ETHIOPIAN PHARMACEUTICAL JOURNAL
Ethiop. Pharm. J. **36,** 11-18 (2020)
http://dx.doi.org/10.4314/epj.v36i1.2

Antibacterial Activity against Clinical Isolates of *Salmonella enterica* Serovar Paratyphi and Brine Shrimp Lethality Assay of *Trichilia megalantha* Harms and *Trichilia welwitschii* C. DC

Philip A. Idowu* **and Tajudeen A. Adegbenle**
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

Increasing resistance of typhoidal *Salmonella enterica* to conventional antibiotics has caused more cases of typhoid, therapeutic failure, morbidity and mortality; creating the need to search for new and effective antimicrobial agents from medicinal plants. The present study aimed to detect antisalmonella activity and cytotoxicity (safety) status of the stem bark and leaves of two Nigerian medicinal plants, *Trichilia megalantha* and *Trichilia welwitschii*. Nine clinical isolates of *Salmonella paratyphi,* whose antibiogram were determined by Kirby-Bauer disc diffusion method were used. Antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the clinical isolates were done by agar cup diffusion and agar dilution methods, respectively. Acute toxicity of the extracts was determined using brine shrimp lethality assay (BSLA). All the nine isolates of *Salmonella paratyphi* were resistant to β-lactam antibiotics (augmentin, cefuroxime, ceftazidime and ampicillin) but susceptible to fluoroquinolones (ciprofloxacin, ofloxacin), nitrofurantoin and gentamicin. The crude extracts of the two plants elicited activity against the nine clinical isolates with the bark extracts being more active than the leaf extracts. *T. welwitschii* was slightly less active than *T. megalantha*. The MIC and MBC ranged 1.25 - 5.0 mg/ml and 2.5 - 10 mg/ml, respectively for the plants. The antisalmonella activity of methanolic extracts of both plants were found to be less than that of ciprofloxacin and ofloxacin. With modal cytotoxicity values of 400 - 500 µg/ml, the plant parts were considered nontoxic. Therefore, *T. megalantha* and *T. welwitschii* could provide a potential source of antibacterial agent(s) for the treatment of *Salmonella* paratyphoid infections.

Keywords: *Trichilia megalantha*; *Trichilia welwitschii,* antisalmonella; cytotoxicity; *Salmonella paratyphi*

INTRODUCTION

Increasing drug resistance of pathogenic microbes to conventional antibiotics has necessitated a search for new and effective antimicrobial agents from natural products (Cowan, 1999; Newmann *et al.,* 2003; Ríos and Recio, 2005). Screening of ethnomedicinal plants for bioactive constituents is the classical way of discovering new drugs or lead compounds (Kinghorn *et al.*, 2011). Many African medicinal plants are yet to be investigated for bioactive constituents (Hostettmann *et al*., 2000). Malaria and typhoid are still prevalent in many African countries, including Nigeria, and the two diseases may occur concurrently (Akinyemi *et al*., 2007). Many Meliaceae plants used to treat malaria in tropical countries also possess anti-microbial property (Omar *et al*., 2003), and their antisalmonella activity could be of additional phytotherapeutic benefit when typhoid is also present. *Salmonella typhi* or *S. paratyphi* are the causative organisms of typhoid or paratyphoid fever, which are still prevalent in many developing countries, including Nigeria (Akinyemi *et al*., 2007). Typhoid fever causes an estimated 21.7 million illnesses and 217,000 death, while paratyphoid fever causes 5.4 million illnesses worldwide (Crump *et al*., 2004; Gibani *et al*., 2018). Unlike typhoid fever, paratyphoid fever is difficult to handle with vaccines owing to the presence of three different forms (paratyphoid A, B, C). Many Meliaceae plants, to which *Trichilia* belongs, are commonly used to treat typhoid fever, as typified by *Azadirachta indica.* Cytotoxicity tests are very important either in the discovery of anticancer agents or in establishing the safety profile of medicinal plant extracts in traditional use (Pisutthanan *et al*., 2004). Brine shrimp lethality assay (BSLA) represents a simple, convenient and rapid method of preliminary assessment of extracts and compounds for cytotoxic property (Meyer *et al*., 1982), and has been used worldwide (Krishnaraju *et al*., 2005; Rahman *et al*., 2008).

