JOBIMB, 2023, Vol 11, No 2, 82-86
https://doi.org/10.54987/jobimb.v11i2.865

HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index

Correlation between Total Phenolic Content and Antibacterial Activities of Some Selected Traditional Plants against Some Species of Pathogenic Bacteria

Ibrahim Abuga[1]*, Shaida Fariza Sulaiman[2], Ibrahim Sani[3], Sufiyanu Abubakar Jiga[3], Adamu Almustapha Aliero[4], Farida Abubakar Tomo[4] and Amina Muhammad[4]

[1]Department of Plant Science and Biotechnology, Faculty of Life Science, Kebbi State University of Science and Technology, P.O. Box 1144, Aliero, Kebbi State, Nigeria.
[2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.
[3]Department of Biochemistry, Faculty of Life Science, Kebbi State University of Science and Technology, P.O. Box 1144, Aliero, Kebbi State, Nigeria.
[4]Department of Microbiology, Faculty of Life Science, Kebbi State University of Science and Technology, P.O. Box 1144, Aliero, Kebbi State, Nigeria.

*Corresponding author:
Ibrahim Abuga
Department of Plant Science and Biotechnology,
Faculty of Life Science,
Kebbi State University of Science and Technology,
P.O. Box 1144,
Aliero, Kebbi State,
Nigeria.
Email: iabubakar128@yahoo.com

HISTORY

Received: 7th Oct 2023
Received in revised form: 21st Nov 2023
Accepted: 29th Dec 2023

KEYWORDS

Plant extracts
80% Methanolic crude extracts
Antibacterial screening
Total phenolic content
Mimusops elengi

ABSTRACT

Plants produce non-nutritive compounds known as secondary metabolites. These compounds can be found in several parts of the plants. Emphases have been placed on these compounds as curative agents in treating different types of diseases. Total phenolic contents of the plant extracts (leaf extract of *Anacardium occidentalle*, bulb extract of *Allium sativum*, bark extract of *Casaurina equisetifolia*, leaf extract of *Dysophylla auriculata* and bark extract of *Mimusops elengi*) were determined by using Folin-Ciocalteu method, and the values were expressed in microgram (µg) gallic acid equivalent (GAE) to 1 milligram (mg) of extract. The antibacterial activities of the 80% methanolic extracts of the plants were determined against nine species of pathogenic bacteria; that were *Escherichia coli* 0157:H7, *Vibrio alginolyticus* (ATCC 17749), *Vibrio parahaemolyticus* (ATCC 17082), *Salmonella paratyphi* (ATCC 9150), *Yersiniae enterocolitica* (ATCC 23715), *Listeria monocytogenes* (ATCC 19115), *Salmonella typhi* (ATCC 14028), *Escherichia coli* (ATCC 0157) and *Staphylococcus aureus* (ATCC 700699). Out of the five selected samples, the highest phenolic content was recorded with the bark extract of *Mimusops elengi* (442.59 ± 2.09). From the antibacterial screening, the most potent extract was observed with the bark extract of *Mimusops elengi* against *L. monocytogenes* (ATCC 19115), with a minimum inhibitory concentration value of 15.63 µg/mL, then followed by *Allium sativum* with 62.5 µg/mL. The correlation determined in this study between the total phenolic content and the antibacterial activity of the bark extract of *Mimusops elengi* confirmed its effectiveness against the screened species of bacteria. This work may be useful for the pharmaceutical industry in developing new natural product-based therapy for pathogenic bacteria.

INTRODUCTION

In other to reduce the rate of antibacterial resistance, the causative agent must be spotted as soon as possible. The increased prevalence of antimicrobial resistance (AMR) has pushed for a continual search for novel antibacterial agents. The world Health Organization has suggested that AMR be included on the National Risk Register, as its potential impact on health is as significant as that of global warming, and that novel antibiotics capable of curtailing the detriment should be developed [1]. The increase in pathogenic resistant microorganisms towards conventional drugs and investigation of the bioactive compounds within folk medicinal plants makes medicinal plants very important in conventional medicine [2]. A lot of investigations have been carried out on medicinal plants resulting in the discovery of novel drugs that are used in developing new curative

agents [3]. For example, a comprehensive review of the antibacterial activity of different plant extracts against different pathogenic bacteria has been reported by [4]. [5] also established various antibacterial activities of 54 extracts from 18 medicinal plants against different strains of bacteria using different solvents of different polarities (ethanol, hexane and ethyl acetate). Many more studies have been conducted on the efficacy of plant extracts against numerous species and strains of pathogenic bacteria [6-10].

