemical Screening of Leaf and Bark of *Blighia sapida*

Ologundudu FA*, Obimakinde ET, Osunyemi OS and Akinnifesi OJ

*Department of Biology, Federal University of Technology Akure, Ondo State, Nigeria.

Received September 18, 2018; Accepted October 04, 2018; Published April 10, 2019

ABSTRACT

Objective: To determine the antimicrobial activity of *Blighia sapida* extracts against some selected strains of microorganisms was evaluated in this study.

Methods: Phytochemical screening of hexane, chloroform, ethanol and aqueous (control) extracts of *B. sapida* leaf and bark was carried out using standard protocols. The antimicrobial activity of the plant material was carried out using the Agar well diffusion method. The extracts were tested against three strains of gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi*) and three strains of gram negative bacteria (*Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella pneumoniae*) isolated from the clinic and characterized.

Results: Phytochemical analysis revealed the presence of saponins, anthraquinones, cardiac glycosides and flavonoids in both leaf and bark extracts. Alkaloids, tannins, phlobatannins and terpenes were also detected in the leaf extract. Results of antimicrobial activity of *B. sapida* extracts confirmed a broad spectrum of activity on all the bacteria tested by aqueous, chloroform, hexane and ethanol. The two extracts did not exert antifungal effect on any of the tested fungal species at all concentrations but exhibited activity against *S. aureus* and *B. subtilis* but not against *E. coli* and *S. dysenteriae*. It was observed that the stem bark extract was more potent than the leaf extract.

Conclusion: The therapeutic potential exhibited by the plant parts of *Blighia sapida* showed greater antimicrobial activity against the tested microorganisms, hence, the plant should be explored for the formulation of drugs to treat infectious diseases caused by microorganisms.

Keywords: Antimicrobial, *Blighia sapida*, Strains, Therapeutic potential

INTRODUCTION

In recent times, emergence of resistance by microorganisms to antibiotics is becoming alarming. Genetic ability of bacteria to transmit and acquire resistance to drugs is a general attribute of bacteria which are utilized against therapeutic agents [1]. Development of new bacteria strains with multi-resistant is a major concern because lots of immunosuppressive patients in hospitals could acquire new infections in hospitals which result in high mortality [2]. Nosocomial, hospital acquired infections caused by microbes could infect people visiting hospitals which may likewise lead to illness and increase in mortality. Apart from infections that can be acquired in the hospitals, there are numbers of infectious diseases that can be caused by microbes which can lead to death.

Increasing rate of resistance of microbes to antimicrobial drugs needs proper monitoring due to the fact that microbes would continually develop strains with multi-resistant against antimicrobial drugs; therefore there is a need for continuous research on development of new medicines (some of which are from natural products such as botanicals) which can be used for treatment of infectious diseases caused by microbes.

Since the ancient times, plants parts have been used as herbal medicines due to their healing properties [3]. Some prominent bioactive compounds such as alkaloids, tannin, flavonoid and phenolic compounds present in plants make them possess medicinal value [4]. The unique medicinal properties of a specific plant depend on the concentrations of

Corresponding author: Ologundudu FA, Department of Biology, Federal University of Technology Akure, Ondo State, Nigeria, E-mail: faologundudu@futa.edu.ng

Citation: Ologundudu FA, Obimakinde ET, Osunyemi OS & Akinnifesi OJ. (2019) Antimicrobial Activity and Phytochemical Screening of Leaf and Bark of *Blighia sapida*. J Genet Cell Biol, 2(1): 36-43.

the bioactive compounds in the plant [3]. Several plants are used for medicinal purposes due to the fact that they contain bioactive compounds. For instance, Sarmiento et al. [5] reported that neem leaves are capable of preventing hepatitis and controlling diabetes, Mukhtar and Ahmed [6] reported that marigold leaf is highly potent in healing of burns and bruises, also, guava plant leaf and bark are well known to treat diarrhea, gastrointestinal disorder, toothaches, colds and swellings [7]. *Blighia sapida* which is the plant of major concern in this study has been reported in previous studies for treatment of various ailments in Africa [8] stated that the bark pulp is used as liniment for edema and intercostal pains in Cote d'Ivoire, while the bark is powdered and grounded with capsicum and rubbed on the body as a stimulant. The ashes of the dried husks and seeds are used in the preparation of soap, because they are rich in potash. The extracts of the leaves are used as eye drop in ophthalmia and conjunctivitis. Locally, various parts of *B. sapida* plants are used either alone or in combination for the treatment of psychosis, cancer, gonorrhea, stomach ache, hernia, backache, diarrhea and constipation [8].

