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Antibacterial-guided isolation of constituents from *Senna alata* leaves with a particular reference against Multi-Drug-Resistant *Vibrio cholerae* and *Shigella flexneri*

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ABSTRACT

Senna alata is widely used in Cameroon for the treatment of several infections which include gonorrhoea, gastro-intestinal and skin diseases. Therefore, its leaves were investigated for antibacterial principles. Extraction of plant material was done with methanol, follow by partition with hexane. Separation and purification of compounds was done using a combination of chromatographic techniques. Isolated compounds were identified by means of spectroscopic methods and comparison with literature data. The antibacterial activity of extracts, fractions and compounds was assessed by evaluating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against Multi-Drug-Resistant **(**MDR) *Vibrio cholerae* and *Shigella flexneri.* Three secondary metabolites namely kaempferol, luteolin and aloe-emodin were isolated from methanol residue active extract. The antibacterial results showed that the MeOH residue extract, fractions A and C, as well as compounds **1-3** exhibited variable MIC values, depending on the bacterial strains. Aloe-emodin (MIC = 4 to 128 µg/mL) exhibited the highest antibacterial activity against MDR *Vibrio cholerae* and *Shigella flexneri.* From these results obtained, *S. alata* leaf could be considered as a natural antibacterial source due to the presence of flavonoid and anthraquinone compounds.

Keywords: *Senna alata*, Fabaceae, kaempferol, luteolin, aloe-emodin, MDR strains.

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INTRODUCTION

Senna alata (Linn.) Roxb. is an ornamental shrub belonging to the Fabaceae family. It grows well in intertropical areas. The leaves are reported to be useful in treating convulsion, gonorrhoea, heart failure, abdominal pains, oedema and are also used as a purgative (Owoyale et al., 2005). In Cameroon, information collected from inhabitants and traditional healers indicated that *S. alata* is used for the treatment of several infections including gonorrhoea, gastro-intestinal and skin diseases. Previous studies demonstrated the presence of antifungal anthraquinones including aloe-emodin, rhein, emodin, and chrysophanol (Agarwal et al., 2000; Manojlovic et al., 2002; Hennebelle et al., 2009); and anthraquinone glycosides (sennosides) with laxative properties (Poppenga, 2006). These later compounds increase gastrointestinal motility, induce fluid movement in the lumen, and have direct irritant effects (Poppenga, 2006). Flavonoids and steroids have also been isolated from leaves, roots and stems of *S. alata* (Hennebelle et al., 2009). Previous works also indicated that compounds from these different classes have antimicrobial activities, especially against *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus spp* and *Candida spp* (Ogwuche et al., 2015; Zintchem et al., 2015; Maloueki et al., 2015)

Antimicrobial activity has formed basis of many applications, including pharmaceutical, row and processed food preservations, alternative medicine and natural therapies. However, overuse of antimicrobial drugs has become the major reason for the emergence and dissemination of multidrug resistant bacteria. Nowadays, antimicrobial resistance is a growing problem that complicates the treatment of important nosocomial and community-acquired infections. In the last few decades, this situation has forced scientists to search for new and effective antimicrobial agents from plants. Higher plants are ancient sources of medicinal agents and an impressive number of modern drugs have been isolated from natural sources; many of them based on their use in traditional medicine (Houria et al., 2014; Ogwuche et al., 2015).

To the best of our knowledge, *S. alata* extracts were tested against different type of microorganisms (Adedayo et al., 2001; Hennebelle et al., 2009), but not yet tested against MDR bacteria strains. In the course of our search of bioactive secondary metabolites from Cameroonian medicinal plants, antibacterial-guided fractionation of *S. alata* MeOH residue extract was carried out. We herein report the isolation and structure elucidation of flavonoids and anthraquinone from leaves of *S. alata*, monitored by antibacterial assays against multi-drug resistant strains of *Vibrio cholerae* and *Shigella flexneri*.

MATERIALS AND METHODS

Plant material

The leaves of *Senna alata* (L.) Roxb. were collected in Maroua, Far-North region, Cameroon, in November 2012. The plant species was identified by Mr. Tapsou, a botanist at IRAD (Institut de Recherche Agricole et de Développement) of Maroua, Cameroon. The identification was confirmed at the National Herbarium, Yaoundé, Cameroon, by comparison with a specimen whose voucher number was 18572 SRF/Cam.