Many novel drugs were discovered from plant sources such as benzoin isolated from Styrax tonkinensis used for oral disinfectant and emetine from *Psychotria ipecacuanha* used for treating amoebic dysentery [11,12]. Phenolic compounds are chemical substances bearing an aromatic ring that is attached to one or more hydroxyl substituents and a functional derivative. The chemical structures of phenolic compounds determine the class of the compounds. Some molecules of the compounds are simple (for example phenolic acids), while some are complex (for example tannins). Simple phenols and polyphenols are the two main subgroups of phenolic acids, which are derivatives of benzoic and cinnamic acid while the third group include the derivatives of phenylacetic acid. Phenolic compounds are found abundant in different parts of plant species. These compounds are found to possess anti-inflammatory, antibacterial and antioxidant activities [13].

Phenolic compounds are ingested, absorbed and conjugated in the small intestine, where three main conjugation reactions take place; methylation, sulfation and glucuronidation [14]. Numerous studies revealed the antibacterial activity of some phenolic compounds against pathogenic bacteria. For example, the activity of the phenolic compounds (coumarin and quercetin) was reported to possess antibacterial effect against *Escherichia coli, Enterobacter aerogenes*, *Salmonella typhimurium* and *Salmonella infantis* [15]. Similarly, a study conducted by [16] showed the antibacterial activity of luteolin-7-glucoside, luteolin, rutin, caffeic acid and rosmarinic acid against three species of diarrheagenic bacteria: *Bacillus cereus*, *Shigella sonnei* and *Staphylococcus aureus.* The results obtained showed that the use of plant materials in treating diseases may reduce or prevent the threat of microbial infections, which may partly be due to their phenolic composition.

MATERIALS AND METHOD

Preparation of extracts and reagents

An electronic balance was used to weigh 1g of crude extract which was dissolved in 1mL of 80% methanol in micro-centrifuge tubes to obtain extracts of 1 g/mL. This was followed by sonicating the tubes and their contents for 5 min. One (1 mL) of Folin-Ciocalteu reagent (from the stock) was added to 9 mL of distilled water to obtain 10% (v/v) of the solution which was used for the analysis [17]. Similarly, to obtain 20% of sodium carbonate (Na_2CO_3), 20 g was weighed and dissolved in 100 mL of distilled water (w/v).

Folin-Ciocalteu assay

Total phenolic content (TPC) was determined using Folin-Ciocalteu's (FC) method [18] modified to the 96-well plate assay, as adopted by [19]. Briefly, 10 µL of the 80% methanolic extracts of each of the 45 plant samples (1 g/mL) were loaded in the 96-well microplate and 25 µL of the reagent (FC) was added, this was kept for about 5 min for pre-incubation at 27 ± 1°C. Subsequently, 25 µL of 20% of a sodium carbonate solution was introduced and then followed by distilled water to a final volume of 200 µL per well, which was then re-incubated for 30 min at 27 ± 1°C. The experiment was conducted in triplicate (n=3). A similar procedure was adopted with 10 different concentrations (9.77-5000 µg/mL) of garlic acid to plot the standard curve of its absorbance. Methanol solvent (80%) was used as the blank. To prepare the blank, 25 µL of the FC reagent was replaced with 25 µL of 80% methanol, and the same procedure was implemented as previously mentioned for FC preparation. The absorbance was then read at 750 nm using a Multiskan Spectrum Micro-plate reader (Thermo Scientific, United States of America) and the absorbance values of the blank (80% methanol) were subtracted from the absorbance values of their corresponding samples.

The results were expressed as micrograms of gallic acid equivalents per milligram extract (µg GAE/mg extract). A standard curve was plotted based on the generated absorbance values. The total phenolic contents of the extracts were calculated by incorporating the absorbance values of the extracts into the standard curve produced by gallic acid. Firstly, the unknown concentration (x) from the calibration curve was calculated from the generated formula:

$$y = 0.000183x + 0.05424$$

Where
y = absorbance of the plant samples.
x = concentration from calibration curve

The total phenolic contents were calculated according to [20]. Thus,

$$TPC = cV/m$$

Where
c = concentration from calibration curve
V= Volume of the extract used
m= Mass of the extract used

The total phenolic contents in microgram (µg) per milligram (mg) were obtained by dividing the TPC content by the mass of the dry plant sample multiply by 1000.