*Correspondence: igboyega@yahoo.com

Trichilia megalantha Harms (Meliaceae) is an evergreen tree of 30 m or more in height, native to Southwest Nigeria and Ivory Coast, while *Trichilia welwitschii* C. DC. is a forest tree of 20 m or more in height, found in Southwest Nigeria, Zaire and Angola (Keay, 1989). The genus *Trichilia* contains many bioactive constituents (Vieira *et al*., 2014). Some bioactivities reported on *Trichilia* species that are found in Nigeria include; antimicrobial in *T. heudelotii* which is a synonym of *T. monadelpha* (Aladesanmi and Odediran, 2000), *T. emetica* (Germano *et al*., 2005) and *T. dregeana* (Eldeen *et al*., 2007) and insect antifeedant in *T. prieuriana* (Lidert *et al*., 1985). Only a few works have been reported on the plants; the antimalarial activity of *T. megalantha* was reported by Fadare *et al*. (2013). Tsamo *et al*. (2013) reported the isolation of limonoids from the seeds of *T. welwitschii,* and the isolated limonoids were found to have low cytotoxic property to Vero cells but good nitric oxide and anticholinesterase inhibitory activities (Dzoyem *et al*., 2015). We hereby report the investigation of the antibacterial activity of *T. megalantha* and *T. welwitchii* against clinical isolates of *Salmonella enterica* serovar Paratyphi and acute toxicity for safety as used orally.

MATERIALS AND METHODS

Collection and authentication of plants:

The leaves, stem barks and root barks of *Trichilia megalantha* and *Trichilia welwitschii* were collected from Olokemeji Reserve Forest (Latitude: 7° 25' north, Longitude: 3° 32' east), Nigeria, during the rainy season. The plants were identified at the Department of Botany, University of Ibadan and authenticated at the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, where voucher specimens of *T. welwitschii* and *T. megalantha* were deposited with herbarium numbers 109860 and 109861, respectively.

Preparation and extraction of plants:

The leaves, stem bark and root bark of *T. megalantha* and *T. welwitschii* were air-dried at room temperature, pulverized and extracted using Soxhlet apparatus. Powdered samples of the leaves, stem bark and root bark of the plants were extracted with absolute methanol. The extracts were concentrated under reduced pressure in a rotary evaporator and stored at 4 ^{o}C.

Test organisms:

Nine clinical isolates of *Salmonella paratyphi* used for the study were collected from the Department of Medical Microbiology, University College Hospital (UCH), Ibadan. Typed *Salmonella typhimurium* (ATCC 14208) used as a reference organism was obtained from the Pharmaceutical Microbiology Laboratory, University of Ibadan. All bacteria were cultured on nutrient agar (No. 2) and nutrient broth (pH 7.4) (Oxoid) and were maintained on agar slope at 4 ^{o}C before use. Brine shrimp (*Artemia salina*, Sanders®) eggs were purchased from Great Salt Lake Company, USA.

Antibiotic discs:

The antibiotic discs (Abtek Biologicals) containing the following antibiotics: c ipro-floxacin (5 µg), ofloxacin (5µg), augmentin (30 µg), nitrofurantoin (300 µg), ampicillin (10 µg), ceftazidime (30 µg), cefuroxime (30 µg) and gentamicin (30 µg) were used.

Antibiotic susceptibility test:

Kirby-Bauer disc diffusion method was used to determine the susceptibility of the clinical isolates and the reference organism to the antimicrobial agents: ampicillin, augmentin, ceftazidime, cefuroxime, cipro-floxacin, gentamicin, nitrofurantoin and ofloxacin. A 0.1 ml of a 10^{-2} dilution of an overnight broth culture of each organism (containing an inoculum size 1.0 x 10^{8} cells/ ml based on comparison of turbidity with 0.5

McFarland standard) was seeded into molten but cooled 20 ml Mueller Hinton (MH) agar. The antibiotic discs were placed on the set agar and allowed to diffuse for 1 h before incubation at 37 ^{o}C for 24 h. The diameter of the zones of inhibition were measured in millimeters and values were interpreted according to standard (CLSI, 2007). The tests were performed in triplicate and average values were recorded.