Extraction and isolation

The air-dried leaves (1.67 kg) of *S. alata* was powdered and extracted thrice with MeOH at room temperature for 48, 96, and 164 h, respectively. The filtrate was evaporated to give 194.9 g of methanol extract. The obtained extract was partitioned with hexane to afford 80.7 g of hexane extract, and 105.1 g of MeOH residue extract.

Both extracts were subjected to antibacterial assays and the MeOH residue extract was found active against the tested strains.

Part of the active MeOH residue extract (105.1 g) was subjected to Si gel flash column chromatography eluted with hexane-acetone gradient [3:1 (6L), 1:1(4L), 1:3 (3L) and acetone-MeOH gradient 10:0 (2.5L), 3:1 (2.5L), 1:1 (3L), 1:3 (1.5L)] to give 46 fractions of 500 mL each. These fractions were combined into six main fractions [A (1–16), B (17–20), C (21–26), D (27–35), E (36–43), F (44–46] based on their TLC profile. These fractions (A-F) were subjected to antibacterial tested and only fractions A and C were found active against the tested strains, and then subjected to further purifications. Fraction A (4.20 g) was subjected to Si gel column chromatography eluted with hexane-acetone gradient [10:0 (1L), 98:2 (4.1L), 95:5 (3.5L), 92:8 (0.5L), 9:1 (0.5L), 4:1 (0.9L), 3:1 (1.1L), 7:3 (0.6L) and 13:7 (0.6L)] to give 124 subfractions of 100 mL each. An orange powder was formed in subfractions 51-58, filtration and recristallization yielded 100 mg of aloe-emodin (**1**). Kaempferol (**2**) was obtained as yellow powder (250 mg) from subfractions 100-113. Fraction C (3.24 g) was also subjected to CC eluted with an isocratic mixture, hexane-acetone-MeOH [6:4:0.1] to give 45 subfractions of 50 mL each. Luteolin (**3**) was obtained as yellow needles in subfractions 9-11.

General procedure of determination of structure

The high-resolution mass spectra were obtained with an LTQ-Orbitrap Spectrometer (Thermo Fisher, USA) equipped with a HESI-II source. The spectrometer was operated in positive mode (1 spectrum s^{-1}; mass range: 200–800) with nominal mass resolving power of 60,000 at *m/z* 400 with a scan rate of 1 Hz with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using an internal standard, Bis(2-ethylhexyl)phthalate: *m/z* = 391.284286.

NMR spectra were measured on Bruker DRX 500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}CNMR, with TMS as internal standard; chemical shifts are given in δ values (ppm).

Flash column chromatography was performed using silica-gel 60 (Merck, 0.040–0.063 mm). Column chromatography was run on Merck silica gel 60 (0.063–0.200 mm) and TLC was carried out on silica gel GF254 pre-coated plates with detection accomplished by visualizing with a UV lamp at 254 and 365 nm, followed by spraying with 50% H_2SO_4 and then heating at 100 ºC.

Antibacterial assay

Bacterial growth conditions

A total of six bacterial strains were tested for their susceptibility to plant extracts/compounds and these strains were taken from our laboratory collection (kindly provided by Dr. T. Ramamurthy, NICED, Kolkata). Among the clinical strains of *Vibrio cholerae* used in this study, strains NB2 and SG24(1) belonged to O1 and O139 serotypes, respectively. These strains were able to produce cholera toxin and hemolysin (Bag et al., 2008; Thakurta et al., 2007). The other strains used in this study were *V. cholerae* non-O1, non-O139 (strains CO6 and PC2) (Bag et al., 2008); and *Shigella flexneri* (Acharyya et al., 2015). The *V. cholerae* non-O1 and non-O139 strains, were positive for hemolysin production but negative for cholera toxin production (Bag et al., 2008). The American Type Culture Collection (ATCC) strain, *Staphylococcus aureus* ATCC 25923, was used for quality control. The bacterial strains were maintained on agar slant at 4 °C and subcultured on a fresh appropriate agar plates 24 h prior to any antibacterial test. The Mueller Hinton Agar (MHA) was used for the

activation of bacteria. The Mueller Hinton Broth (MHB) and nutrient agar (Hi-Media) were used for the MIC and MBC determinations respectively.