Method for bacteria culture

One loopful of pure bacterial colonies from the agar slant was streaked onto the surface of fresh nutrient agar. The inoculated plates were then incubated at 37 °C for 24 h. Then, one loopful of pure bacterial colonies from the agar plates was inoculated in fresh nutrient broth. The inoculated plates were then incubated at 37 °C for 24 h. The bacterial suspension was then prepared by pipetting the suspension into the sterile universal bottle. Sterile broth was then added into the universal bottle and the turbidity of the solution was then adjusted to 0.5 McFarland turbidity standards equivalent to 1.5 x 10^8 CFU/mL (Colony Forming Unite). Then, the solution was diluted 1:100 with sterile broth. The initial inoculum size was approximately 1.5 x 10^6 CFU/mL for bacteria.

Plant materials

The plant samples used in this research were identified by Professor Shaida Fariza Sulaiman (a professor and a botanist at the School of Pharmaceutical Sciences, USM, Penang). The vouchered specimens were prepared and deposited in the school of Biological Science, USM. The samples were collected from various places within Penang, Malaysia during the rainy season.

Plant extracts preparation using 80% methanol

The extraction methodology was implemented as described by [21]. Plant parts were washed with water to remove the debris,

and then dried in an oven at 50 °C for two to three days. This was followed by grinding the parts into powder using a blender (JS Kitchenware, Malaysia). The powdered plant parts were soaked in 80% methanol solvent and then boiled for 2 h. It was then filtered using Whatman filter paper No. 1 to obtain the filtrates. The remaining specimens were again boiled, and the filtrates were obtained. The process was repeated until all had been extracted, and finally, the filtrates of the extracts were kept in an oven at 50 °C to dry and were stored at 4 °C.

The concentration of 40 mg/mL of each extract was subjected to six two-fold serial dilution and different concentrations were obtained; 40 mg/mL, 20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL respectively. The same serial dilution was obtained for the positive control (tetracycline); 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.362.5 mg/mL and 0.15625 mg/mL respectively. Both 80% methanol extracts of the plant samples and the positive control were dissolved in 99.9% (v/v) dimethyl sulfoxide (DMSO) to obtain the concentrations. Lastly, the different concentrations of the extracts obtained from the serial dilution were subjected to antibacterial screening.

Antibacterial screening of the 80% methanolic extracts

The minimum inhibitory concentration (MIC) of the extracts was determined using microdilution broth method. The methodology was adopted as described by [22] with some modifications. The parameters used for the microdilution assay are in accordance with that of [23]. The bacteria were cultured from stock into 10 mL fresh nutrient broth and incubated at 37 ºC for 18-22 h. The colonies of the cultured bacteria were inoculated in normal saline. The turbidity of the grown bacteria (inoculum) was then adjusted to 0.5 McFarland standard [approximately 1.5 $\times 10^8$ colony-forming unit per milliliter (CFU/mL)]. A final concentration of 2.5% (v/v) of DMSO was obtained.

The final density of the inoculums was obtained at approximately 1.5 x 10^6 CFU/mL. The plant samples (5 µL) were loaded on the sterile microplates, and then followed by 195 µL of the inoculums. Dimethylsulfoxide (DMSO) solvent (5 µL) with 195 µL of the inoculums was loaded, and this served as the negative control; consequently, 5 µL of tetracycline with 195 µL of inoculums was loaded which was used as the positive control. Two hundred microliter (200 µL) of sterile nutrient broth was loaded for sterility control and 200 µL of the inoculum for inoculum control. The loaded microplates were covered and sealed with paraffin film to avoid contamination and then incubated at 37 °C for 18-22 h. A solution of Iodonitrotetrazolium chloride (INT) was prepared at a concentration of 0.3 mg per 1 mL of sterile distilled water, which was used to analyze the result of the bioassay. Iodonitrotetrazolium chloride (40 µL) was loaded to the incubated plate, and the result was observed and recorded after 30 min of re-incubation period at 37 ºC. The appearance of pink colour indicates the absence of inhibition, and the lacking pink color indicates the presence of inhibition. Each of the tests was replicated three times. The MIC was ascertained by choosing the least concentration of the plant extract that totally hinders the survival of the organism using INT as an indicator [24].