Determination of antisalmonella activity:

The antisalmonella activity of the leaf extracts were determined using agar well diffusion using the method of Perez *et al.* (1990) with slight modifications. A 10^{-2} dilution of an overnight broth culture of each organism (containing an inoculum size 1.0 x 10^{8} cells/ml as compared with the turbidity of 0.5 McFarland) was evenly spread on the surface of agar plates by sterile swab sticks. Sterile cork borer (6 mm in diameter) was used to bore wells in the nutrient agar. The plant extracts in 40% methanol or 2% dimethyl sulfoxide (DMSO) at various concentrations (100, 50 and 25 mg/ml) were added in each well. Gentamicin (10 µg/ml) served as a positive control while 40% methanol or 2% DMSO was used as a negative control. The plates were allowed to stand for 1 h for proper diffusion of the extract into the agar and incubated at 37 ^{o}C for 24 h. The plates were observed for presence of bacterial growth, and zone of growth inhibition around the well (≥ 10 mm) were used as a measure of activity of extracts.

Determination of minimum inhibitory concentrations (MICs):

The MIC of each extract was determined using agar dilution method of Andrews (2001) with slight modifications. Each extract was serially diluted to obtain concentrations of 50.0, 25.0, 12.5, 6.25, 3.13, 1.65, 0.82 mg/ml in the agar and 2 ml of each concentration of the extract was incorporated into 18 ml of MH agar and each of the test isolates was inoculated on the plates by streaking method. Plates were incubated at 37 °C for 24 h, after which they were observed for growth. The least concentration in which there was no visible growth was noted as the MIC.

Determination of minimum bactericidal concentrations (MBCs).

The MBC of each extract was determined by inoculating freshly prepared MH broth in test tubes with an inoculum from the MIC plates showing no visible growth. The tubes were then incubated at 37 ^{o}C for 24 h. The tube with the minimum concentration of the extract showing absence of growth was recorded as the MBC (CLSI, 2007).

Determination of cytotoxicity (Brine shrimp lethality assay):

The cytotoxicity of *T. megalantha* and *T. welwitschii* methanolic extracts was done according to the methods of Meyer *et al.* (1982) and Quazi *et al.* (2017). Brine shrimp eggs (*Artemia salina*, Sanders®, USA) were hatched in natural sea water collected from Badagry Beach in Lagos, in a plastic chamber. The eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 - 72 h incubation at room temperature (25 - 29 ^{o}C), hatched nauplii (larvae) were collected by pipette from the lighter side while their shells were left in another side. The crude extracts were dissolved in DMSO at a maximum concentration of not exceeding 0.05% and then diluted with sea water (4.5 ml of sea water + 0.5 ml of dissolved extract) to final concentrations of 1000, 100 and 10 ppm, successively. Ten nauplii in sea water with quinidine sulphate (Sigma) at 200 µg/ml and those without extract were set up as positive and negative controls, respectively. Each concentration was run in triplicate and vincristine sulphate and DMSO were used as positive and negative controls, respectively. After 24 h, the number of nauplii that survived was counted under a magnifying glass, and these were used to determine the numbers killed. The numbers of dead nauplii

were recorded and concentration response curve (AAT Bioquest, 2020) was used to determine the lethal concentration to half of the test organisms (LC_{50}).

$$\text{Percent death} = \frac{\text{Total naupii} - \text{Alive naupii}}{\text{Total naupii}} \times 100\%$$

RESULTS AND DISCUSSION

Table 1 shows the antibiogram results with interpretation based on CLSI (2007) showing: resistance (R), where there is no zone of inhibition or where the value is low, intermediate (I) for low sensitivity, or susceptible (S) where the zone of inhibition is high. All the nine isolates and the standard strain were completely resistant (100%) to ampicillin, amoxicillin-clavulanic acid and ceftazidime, cefuroxime (all of which are β-lactam antibiotics), but showed 100% susceptibility to ciprofloxacin, gentamicin, nitrofurantoin, ofloxacin. It is interesting to discover that the clinical isolates have resistance pattern that is similar to that of *S. typhimurium* standard. The recorded 100% resistance of *Salmonella* strains to penicillins and cephalosporins tested, which was also noted by Eguale *et al*. (2017), is worrisome. The isolates were mostly susceptible to ciprofloxacin and ofloxacin, which are now the current standard antibiotics for treating typhoid and paratyphoid fever in Nigeria. However, it should be noted that *Salmonella* isolates with reduced susceptibility to fluoroquinolones have been identified in Nigeria (Akinyemi *et al*., 2007), Ethiopia (Eguale *et al*., 2017) and elsewhere.