Having been aware of the great medicinal importance of plants for treatment of various diseases, especially those related to have antimicrobial activities; this research is therefore aimed at investigating the antimicrobial activities of *B. sapida* against selected microorganism.

MATERIALS AND METHODS

Collection and authentication of plant materials

The leaf and bark of *Blighia sapida* were collected from Ikare Akoko, Ondo State, Nigeria. The plant materials were identified and authenticated by a taxonomist at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko.

Preparation of plant extracts

The leaves and bark of *Blighia sapida* were washed and dried at room temperature for 28 days and then crushed into fine powder using an electric blender and later sieved. 20 g of the powder was used for the hexane extraction, chloroform, ethanol and water and was added to a thimble and then placed in a Soxhlet extractor.

Fractionation of extract

Leaf and Bark extracts were fractionated using N-hexane, chloroform, ethanol and water. About 20 g of each dried extract was ground in a mortar and dissolved in 200 ml of water and later filtered through a Whatmann No. 1 filter paper. 200 ml of N-hexane was added to the mixture, shaken vigorously and allowed to settle. The other fractions were removed and concentrated. 200 ml of chloroform was added to the aqueous layer and also vigorously shaken and allowed to settle. The aqueous and the chloroform layers were further separated while the chloroform portion was concentrated to dryness by allowing to standing on the laboratory bench while the solvent evaporated.

Preparation of the medium

39 g of potato dextrose agar powder were weighed into clean conical Flask and 100 ml of sterilized distilled was dispensed into the conical flask to form homogenized solutions. It was properly homogenized on hot plate using magnetic stirrer before it was sterilized in an autoclave at 121°C for 15 min. Later it was cooled in water bath at 45°C and 500 mg of antibiotics (Chloramphenicol) was added.

Phytochemical analysis

All the fractions from the extracts were subjected to phytochemical screening to test for the presence of saponin, alkaloids, flavonoids, glycosides, tannins, phenol, carbohydrates, phytosterols, quinone, steroids and phytosteroids, terpenoids, cardiac glycosides, coumarins and anthraquinone among other secondary metabolites [9,10].

Test organisms

Source of microorganisms: Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi*) and gram negative bacteria (*Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella pneumoniae*) were obtained from the Department of Microbiology, Federal University Of Technology Akure, Nigeria.

Purification of test organisms: The purity of the test organisms were confirmed by sub-culturing into nutrient broth incubated at 37°C for 18 h after which they were streaked unto sterile nutrient agar plate and later incubated. The developed colonies were observed under the microscope after simple staining after which they were later sub-cultured.

Standardization of inoculum: The inocula were prepared from the stock cultures which were maintained in nutrient agar at 4°C and sub-cultured in nutrient broth using a sterilized wire loop. The density of suspension inoculated unto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution [11].

Susceptibility test: Agar well diffusion method was employed for antibacterial assay following established protocols. The preparation was incubated at appropriate temperature. The zone of inhibition diameter formed in the medium was measured to determine antibacterial effectiveness of the different concentrations of the extracts.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration for bacterial isolates was carried out using tube dilution as described by Akinyemi et al. [12]. Stock solution of 50,000 µg in 10 ml sterilized distilled water was serially diluted to arrive at

concentrations of 500 µg/ml, 1000 µg/ml, 2000 µg/ml and 4000 µg/ml, respectively.

Positive and negative control

Chloramphenicol and N-hexane were used as positive control for *Bacillus subtilis*, *Salmonella typhi*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *E. coli* and *Kleibsella pneumonia* sterilized distilled water was used as negative control.