RESULTS

Table 1 shows the total phenolic contents of the 5 species of plant extracts employed in this study. The total phenolic contents of these extracts were determined by using Folin-Ciocalteu method. The evaluation of phenolic content in each extract was conducted by extrapolating from the gallic acid standard curve. These values are expressed in terms of microgram (µg) gallic acid equivalent (GAE) to 1 milligram (mg) of extract. The absorbance was detected at 750 nm using UV-Visible spectrophotometer. Out of the five samples, the highest phenolic content was recorded with the bark extract of *Mimusops elengi* (442.59 ± 2.09).

Table 1. Total phenolic content of the 5 plant extracts.

		(µgGAE/mg extract)				
Scientific name	Voucher ID	1	2	3	Mean	SD
Allium sativum	SFS/IB/2	354.07	351.34	355.42	353.61	1.69
Anacardium occidentalle	SFS/IB/5	34.29	36.41	36.51	34.80	1.02
Casaurina equisetifolia	SFS/IB/8	54.27	57.07	55.00	55.45	1.19
Dysophylla auriculata	SFS/IB/12	32.54	31.05	33.39	32.33	0.97
Mimusops elengi	SFS/IB/24	445.45	440.52	441.77	442.59	2.09

Note: Result of total phenolic contents are expressed as means of three determinations of microgram Gallic acid equivalent/miligram (ugGAE/mg).

Antibacterial activities against different strains of bacteria of the 5 plant extracts are shown in **Table 2**. Most of the plant extracts displayed antibacterial activities against two or more tested bacteria with MIC values ranging from 1000 µg/mL to 7.81 µg/mL. The MIC were the final concentrations of the tested samples, which were prepared from the initial concentrations of 40 mg/mL to 1.25 mg/mL. However, none of the extracts was found to exhibit an antibacterial effect against all the species of the studied bacteria.

Table 2. Minimum inhibition concentration (µg/mL) of the methanolic (80%) extracts.

	Gram-positive		Gram-negative						
Plant name	*L. monocytogenes*	*S. aureus*	*E. coli* 0157: H7	*E. coli*	*S. paratyphi*	*S. typhi*	*V. alginolyticus*	*V. paraheamolyticus*	*Y. enterocolitica*
Tetracycline	0.24	0.06	0.49	1.95	0.12	15.63	15.63	15.63	3.97
Anacardium occidentalle	1000	500	-	1000	1000	500	-	-	1000
Allium sativum	-	62.5	-	-	-	-	1000	1000	-
Casaurina equisetifolia	-	1000	-	-	-	-	-	-	-
Dysophylla auriculata	1000	-	-	-	-	-	-	-	500
Mimusops elengi	15.63	-	-	-	-	-	-	-	250

DISCUSSION

Several studies have established the significance of phenolic compounds against different strains of pathogenic bacteria. The therapeutic action of these compounds against various strains of bacteria has been investigated by many researchers [25 and 26]. The results obtained from this research disclosed vital evidence about these compounds as potential natural therapies. The work of this finding is in conformity with that of [27] which confirmed the total phenolic content of 375.9 mg/g of the chloroform bark extract of *M. elengi* using Folin-Ciocalteau reagent. Another research conducted by [28] also exhibited higher total phenolic content (698.7 ± 0.93 mg/g) of the methanolic bark extract of the plant (*M. elengi*).

The antibacterial activities of the tested plant extracts against nine different species of bacteria that are reported to cause infection were determined in this study. The difference in susceptibility of the bacteria might be due to the distinction in the

composition of the cell walls of Gram-negative and Gram-positive bacteria. Gram-positive bacteria are more susceptible to plant extracts due to the presence of a thin cell wall layer of peptidoglycan, which may allow easy penetration of compounds compared to Gram-negative bacteria which contains a lipid membrane bounded by a thick cell wall layer of peptidoglycan, this can restrict the passage of compounds into the cell of the host bacteria [29].