Inocula preparation

Suspensions of bacteria were prepared in MHB from cells arrested during their logarithmic phase growth (4h) on MHB at 37 °C. The turbidity of the microbial suspension was read spectrophotometrically at 600 nm and adjusted to an OD of 0.10 with MHB, which is equivalent to 1.5 $\times 10^8$ CFU/mL. From this prepared solution, other dilutions were made with MHB to yield 1 $x10^6$ CFU/mL (Mabou et al., 2016).

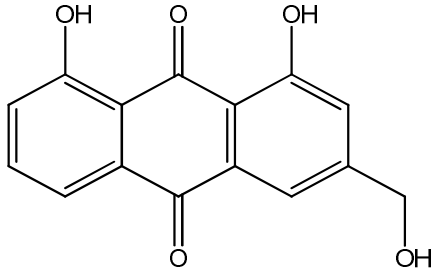
Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC and MBC of extracts and compounds **1-3** were assessed using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI, 1997, 1999) with slight modifications. Each test sample was dissolved in dimethylsulfoxide (DMSO) to give a stock solution. The 96-well round bottom sterile plates were prepared by dispensing 180 µl of the inoculated broth ($1x10^6$ CFU/mL) into each well. Twenty microliters aliquot of the compounds were added. The final concentrations of the tested samples varied from 16 to 2048 µg/mL for extracts and from 0.125 to 512 µg/mL for pure compounds. The final concentration of DMSO in each well was <1% [preliminary analyses with 1% (v/v) DMSO did not inhibit the growth of the test organisms]. Dilutions of ampicillin and tetracycline served as positive controls, while broth with 20 µL of DMSO + medium were used as negative control. Plates were covered and incubated for 24 h at 37 °C. After incubation, minimum inhibitory concentrations (MICs) were read by observing and comparing the test wells with the positive and negative controls. The MIC value was the lowest concentration of the test substances that prevented the visible growth of the microorganism. Minimum bactericidal concentration (MBC) values were determined by plating 10 µL from each negative well and from the positive growth control on nutrient agar (Hi-Media) and incubated for 24 h at 37 °C. The lowest concentrations that yielded no growth after this subculturing were taken as the MBC values.

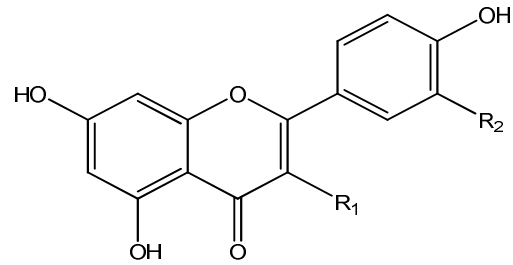
RESULTS

Hexane and MeOH residue extracts of *S. alata* leaves were subjected to antibacterial assays against MDR strains. Only MeOH residue extract was sensitive to the different microorganisms with MIC and MBC ranging from 512 to 2048 µg/ml (Table 1), and was therefore selected for further studies. MeOH residue extract was subjected to flash column chromatography and fractions obtained were tested. Fractions A and C were active to the tested microorganisms with MIC and MBC values ranging from 64 to 1024 µg/ml (Table 1), and were selected for further studies. However, no activity was noted with fractions B, D, E and F against all the bacterial strains at the concentrations up to 2048 µg/mL. This finding suggests that fractionation enhanced the antibacterial properties of fractions A and C and diluted those of fractions B, D, E and F.

Fractionation and purification of A and C yielded to the isolation of three bioactive compounds namely Aloe-emodin (**1**) (Panichayupakaranant et al., 2009), Kaempferol (**2**) (Melo et al., 2009) and Luteolin (**3**) (Lie-Chwen et al., 2015) (Figure 1). The antibacterial activity of the isolated compounds was examined by broth microdilution assay against six bacterial strains. The results showed that all the isolated compounds exhibited variable MIC values, depending on the bacterial strains (Table 1).



Aloe-emodin (**1**)



R_1 = OH, R_2 = H: kaempferol (**2**)
R_1 = H, R_2 = OH: luteolin (**3**)

Figure 1: Structures of isolated compounds from the MeOH residue extract of *S. alata* leaves.

Table 1: Antibacterial activity (MIC and MBC in µg/ml) of *S. alata* leaf extracts and their isolated compounds.