Table 2 shows the antisalmonella activity of *T. megalantha* and *T. welwitschii* and the stem bark of the former plant being the most active having inhibition zone range of 9 - 18 mm. The extracts of *T. megalantha* and *T. welwitschii* were active against the nine isolates *S. paratyphi* at 100 mg/ml with a decreased activity as the concentrations were reduced to 25 mg/ml. *T. welwitschii* and *T. megalantha* stem bark extracts were the most active with inhibition zone of 16 mm and 18 mm, respectively. Zone of growth inhibition of *T. welwitschii* against the test organism ranged between 9 - 16 mm, while that of *T. megalantha* ranged between 9 - 18 mm. At 50 mg/ml, the root bark extract of *T. megalantha* was active against eight isolates out of nine, the root bark and leaf extracts of *T. welwitschii* with the stem bark extract of *T. megalantha* elicited activity on seven isolates, while the leaf extract of *T. megalantha* was active on only two isolates. At 25 mg/ml, *T. megalantha* leaf extract was active on Sal 1 but was inactive on other *Salmonella* isolates. At 100 mg/ml, crude methanol extracts of both the root bark and leaf of *T. welwitschii* were comparable to 10 µg gentamicin.

The methanol extracts of the root bark, stem bark and leaf of both *T. megalantha* and *T. welwitschii* possess activity against the clinical isolates of *S. paratyphi*, which were

Table 1. Susceptibility of *Salmonella paratyphi* isolates to antibiotics with CLSI interpretation

Antibiotics	Zones of inhibition (mm)/Susceptibility of tested microorganisms									
	Sal 1	Sal 2	Sal 3	Sal 4	Sal 5	Sal 6	Sal 7	Sal 8	Sal 9	Sal ATCC
CPR (5 µg)	22/S	21/S	25/S	24/S	25/S	27/S	26/S	25/S	22/S	22/S
OFL (5 µg)	22/S	22/S	19/S	25/S	25/S	20/S	25/S	22/S	20/S	21/S
AUG (30 µg)	R	R	R	R	R	R	R	R	R	R
NIT (300 µg)	21/S	22/S	22/S	22/S	20/S	20/S	22/S	20/S	21/S	20/S
AMP (10 µg)	R	R	R	R	R	R	R	R	R	R
CAZ (30 µg)	R	R	R	R	R	R	R	R	R	R
CRX (30 µg)	R	R	R	R	R	R	R	R	R	R
GEN (10 µg)	14/I	15/S	13/I	14/I	14/I	15/S	15/S	14/I	13/I	13/I

CLSI: Clinical and Laboratory Standards Institute; CPR: Ciprofloxacin, OFL: Ofloxacin, AUG: Augmentin, NIT: Nitrofurantoin, AMP: Ampicillin, CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamicin; R: Resistance/No zone of inhibition; I: Intermediate, S: Susceptible; Sal ATCC: *Salmonella typhimurium* ATCC 14208.

Table 2. Susceptibility of *Salmonella paratyphi* isolates to extracts of *Trichilia megalantha* and *Trichilia welwitchii*

Extract	Conc. in mg/ml	Zones of inhibition (mm) of *S. paratyphi* strains								
		Sal 1	Sal 2	Sal 3	Sal 4	Sal 5	Sal 6	Sal 7	Sal 8	Sal 9
TWRB	100	13	15	10	14	11	11	13	12	12
	50	12	11	-	11	10	-	10	11	10
	25	9	10	-	9	9	-	-	-	9
TWSB	100	11	14	12	16	15	10	14	11	12
	50	10	12	11	13	11	9	12	9	11
	25	9	11	9	10	10	-	-	-	9
TWL	100	13	12	13	14	13	14	12	13	13
	50	-	10	10	-	9	12	11	10	10
	25	-	-	9	-	-	10	-	9	9
TMRB	100	12	11	11	10	11	16	11	14	9
	50	11	10	10	9	10	10	9	12	-
	25	10	9	-	-	9	-	-	10	-
TMSB	100	15	11	12	9	10	14	10	18	15
	50	11	10	11	-	9	10	-	14	12
	25	9	9	-	-	-	-	-	10	-
TML	100	12	10	11	9	10	9	10	10	11
	50	11	-	-	-	-	-	-	9	-
	25	10	-	-	-	-	-	-	-	-