Tetracycline was used as a positive control in the screening. The drug is used as an antibacterial agent for treating bacterial infections in many parts of the world [30 and 31]. From the screening, the methanolic bark extract of *Mimusops elengi*, is the most effective extract against *L. monocytogenes* (ATCC 19115), with a minimum inhibitory concentration of 15.63 µg/mL. The result is in agreement with the findings of [32] that reviewed the antibacterial activity of the plant (*M. elengi*) using various methods of extraction against numerous strains of pathogenic bacteria.

CONCLUSION

The present study highlights the significance of plant extracts, particularly those derived from *Mimusops elengi*, in the context of their phenolic content and antibacterial activities. This research quantitatively assessed the total phenolic contents of five species of plant extracts, showcasing *Mimusops elengi* bark extract as possessing the highest phenolic contents with significant therapeutic properties. Furthermore, the antibacterial screening of 5 plant extracts against various bacterial strains revealed a broad spectrum of activity, with MIC values ranging significantly, indicating the extracts' variable efficacy against different bacterial pathogens. Notably, the methanolic bark extract of *Mimusops elengi* exhibited a compelling inhibitory effect against *L. monocytogenes*, positioning it as a potent antibacterial agent among the tested extracts. In conclusion, this study contributes valuable data to the growing body of evidence supporting the use of plant-derived phenolic compounds as natural alternatives to synthetic antibiotics. The demonstrated antibacterial efficacy of *Mimusops elengi*, alongside its high phenolic content, emphasizes the potential of such natural resources in developing effective, sustainable strategies for managing bacterial infections.

REFERENCES

1. Zainuri, D.A., Arshad, S., Khalib, N.C., Razak, I.A., Pillai, R.R., Sulaiman, S.F., Hashim, N.S., Ooi, K.L., Armaković, S., Armaković, S.J. and Panicker, C.Y., 2017. Synthesis, XRD crystal structure, spectroscopic characterization (FT-IR, 1H and ^{13}C NMR), DFT studies, chemical reactivity and bond dissociation energy studies using molecular dynamics simulations and evaluation of antimicrobial and antioxidant activities of a novel chalcone derivative,(E)-1-(4-bromophenyl)-3-(4-iodophenyl) prop-2-en-1-one. J Mol Struct. 2017; 1128: 520-533.
2. Nath K, Talukdar AD, Bhattacharya MK, Bhowmik D, Chetri S, Choudhury D, Mitra A, Bhattacharjee A. Antibacterial activity of certain ferns against multi drug resistant organisms. J Nat Rem. 2017; 17:144-53.
3. Harvey A, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov. 2015; 14: 111–129.
4. Al-Snai AE. Iraqi medicinal plants with antibacterial effect-A review. IOSR J Pharm. 2019; 9(8):22-103.
5. Hossan MS, Jindal H, Maisha S, Samudi Raju C, Devi Sekaran S, Nissapatorn V, Kaharudin F, Su Yi L, Khoo TJ, Rahmatullah M, Wiart C. Antibacterial effects of 18 medicinal plants used by the Khyang tribe in Bangladesh. Pharm Biol 2018; 56(1):201-8.
6. Amber R, Adnan M, Tariq A, Khan SN, Mussarat S, Hashem A, Al-Huqail AA, Al-Arjani AB, Abd_Allah EF. Antibacterial activity of selected medicinal plants of northwest Pakistan traditionally used against mastitis in livestock. Saudi J Biol Sci. 2018; 25(1):154-61.
7. Bereksi MS, Hassaïne H, Bekhechi C, Abdelouahid DE. Evaluation of antibacterial activity of some medicinal plants extracts commonly used in Algerian traditional medicine against some pathogenic bacteria. Pharmacogn J. 2018; 10(3): 507-512.
8. Ceylan S, Cetin S, Camadan Y, Saral O, Ozsen O, Tutus A. Antibacterial and antioxidant activities of traditional medicinal plants from the Erzurum region of Turkey. Ir J Med Sci. (1971-). 2019; 188:1303-9.
9. Mishra M, Mishra VK, Kashaw V, Iyer AK, Kashaw SK. Comprehensive review on various strategies for antimalarial drug discovery. Eur J Med Chem. 2017; 125:1300-20.
10. Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology 1999 Jan 1 (Vol. 299, pp. 152-178). Academic press.
11. Wichelns D, Milan F, Hoanh CT, Phuong ND. Household opportunity costs of protecting and developing forest lands in Son La and Hoa Binh Provinces, Vietnam. Int J Commons. 2016;10(2): 902-928.
12. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008; 3(2):163-75.
13. Junqueira-Gonçalves MP, Yáñez L, Morales C, Navarro M, Contreras RA, Zúñiga GE. Isolation and characterization of phenolic compounds and anthocyanins from murta (Ugni molinae Turcz.) fruits. Assessment of antioxidant and antibacterial activity. Molecules. 2015; 20(4):5698-713.
14. Heleno SA, Martins A, Queiroz MJ, Ferreira IC. Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. Food Chem. 2015; 173: 501-13.
15. Nitiema LW, Savadogo A, Simpore J, Dianou D, Traore AS. In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains. Int J Microbiol Res. 2012; 3(3):183-7.
16. Sharma A, del Carmen Flores-Vallejo R, Cardoso-Taketa A, Villarreal ML. Antibacterial activities of medicinal plants used in Mexican traditional medicine. J Ethnopharmacol. 2017; 208:264-329.
17. Sulaiman SF, Sajak AA, Ooi KL, Seow EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. J Food Compost Anal. 2011; 24(4-5):506-15.
18. Sulaiman SF, Ooi KL, Supriatno. Antioxidant and α-glucosidase inhibitory activities of cucurbit fruit vegetables and identification of active and major constituents from phenolic-rich extracts of Lagenaria siceraria and Sechium edule. J Agric Food Chem. 2013; 61(42):10080-90.
19. Tsering J, Gogoi BJ, Tag H. Ethnobotany and phytochemical analysis of Pyrus pashia leaves. Int J Pharm Sci Res. 2012; 3(8):2721.
20. Wagner VP, Martins MA, Martins MD, Warner KA, Webber LP, Squarize CH, Nör JE, Castilho RM. Overcoming adaptive resistance in mucoepidermoid carcinoma through inhibition of the IKK-β/IκBα/NFκB axis. Oncotarget. 2016; 7(45):73032.
21. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Third ed. Springer science & business media: 1998.
22. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998; 64(08):711-3.
23. World Health Organization. Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline, including tuberculosis. WHO; 2017.
24. Chidan Kumar CS, Then LY, Chia TS, Chandraju S, Win YF, Sulaiman SF, Hashim NS, Ooi KL, Quah CK, Fun HK. Benzofuranyl esters: synthesis, crystal structure determination, antimicrobial and antioxidant activities. Molecules. 2015; 20(9):16566-81.
25. Panghal M, Kaushal V, Yadav JP. In vitro antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Ann Clin Microbiol Antimicrob. 2011; 10(1):1-1.