Samples	**Inhibition parameters**	***V. cholerae* SG24 (1)**	***V. cholerae* CO6**	***V. cholerae* NB2**	***V. cholerae* PC2**	***S. flexneri* SDINT**	***S. aureus* ATCC 25923**
MeOH residue extract	MIC	2048	2048	1024	2048	1024	512
	MBC	2048	2048	2048	2048	1024	512
	MBC/MIC	1	1	2	1	1	1
Fraction A	MIC	256	512	512	1024	1024	64
	MBC	256	512	1024	1024	1024	128
	MBC/MIC	1	1	2	1	1	2
Fraction C	MIC	256	512	1024	1024	1024	256
	MBC	512	512	1024	1024	1024	256
	MBC/MIC	2	1	1	1	1	1
1	MIC	64	32	128	32	16	4
	MBC	64	32	128	64	16	4
	MBC/MIC	1	1	1	2	1	1
2	MIC	32	32	128	32	128	16
	MBC	64	32	128	64	128	16
	MBC/MIC	2	1	1	2	1	1
3	MIC	128	64	128	>512	128	32
	MBC	256	64	128	>512	256	32
	MBC/MIC	2	1	1	/	2	1
Tetracycline	MIC	0.50	2	0.50	0.50	16	2
	MBC	1	8	2	1	32	8
	MBC/MIC	2	4	4	2	2	4
Ampicillin	MIC	16	16	>512	>512	>512	4
	MBC	16	16	>512	>512	>512	4
	MBC/MIC	1	1	/	/	/	1

/: not determined; Hexane fraction, fractions B, D, E and F were not active at concentrations up to 2048 µg/ml. MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

DISCUSSION

The current findings revealed isolation of bioactive compounds of flavonoid and anthraquinone type, in accordance to literature review. Similar secondary metabolites were found by Zintchem et al. (2013) who concluded that their major biological effect on human health was against chronic diseases.

The lowest MIC and MBC values of 4 µg/mL (corresponding to the most active sample) were recorded on *Staphylococcus aureus* with compound **1**. However, the highest MIC value of 2048 µg/mL was obtained against *Vibrio cholerae* SG24 (1), *Vibrio cholerae* CO6 and *Vibrio cholerae* PC2 with MeOH residue extract whereas the highest MBC value of 2048 µg/mL was recorded on *Vibrio cholerae* SG24 (1), *Vibrio cholerae* NB2, *Vibrio cholerae* CO6 and *Vibrio cholerae* PC2 with the MeOH residue extract. A lower MBC/MIC (≤4) value signifies that a minimum amount of plant extracts/compound is used to kill the bacterial species, whereas, a higher value signifies the use of comparatively more amount of sample for the control of any microorganism (Djouossi et al., 2015; Tamokou et al., 2016; Mabou et al., 2016).

The strains of *Vibrio cholerae* NB2, PC2 (Thakurta et al., 2007; Bag et al., 2008) and *Shigella flexneri* (Acharyya et al., 2015) included in the present study were MDR strains and these were resistant to commonly used drugs such as ampicillin, streptomycin, tetracycline, nalidixic acid, furazolidone, co-trimoxazole, etc. However, these bacterial strains were found to be sensitive to most of the tested samples, suggesting that their administration may represent an alternative treatment against the *V. cholerae*, the causative agent of dreadful disease cholera and *S. flexneri*, the causative agent of shigellosis. Taking into account the medical importance of the tested bacteria, this result can be considered as promising in the perspective of new antibacterial drugs development. Although these compounds have been reported to possess antibacterial activities (Coopoosamy and Magwa, 2006; Teffo et al., 2010; Su et al., 2014; Liu et al., 2015; Del Valle et al., 2016; Joung et al., 2016), no study has been reported on the activity of these plant extracts/compounds against these types of MDR pathogenic strains.

Conclusion

The antibacterial guided isolation and structure elucidation of three known compounds namely aloe-emodin, kaempferol and luteolin. Aloe-emodin exhibited the largest antibacterial activity against MDR *Vibrio cholerae* and *Shigella flexneri.* From these results obtained, *S. alata* leaf could be considered as a natural antibacterial source due to the presence of flavonoid and anthraquinone compounds. Further, *in vivo* experiments are needed for clinical application in MDR *Vibrio cholerae* / *Shigella flexneri* infection.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

JSNT designed the study, did the isolation and structure elucidation part with the help of VTT, ML, under the supervision of MS. JDT and PS did the biological part under the supervision of PKB. JDT also participated in the study design, manuscript writing and editing. All authors read and approved the final manuscript.