TWRB: *T. welwitchii* root bark, TWSB: *T. welwitchii* stem bark, TWL: *T. welwitchii* leaf; TMRB: *T. megalantha* root bark, TMSB: *T. megalantha* stem bark, TML: *T. megalantha* leaf; -: No zone of inhibition; Sal 1-9: *Salmonella paratyphi* isolates number 1 to 9.

resistant to ampicillin, augmentin, ceftazidime and cefuroxime. The greatest antibacterial activity was found in the stem bark extracts of both plants. The antisalmonella activity of methanol extracts of the two plants were less than those of ciprofloxacin and ofloxacin which are the current drugs of choice for severe cases of typhoid and paratyphoid fever in Nigeria (Akinyemi *et al*., 2007), this may be due to the presence of a very minimal concentration of active constituents in the crude extracts. The antibacterial activity of the extracts is expected to be greater when the active constituents are isolated. Hence, the two plants can generate leads through isolation and characterization of their active constituents especially from the stem barks, which can be modified structurally to obtain antibiotic of choice for treating typhoid and paratyphoid fever and other bacterial infections caused by susceptible organisms.

Table 3 shows MIC and MBC of the two plants' extracts on selected *S. paratyphi* strains. The MIC range of 1.25 - 5.0 mg/ml was close to the MBC value of 2.5 - 10 mg/ml. MIC and MBC provide quantitative assessment of antimicrobial activity unlike agar diffusion method where zones of inhibition give qualitative evaluation. The lower the MIC (and MBC), the higher the activity of the agent, therefore, the roots of *T. megalantha* with MIC of 1.25 mg/ml was slightly more active than the others. Further, the MIC index (MBC/MIC) values were less than 4, indicating that the extracts exhibited bactericidal action on the organisms (Koné *et al*., 2007; Mogana *et al*., 2020). When the MIC index value is greater than 4, bacteriostatic action is indicated. In chemotherapy of infectious diseases, bactericidal drugs are preferable in immunocompromised patients. Therefore, the extracts of the *Trichilia* species with MIC index of 1 - 2 are bactericidal in action and can be of therapeutic value.

Table 4 shows brine shrimp lethality assay (BSLA) result of the extracts. Five of the six extracts showed LC_{50} of 440 - 560 µg/ml while the 6th (*T. megalantha* stem bark) was 128 µg/ml. Sahgal *et al*. (2010) reported LC_{50} of 680 µg/ml for *Swietenia*

Table 3. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the plant extracts on selected *Salmonella paratyphi* isolates

Plant	Plant part	*Salmonella paratyphi* 1		*Salmonella paratyphi* 8	
		MIC	MBC	MIC	MBC
Trichilia megalantha	Leaf	2.50	5.00	5.00	10.00
	Stem bark	2.50	5.00	2.50	5.00
	Root bark	1.25	2.50	1.25	2.50
*Trichilia welwitschi*i	Leaf	2.50	5.00	5.00	5.00
	Stem bark	2.50	5.00	5.00	10.00
	Root bark	5.00	I0.0	2.50	5.00

mahagoni of the same family Meliaceae. The higher the value of LC_{50} the lower the cytotoxicity, and according to Pisutthanah *et al.* (2004) and Krishnaraju *et al*. (2005), LC_{50} higher than 100 µg/ml is considered nontoxic and not potent enough to be considered for anticancer use. For a significant cytotoxicity useful as antitumor, the LC_{50} must be close to the vincristine standard of 0.91 µg/ml. Generally, cytotoxic antitumor activities are low among extracts of *Trichilia* species (Vieira *et al*., 2014; Dzoyem *et al*., 2015), therefore the high LC_{50} values may not favor anticancer property but surely represent nontoxicity when consumed. It is an advantage when an extract of low cytotoxicity possesses good antimicrobial activity, since the antibacterial mechanism is not based on cytotoxicity. Although McLaughlin *et al.* (1998), Carballo *et al.* (2002), and Sahgal *et al*. (2010) showed correlation between BSLA and cell-line cytotoxicity values, BSLA is considered a preliminary test that requires confirmation with cell-line or animal model (Quazi *et al.*, 2017).