RESULTS

Qualitative phytochemical analysis of *B. sapida* leaf and bark in different solvents

On the dried leaf and bark samples of *B. sapida* collected, qualitative phytochemical screening was carried out using ethanol, chloroform, water and N-hexane as solvents. The following phyto-constituents such as saponin, alkaloids, flavonoids, glycosides, tanins, phenol, carbohydrates, phytosterols, quinone, steroids, phytosteroids, terpenoids, cardiac glycosides, coumarins, anthraquinone and protein were extracted to varying degree.

Alkaloids saponins, tannins, phlobatannins, flavonoids, terpenes, cardiac glycosides and combined anthraquinones were detected in the leaf of *B. sapida*, however, only saponins, flavonoids, combined anthraquinones and cardiac glycosides were detected in the stem bark **(Tables 1 and 2)**.

Table 1. Qualitative phytochemical analysis of *B. sapida* leaf extract in different solvents.

Phytochemical test	Hexane Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract
Saponin	+	-	++	+
Alkaloids	+	-	-	-
Flavonoids	+	+	-	-
Glycosides	-	-	-	-
Tanins	-	-	+	-
Phenol	-	-	-	-
Carbohydrates	-	++	-	++
Phyto sterols	++	-	-	-
Quinone	-	-	++	-
Steroids and Phytosteroids	+	-	+	-
Terpenoids	-	+	++	+
Cardiac glycosides	+	++	+	++
Coumarins	-	+	-	++
Anthraquinone	-	-	-	++
Protein	-	-	-	-

Key: + = present, - = absent, ++ = 50% inhibition, + = 75% inhibition, - = 100% inhibition

Table 2. Qualitative phytochemical analysis of *B. sapida* bark in different solvents.

Phytochemical test	Hexane Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract
Saponin	-	-	++	++
Alkaloids	+	-	-	-
Flavonoids	+	++	-	-
Glycosides	-	-	-	-
Tanins	-	-	+	-
Phenol	-	-	+	+
Carbohydrates	-	+	+	++
Phytosterols	-	-	-	-
Quinone	+	++	++	-
Steroids and Phytosteroids	-	-	+	+
Terpenoids	-	++	++	++
Cardiac glycosides	+	++	+	++
Coumarins	-	+	-	++
Anthraquinone	-	-	++	++
Protein	-	-	-	-

Key: + = present, - = absent, ++ = 50% inhibition, + = 75% inhibition, - = 100% inhibition

Yield of leaf and bark extract of *Blighia sapida*

Table 3 shows the percentage yield of extract in each solvent used to ferment the extract. It was observed that leaf of the plant had the highest yield of 5.2% using hexane for extraction and the lowest yield of 0.4% using chloroform for extraction while the highest yield of 0.7% and lowest yield of 0.3% was obtained for the plant bark using Hexane and chloroform respectively for extraction.

Table 3. Yield of leaf and bark extract of *Blighia sapida*.

Parts	Hexane	Chloroform	Ethanol	Aqueous
Leaf	5.2%	0.4%	3.6%	0.6%
Bark	0.7%	0.3%	0.5%	0.4%

Antibacterial activity of the extracts of the leaf of *B. sapida* against the test bacterial isolates

Table 4 shows the antibacterial activity of the leaf extract and the standard drug streptomycin. Result obtained showed that the leaf extract had no activity against the two test Gram negative bacteria (*E. coli* and *S. dysenteriae*) at the various test concentrations, but had activity against the Gram positive bacteria (*S. aureus* and *B. subtilis*) only at 200 mg/ml.

Table 4. Antibacterial activity of the extracts of the leaf of *B. sapida* against the test bacterial isolates.