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REFERENCES

Acharyya S, Sarkar P, Saha DR, Patra A, Ramamurthy T, Bag PK. 2015. Intracellular and membrane damaging activities of methyl gallate isolated from *Terminalia chebula* against multi-drug resistant *Shigella species*. *J. Med.*

Microbiol., **64**(8): 901-909. Doi: 10.1099/jmm.0.000107.

Adedayo O, Anderson WA, Moo-Young M, Snieckus V, Patil PA, Kolawole DO. 2001. Phytochemistry and Antibacterial Activity of *Senna alata* Flower. *Pharmaceutical Biology*, **39**(6): 408–412. Doi: http:// dx.doi.org/10.1076/phbi.39.6.408.5880.

Agarwal SK, Singh SS, Verma S, Kumar S. 2000. Antifungal activity of anthraquinone derivatives from *Rheum emodi*. *J Ethnopharmacol*., **72**: 43–46.

Bag P, Bhowmik P, Hajra TK, Ramamurthy T, Sarkar P, Majumder M, Chowdhury G, Das S C. 2008. Putative virulence traits and pathogenicity of *Vibrio cholerae* non-O1, non-O139 isolated from surface waters in Kolkata, India. *Appl Environ Microbiol.*, **74**(18): 5635-5644. Doi: 10.1128/AEM.00029-08

CLSI. Methods for determining bactericidal activity of antimicrobial agents. Approved guideline, M26-A. Clinical and Laboratory Standards Institute, Wayne, Pa; 1999.

CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standards, M7-A4. Clinical and Laboratory Standards Institute, Wayne, PA; 1997.

Coopoosamy RM, Magwa ML. 2006. Antibacterial activity of aloe emodin and aloin A isolated from *Aloe excelsa. Afr. J. Biotechnol*., **5**(11): 1092-1094. http://www.academicjournals.org/AJB

De Melo GO, Malvar DDC, Vanderlinde FA, Rocha FF, Pires PA, Costa E A, Matos LG, Kaiser CR, Costa SS. 2009. Antinociceptive and anti-inflammatory kaempferol glycosides from *Sedum dendroideum*. *J. Ethnopharmacol.*, **124**(2): 228–232. DOI: 10.1016/j.jep.2009.04.024

Del Valle P, García-Armesto MR, de Arriaga D, González-Donquiles C, Rodríguez-Fernández P, Rúa J. 2016. Antimicrobial activity of kaempferol and resveratrol in binary combinations with parabens or propyl gallate against *Enterococcus faecalis. Food Control*, **61**: 213–220. DOI: 10.1016/j.foodcont.2015.10.001

Elujoba AA, Ajulo OO, Iweibo GO. 1989. Chemical and biological analyses of Nigerian *Cassia* species for laxative activity. *J. Pharm. Biomed. Anal.,* **7**(12): 1453–1457.

Hennebelle T, Weniger B, Joseph H, Sahpaz S, Bailleul F. 2009. *Senna alata*. *Fitoterapia*, **80**: 385–393. DOI: 10.1016/j.fitote.2009.05.008.

Houria A, Esma F, Rabah D, Dounia M. 2014. In vitro antibacterial activity of *Pituranthos scoparius* from Algeria. *Int. J. Biol. Chem. Sci*., **8**(5): 2095-2108. DOI: http://dx.doi.org/10.4314/ijbcs.v8i5.15

Joung D-K, Lee Y-S, Han S-H, Lee S-W, Cha S-W, Mun S-H, Kong R, Kang O-H, Song H-J, Shin D-W, Kwon D-Y. 2016. Potentiating activity of luteolin on membrane permeabilizing agent and ATPase inhibitor against methicillin-resistant *Staphylococcus aureus*. *Asian Pac. J. Trop. Med.*, **9**(1): 19-22. DOI: 10.1016/j.apjtm.2015.12.004

Lie-Chwen L, Yu-Feng P, Tung-Hu T. 2015. Isolation of Luteolin and Luteolin-7-*O*-glucoside from *Dendranthema morifolium* Ramat Tzvel and Their Pharmacokinetics in Rats. *J. Agric. Food Chem.*, **63** (35): 7700–7706. DOI**:** 10.1021/jf505848z

Liu J, Wu F, Chen C. 2015. Design and synthesis of aloe-emodin derivatives as potent anti-tyrosinase, antibacterial and anti-inflammatory agents. *Bioorganic & Medicinal Chemistry Letters*, **25**(22): 5142–5146. DOI: 10.1016/j.bmcl.2015.10.004.