Table 4. Results of brine shrimp lethality assay (BSLA) of the plant extracts

Extract	1000 ppm		100 ppm		10 ppm		LC_{50} (µg/ml)
	0 min	24 h	0 min	24 h	0 min	24 h	
TWL	10	8	10	10	10	10	559.544
	10	9	10	10	10	10	
	10	9	10	10	10	10	
TWSB	10	4	10	10	10	10	498.534
	10	5	10	10	10	10	
	10	4	10	10	10	10	
TWRB	10	4	10	10	10	10	458.759
	10	6	10	10	10	10	
	10	7	10	10	10	10	
TML	10	5	10	9	10	9	441.853
	10	7	10	10	10	9	
	10	6	10	9	10	9	
TMSB	10	0	10	5	10	9	127.762
	10	0	10	7	10	10	
	10	0	10	6	10	9	
TMRB	10	0	10	10	10	10	448.559
	10	1	10	10	10	10	
	10	0	10	10	10	10	

TWL: *T. welwitchii* leaf, TWSB: *T. welwitchii* stem bark, TWRB: *T. welwitchii* root bark; TML: *T. megalantha* leaf, TMSB: *T. megalantha* stem bark, TMRB: *T. megalantha* root bark; -: No zone of inhibition; Sal 1 - 9: *Salmonella paratyphi* isolates number 1 to 9.

CONCLUSION

The methanolic extracts of the various parts of *T. megalantha* and *T. welwitschii* showed good antibacterial activity against *S. enterica* serovar Paratyphi to justify the traditional uses of the plants in typhoidal diseases. Brine shrimp lethality assay showed the nontoxic nature of the plants hence should be safe when consumed for therapeutic purposes. We recommend a bioassay-guided isolation of the active compound(s) which may serve as a therapeutic agent or as a lead compound.

Acknowledgements

The authors appreciated Mr. Samuel Odewor (FRIN) and Kayode Owolabi (Botanical Garden, UI) for their roles in collection, identification and authentication of the plants. We also acknowledge Mr. Odekanmi of Medical Microbiology Unit (UCH) for collection of organisms and Pharmaceutical Microbiology (UI) staff for technical assistance.

REFERENCES

AAT Bioquest, Inc. (2020). Quest Graph™ IC50 Calculator. Retrieved from https://www.aatbio.com/tools/ic50-calculator.

Akinyemi KO, Bamiro BS, Coker AO (2007) Salmonellosis in Lagos, Nigeria: incidence of *Plasmodium falciparum*-associated co-infection, patterns of antimicrobial resistance, and emergence of reduced susceptibility to fluoroquinolones. *J Health Popul Nutr* **25**: 351-358.

Aladesanmi AJ, Odediran SA (2000). Antimicrobial activity of *Trichilia heudelotii* leaves *Fitoterapia* **71**: 179-182.

Andrews JM (2001). Determination of minimum inhibitory concentrations. *J Antimicrob Chemother* **48** (**Suppl 1**): 5-16.

Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD (2002). A comparison between two brine shrimp assays to detect *in vi*tro cytotoxicity in marine natural products. *BMC Biotechnol* **2**: 17.

Clinical and Laboratory Standards Institute (CLSI) (2007). Performance standard for antibiotic susceptibility testing; Seventeenth Informational Supplement. Document M100 -S17, Vol. 27 No. 1 Wayne, PA USA.

Cowan MM (1999). Plant products as antimicrobial agents. *Clin Microbiol Rev* **12**: 564-582

Crump JA, Luby SP, Mintz ED (2004). The global burden of typhoid fever. *Bull World Health Organ* **82**: 346-353.

Dzoyem JP, Tsamo AT, Melong R, Mkounga P, Nkengfack AE, McGaw LJ, Eloff JN (2015). Cytotoxicity, nitric oxide and acetylcholinesterase inhibitory activity of three limonoids isolated from *Trichilia welwitschii* (Meliaceae). *Biol Res* **48:** 57.