	Zone of Inhibition (mm)			
	HE	**AQ**	**CE**	**EE**
Bacteria strain (Gram positive)				
Bacillus subtilis	2.00 ± 0.20^{d}	1.00 ± 0.20^{d}	1.80 ± 0.20^{b}	2.70 ± 0.20^{d}
Staphylococcus aureus	1.40 ± 0.20^{c}	1.60 ± 0.20^{c}	0.80 ± 0.20^{a}	2.00 ± 0.20^{c}
Salmonella typhi	1.10 ± 0.20^{a}	0.80 ± 0.20^{b}	1.90 ± 0.20^{c}	1.90 ± 0.20^{e}
Bacteria strain (Gram negative)				
Streptococcus pneumonia	1.30 ± 0.20^{b}	1.60 ± 0.20^{e}	1.20 ± 0.20^{a}	0.90 ± 0.20^{a}
Escherichia coli	1.10 ± 0.00^{d}	0.90 ± 0.20^{d}	1.30 ± 0.20^{b}	0.90 ± 0.20^{a}
Klebsiella pneumonia	1.20 ± 0.10^{e}	1.50 ± 0.20^{d}	1.60 ± 0.20^{e}	1.90 ± 0.20^{d}
LSD (0.50)				

Key: HE: Hexane Extract; CH: Chloroform Extract; EE: Ethanol Extract; AQ: Aqueous Extract
Means with the same letter in superscript along the same column are not significantly different at P<0.05

Antibacterial activity of the extracts of stem bark of *B. sapida* against the test bacterial isolates

The antibacterial activities of the stem bark extract of *B. sapida* and streptomycin are presented the **Table 5**. The stem bark extract inhibited the growth of *B. subtilis* at concentrations 60 mg/ml and higher whereas it inhibited the growth of *S. aureus* only at 100 and 200 mg/ml. No inhibition was observed for *E. coli* and *S. dysenteriae* at the various test concentrations.

There was no antifungal activity against any of the fungal species when the leaf and stem bark extracts of *B. sapida* were assayed. Only the standard drug nystatin inhibited the growth of the fungal species with mean values of inhibition zone of 22 mm, 18 mm, 20 mm and 22 mm for *C. albicans*, *M. canis*, *A. niger* and *A. fumigatus*, respectively. In all the control experiments (without extract and standard drug) there was steady growth.

Table 5. Antibacterial activity of the extracts of the bark of *B. sapida* against the test bacterial isolates.

	Zone of Inhibition (mm)			
	HE	**AQ**	**CE**	**EE**
Bacteria strain (Gram positive)				
Bacillus subtilis	2.80 ± 0.10^{e}	1.30 ± 0.10^{c}	2.17 ± 0.12^{c}	1.73 ± 0.20^{c}
Staphylococcus aureus	2.13 ± 0.58^{c}	1.00 ± 0.10^{a}	2.47 ± 0.12^{d}	1.73 ± 0.15^{c}
Salmonella typhi	2.26 ± 0.21^{e}	2.50 ± 0.10^{e}	2.10 ± 0.10^{d}	1.37 ± 0.25^{c}
Bacteria strain (Gram negative)				
Streptococcus pneumonia	2.60 ± 0.00^{b}	2.60 ± 0.20^{f}	1.13 ± 0.58^{a}	2.40 ± 0.20^{e}
Escherichia coli	2.00 ± 0.17^{d}	1.07 ± 0.15^{b}	2.73 ± 0.57^{f}	1.70 ± 0.20^{a}
Klebsiella pneumonia	3.10 ± 0.10^{f}	1.70 ± 0.10^{d}	1.77 ± 0.12^{b}	1.40 ± 0.20^{a}
LSD (0.50)				

Key: HE: Hexane extract; CH: Chloroform extract; EE: Ethanol extract; AQ: Aqueous extract
Mean with the same letter in superscript along the same column are not significantly different at P<0.05

The minimum inhibitory concentrations (MIC) of extract of *B. sapida* stem bark against *Staphylococcus aureus* and *Bacillus subtilis*

The Minimum Inhibitory Concentration (MIC) of the stem barks extract of *B. sapida* for susceptible bacterial species. The MIC values obtained were 12.5 mg/ml and 100 mg/ml for *B. subtilis* and *S. aureus*, respectively **(Table 6)**.

Table 6. MIC of extract of *B. sapida* stems bark against *Staphylococcus aureus* and *Bacillus subtilis*.