Mabou FD, Tamokou JDD, Ngnokam D, Voutquenne-Nazabadioko L, Kuiate JR, Bag PK. 2016. Complex secondary metabolites from *Ludwigia leptocarp*a with potent antibacterial and antioxidant activities. *Drug Discov Therapeutics,* **10**(3):141-149.

Maloueki U, Kapetshi J, Kabena NO, Ndimbo KS-P, K-t-NJ-P Ngbolua, Mbomba NB, Muganza C-DM. 2015. Activités

antimicrobiennes et antioxydantes des extraits aqueux totaux des fruits de Dialium orbisieri et Dialium gossweilerii (Fabaceae - Caesalpinioideae), consommés par les bonobos, Bolobo, République Démocratique du Congo. *Int. J. Biol. Chem. Sci*., **9**(2): 633-642. DOI: http://dx.doi.org/10.4314/ijbcs.v9i2.5

Manojlovic NT, Solujic S, Sukdolak S. 2002. Antimicrobial activity of extract and anthraquinones from *Caloplaca schaereri*. *Lichenologist* **34**: 83–85. DOI: 10.1006/lich.2001.0365

Ogwuche CE, Amupitan JO, Ndukwe GI. 2015. Antimicrobial activity of the leaf of the white species of *Sesamum indicum* from Benue State, Nigeria. *Int. J. Biol. Chem. Sci*. **9**(2): 996-1003. http://dx.doi.org/10.4314/ijbcs.v9i2.35

Owoyale JA, Olatunji GA, Oguntoye SO. 2005. Antifungal and antibacterial activities of an alcoholic extract of *Senna alata* leaves. *J. Appl. Sci. Environ. Mgt.* **9**(3): 105-107. http:// www.bioline.org.br/ja

Panichayupakaranant P, Sakunpak A, Sakunphueak A. 2009. Quantitative HPLC determination and extraction of anthraquinones in *Senna alata* leaves. *J Chromatogr Sci.*, **47**(3):197-200.

Poppenga RH. 2006. Hazards Associated with the Use of Herbal and Other Natural Products. In *Small Animal Toxicology* (2nd Edn). W.B. Saunders: Saint Louis; 312–344.

Su Y, Ma L, Wen Y, Wang H and Zhang S. 2014. Studies of the *in Vitro* Antibacterial activities of several polyphenols against clinical isolates of Methicillin-Resistant *Staphylococcus aureus*. *Molecules,* **19**(8): 12630-12639. DOI: 10.3390/molecules190812630

Tamokou JDD, Tsemeugne J, Sopbué Fondjo, E, Sarkar P, Kuiate J, Djintchui NA, Sondengam BL, Bag PK. 2016. Antibacterial and cytotoxic activities and SAR of some azo compounds containing thiophene backbone. *Pharmacologia,* **7**(4):182-192. DOI: 10.5567/pharmacologia.2016.182.192

Teffo LS, Aderogba MA, Eloff JN. 2010. Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. var. *angustifolia* leaf extracts. *South African Journal of Botany,* **76**(1):25-29. DOI: 10.1016/j.sajb.2009.06.010.

Thakurta P, Bhowmik P, Mukherjee S, Hajra TK, Patra A, Bag PK. 2007. Antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica* used to treat cholera and diarrhea in India. *Journal of Ethnopharmacology,* **111** (3): 607-612. DOI: 10.1016/j.jep.2007.01.022

Zintchem R, Njinkio B, Kamgang R, Fokunnga C, Tsala DE, Biwole Sida M. 2013. Antioxidative properties of *Mallotus oppositifolium* decoction leaves extract using *in vitro* models. *Int. J. Biol. Chem. Sci*., **7**(6): 2396-2408. DOI: http://dx.doi.org/10.4314/ijbcs.v7i6.18

Zintchem R, Fankem GO, Fokunang C. Mkounga P, Tembe EA, Kamgang R, Gatsing D, Dimo T. 2015. *In vitro* evaluation of the antimicrobial properties of *Mallotus oppositifolium* decoction leaf extracts and fractions. *Int. J. Biol. Chem. Sci*., **9**(2): 815-824. DOI: http://dx.doi.org/10.4314/ijbcs.v9i2.21