Eguale T, Birungi J, Asrat D, Njahira MN, Njuguna J, Gebreyes WA, Gunn JS, Djikeng A, Engidawork E (2017). Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal *Salmonella* isolates from humans and animals in central Ethiopia. *Antimicrob Resist Infect Control* **6**: 13.

Eldeen IM, van Heerden FR, van Staden J (2007). Biological activities of cycloart-23-ene-3,25-diol isolated from the leaves of *Trichilia dregeana*. *South Afr J Bot* **73**: 366-371.

Fadare DA, Abiodun OO, Ajaiyeoba EO (2013). *In vivo* antimalarial activity of *Trichilia megalantha* Harms extracts and fractions in animal models. *Parasitol Res* **112**: 2991-2995.

Germanò MP, D'Angelo V, Sanogo R, Catania S, Alma R, De Pasquale R, Bisignano G (2005). Hepatoprotective and antibacterial effects of extracts from *Trichilia emetica* Vahl. (Meliaceae). *J Ethnopharmacol* **96**: 227-232

Gibani MM, Britto C, Pollard AJ (2018). Typhoid and paratyphoid fever: a call to action. *Curr Opin Infect Dis* **31**: 440-448.

Hostettmann K, Marston A, Ndjoko K, Wolfender J-L (2000). The potential of African plants as a source of drugs. *Curr Org Chem* **4**: 973-1010.

Keay RWJ (1989). *Trees of Nigeria.* Oxford Science Publications, Clarendon Press Oxford., pp 351.

Kinghorn AD, Chai H-B, Sung CK, Keller WJ (2011). The classical drug discovery approach to defining bioactive constituents of botanicals. *Fitoterapia* **82**: 71-79.

Koné MW, Atindehou KK, Kacou-N'Douba A, Dosso M (2006). Evaluation of 17 medicinal plants from northern Côte d'ivoire for their *in vitro* activity against *Streptococcus pneumoniae. Afr J Tradit Complement Altern Med* **4**: 17 - 22.

Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay H-S, Subbaraju GV (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci Eng* **3**: 125-134.

Lidert Z, Taylor DAH, Thirugnanam M (1985). Insect antifeedant activity of four prieurianin -type limonoids. *J Nat Prod* **48**: 843-845.

McLaughlin JL, Rogers LL, Anderson JE (1998). The use of biological assays to evaluate botanicals. *Drug Inform J* **32**: 513-24.

Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE, McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med* **45**: 31-34

Mogana R, Adhikari A, Tzar MN, Ramliza R, Wiart C (2020). Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. *BMC Complement Med Ther* **20**:55.

Newman DJ, Cragg GM, Snader KM (2003). Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* **66**: 1022-1037.

Omar S, Zhang J, MacKinnon S, Leaman D, Durst T, Philogene BJR, Arnason JT, Sanchez-Vindas PE, Poveda L, Tamez PA, Pezzuto JM (2003). Traditionally-used antimalarials from Meliaceae. *Curr Top Med Chem* **3**: 133-139.

Perez C, Pauli M, Barzerque P (1990). An antibiotic assay by agar well diffusion method. *Acta Biol Med Exp* **15**: 113-115.

Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S, Muanrit O (2004). Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae. *Naresuan Univ J* **12**: 13-18.

Quazi SS, Fatema CA, Mir M (2017). Brine shrimp lethality assay. *Bangladesh J Pharmacol* **12**: 186-189.

Ríos J-L, Recio MC (2005). Medicinal plants and antimicrobial activity. *J Ethnopharmacol* **100**: 80-84.

Rahman MS, Begum B, Chowdury R, Rahman MK, Rashid MA (2008). Preliminary cytotoxicity screening of some medicinal plants of Bangladesh. *Dhaka Univ J Pharm Sci* **7**: 47-52.

Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM (2010). Brine shrimp lethality and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract. *Pharmacognosy Res* **2**: 215-220.

Tsamo A, Langat MK, Nkounga P, Waffo AFK, Nkengfack AE, Mulholland DA (2013). Limonoids from the West African *Trichilia welwitschii* (Meliaceae). *Biochem Syst Ecol* **50**: 368-370.

Vieira IJC, Terra W, Gonçalves MdS, Braz-Filho R (2014). Secondary metabolites of the genus *Trichilia*: contribution to the chemistry of Meliaceae family. *Am J Anal Chem* **5**: 91-121.