26. Menezes IR, Santana TI, Varela VJ, Saraiva RA, Matias EF, Boligon AA, Athayde ML, Coutinho HD, Costa JG, Rocha JB. Chemical composition and evaluation of acute toxicological, antimicrobial and modulatory resistance of the extract of Murraya paniculata. Pharm Bio 2015; 53(2):185-91.
27. Rao KS, Munjuluri PR, Kumar BR, Keshar NK. Evaluation of in vitro antioxidant activity and total phenolic content of methanol bark extract of *Mimusops elengi*. Free Radic Antioxid. 2011; 1(2):62-71.
28. Rao KS, Munjuluri PR, Keshar NK. Invitro antioxidant activity and total phenolic content of Mimusops elengi bark. Ind J Pharm Edu Res. 2011; 45(4):317.
29. Brown L, Wolf JM, Prados-Rosales R, Casadevall A. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. Nat Rev Microbiol. 2015; 13(10): 620-30.
30. Mukherjee R, Patra M, Dutta D, Banik M, Basu T. Tetracycline-loaded calcium phosphate nanoparticle (Tet-CPNP): rejuvenation of an obsolete antibiotic to further action. Biochim Biophys Acta Gen Subj. 2016; 1860(9):1929-41.
31. Qadri F, Svennerholm AM, Faruque AS, Sack RB. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin Microbiol Rev. 2005; 18(3):465-83.
32. Kadam PV, Yadav KN, Deoda RS, Shivatare RS, Patil MJ. Mimusops elengi: A review on ethnobotany, phytochemical and pharmacological profile. J Pharmacogn Phytochem. 2012; 1(3):64-74.