Concentration (mg/ml)	Test Organisms	
	Staphylococcus aureus	***Bacillus subtilis***
100	-	-
50	+	-
25	+	-
12.5	+	+
6.25	+	+
3.125	+	+
1.5625	+	+

Key: - = Clear (no growth); + = turbid (growth)

DISCUSSION

The antimicrobial activities of *B. sapida* against selected microorganisms were investigated in this study. Form the results obtained from the qualitative phytochemical screening of *B. sapida* using ethanol, chloroform, water and N-hexane as solvents for extraction, result showed that phytochemicals such as alkaloids saponins, tannins, phlobatannins, flavonoids, terpenes, cardiac glycosides and combined anthraquinones were detected in the leaf of *B. sapida*, however, only saponins, flavonoids, combined anthraquinones and cardiac glycosides were detected in the stem bark. The antimicrobial activities reported in this research could be attributed to the presence of these phytochemical constituents of the plant. It is interesting to note that fewer phytochemical constituents were detected in the stem bark extract than the leaf extract, yet it performed better than the leaf extract. The stem bark of the plant demonstrating high inhibitory activity is in agreement with the report of Sukumar et al. [13] that the activity of phytochemical compounds on target species varies with respect to plant parts from which they are extracted. The stem bark extracts demonstrating higher inhibitory activities than the leaf extract could be as a result of the phytoconstituents in the leaf were present in trace amount or could be different in type compared to those detected in the stem bark. For instance, saponins often occur as complex mixtures and according to the structure of the aglycone or sapogenin, two kinds of saponins are recognized, the steroidal and the pentacyclic type [9]. Also, there are various classes of alkaloids, but basic nitrogen is the unifying factor, each phytoconstituent is a group of compounds, each compound differing in structure and chemical properties [9,14].

The results of antimicrobial sensitivity test revealed that there was no activity against the tested fungal species at all concentration of the extracts used. This indicates that the fungal species used in this study demonstrated physiological resistance to the leaf and stem bark extracts of *B. sapida*. The absence of antifungal activity using the extracts agrees with the report of Duraipandiyan et al. [15], who documented that ethanolic leaf extract of *B. sapida* had no antifungal activity against *C. albicans*. However, this finding disagrees with the finding of Nascimento et al. [2] who reported the susceptibility of *C. albicans* to extracts from basil, clove, guava, jambolan, lemon balm, pomegranate, rosemary and thyme. This difference could be as a result of the variation in the phytochemical constituents of the different plants used in the various study.

From the results obtained from this study, it was observed that the ethanolic leaf and stem bark extracts of *B. sapida* demonstrated inhibitory activity against Gram positive bacteria (*S. aureus* and *B. subtilis*). Farjana et al. [16] also reported that ethanol extracts of guava leaf showed antibacterial activity against *S. aureus* and *Staphylococcus*

epidermidis in their study. However, the Gram negative bacteria (*E. coli* and *S. dysenteriae*) were resistant to the two extracts. In agreement to this finding is the report of Nascimento et al. [2] who also reported that *E. coli* is resistant to all the extracts gotten from plants used in their study. Also, previous researches have reported *E. coli* to have multi-resistance against drugs. Resistance of Gram negative bacteria to these plant extracts could be as a result of the possession of sophisticated cell wall by the bacteria which does not allow permeation of external agents [17].

The plant extracts, though active to some extent against the Gram positive bacterial species used in the study but was not as active when compare with the effect of streptomycin, the standard drug used. Streptomycin is a broad spectrum antibiotic, which is active against both Gram positive and Gram negative bacteria. The lower inhibitory activity of the extracts when compared with the standard drug may be attributed to the fact that the extracts used were in their crude form. Also, the active phytochemical constituents of the plant extracts acting against the bacteria could be present in trace amount while the active constituents of streptomycin could be present in very high amount. It is anticipated that better results could be obtained with purified fractions of the extracts.

Statistical analysis revealed significant difference ($P<0.05$) between the potency of leaf and stem bark extracts of *B. sapida* against bacteria with the stem bark extract being more potent. Only the minimum inhibitory concentration (MIC) of the stem bark was determined, because of aforementioned results earlier obtained. In coincidence with the negative effect of the extract on Gram negative bacteria. Sharma et al. [18] reported that the antidiarrheal activity of aqueous and ethanolic stem bark extracts of *B. sapida* was as a result of the ability of the extracts to inhibit intestinal motility and enter pooling effect. This implies that the usefulness of the leaf and stem bark extracts of *B. sapida* in the treatment of dysentery in folklore is not due to its antibacterial effect on toxin producing bacteria that are associated with diarrhea, such as *E. coli* and *S. dysenteriae* **(Table 7)**.

Table 7. The minimum bactericidal concentration (MBC) of hydro-ethanolic extract of *B. sapida* stem bark assayed against *Staphylococcus aureus* and *Bacillus subtilis*.

Concentration (mg/ml)	Test Organisms	
	Staphylococcus aureus	***Bacillus subtilis***
100	-	-
50	+	-
25	+	-
12.5	+	+

Key: - = Clear (no growth); + = turbid (growth)

CONCLUSION

This research has revealed that *Blighia sapida* extracts showed antimicrobial activity against the tested microorganisms at varying levels. Both plant parts could be the best form of treatment to reduce the prevalence of infections caused by microorganisms. In addition, more research can be carried out on this plant to know the most active constituents of the plant responsible for antimicrobial activity; these active constituents can be isolated to develop new drugs which can be used for treatment of infections caused by microbes. Therefore being able to identify plants and their active constituents that are potent against microorganism will be a break through to solving the problem of emergence of resistance by microorganisms to antibiotics as new drugs could be formulated.

CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

REFERENCES

1. Cohen ML (1992) Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 257: 1050-1055.
2. Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol 31: 247-256.
3. Farjana A, Zerin N, Kabir MS (2014) Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. Asian Pac J Trop Dis 4: S920-S923.
4. Shihabudeen MH, Priscilla DH, Thirumurugan K (2010) Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Int J Pharma Sci Res 1: 430-434.

5. Sarmiento WC, Maramba CC, Gonzales MLM (2011) An in vitro study on the antibacterial effect of neem (*Azadirachta indica*) leaf extract on methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. PIDSP J 12: 40-45.

6. Mukhtar H, Ahmed N (2000) Tea polyphenols: Prevention of cancer and optimizing health. Am J Clin Nutr 71: 1698-1702.

7. Richard FT, Joshua AT, Phillips AJ (2013) Effect of aqueous extract of leaf and bark of guava (*Psidium guajava*) on fungi *Microsporum gypseum* and *Trichophyton mentagrophytes* and bacteria *Staphylococcus aureus* sand *Staphylococcus epidermidis*. Adv Med Plant Res 1: 45-48.

8. Okogun JI (1996) Medicinal plant research in Nigeria. Chem Nigerian Med Plants 10: 31-45.

9. Evans WC (2000) Trease and Evans Pharmacognosy (15th Edn). W.B. Saunders Company Ltd., pp: 135-150.

10. Sofowora A (2001) Medicinal plants and traditional medicine in Africa. J Phytochem 34: 223-230.

11. Cheesebrough M (2000) District laboratory practice in tropical African countries. Cambridge University Press, London.

12. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure AK (2005) Screening of crude extracts of some medicinal plants used in South-West Nigerian Unorthodox medicine for anti-methicilin resistant *Staphylococcus aureus*. BMC Complement Altern Med 5: 6.

13. Sukumar K, Perich MJ, Boobar LR (1991) Botanical derivatives in mosquito control: A review. J Am Mosq Control Assoc 7: 210-237.

14. Sofowora A (1993) Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp: 191-289.

15. Duraipandiyan V, Ayyaner M, Ignacimuthus S (2006) Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med 6: 35.

16. Farjana A, Zerin N, Kabi S (2014) Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. Asian Pac J Trop Dis 4: 920-923.

17. Brown WL (1975) Contributions toward a reclassification of the formicidae, V. ponerinae, tribes Platythyreini, Cerepacchyini, Cylindromyrmecini, Acanthostichini and Aenictogitini. Cornell University 5: 1-116.

18. Sharma DK, Gupta VK, Kumar S, Joshi V, Mandal RS, et al. (2015) Evaluation of antidiarrheal activity of ethanolic extract of *Holarrhena antidysenterica* seeds in rat. Vet World 8: 1392-1395.