ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 8, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

BRINE SHRIMPS LETHALITY TEST OF ETHANOL EXTRACT AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF ETHYL ACETATE FRACTION OF *BLIGHIA SAPIDA*

ADEKOLA MB[1]*, AREOLA JO[2], ORIYOMI OV[3], APATA JT[4], APALOWO OE[4], ADESINA AF[4], BABALOLA OO[4]

[1]Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta. [2]Department of Medical Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria. [3]Institute of Ecology and Environmental Studies, Obafemi Awolowo University, Ile-Ife, Nigeria. [4]Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Nigeria. Email: adekolamb@funaab.edu.ng

Received: 23 March 2020, Revised and Accepted: 01 June 2020

ABSTRACT

Objective: The objective of the study was to evaluate brine shrimp lethality of *Blighia sapida* stem-bark extract and its fractions and identify the bioactive constituents in the ethyl acetate fraction (EAF) using **g**as **c**hromatography-mass spectrometry (GC-MS) technique.

Methods: The ethanol extract (EE) and its fractions were subjected to lethality assay, and GC-MS analysis of EAF was carried out.

Results: The lethality test showed a concentration-dependent mortality rate in the brine shrimp nauplii for the EE and its fractions. GC-MS analysis of EAF of the extract revealed the existence of 13 peaks of the GC-MS chromatogram with only one prominent compound, n-hexadecanoic acid (peak area of 10.13%).

Conclusion: The result revealed the presence of 13 bioactive components in the EAF of the extract, the majority of which have been reported for different biological activities, hence, justifies the use of the plant in the treatment and management of different diseases ethnomedicinally.

Keywords: Brine shrimp, Lethality, *Blighia sapida*, Crude extract, Ethyl acetate, Gas **c**hromatography-mass spectrometry.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i8.37613

INTRODUCTION

The idea that herbal medicines are safe and not toxic has not been correct because many plants in their natural states are toxic, and it has been documented that some plants used in herbal medicine are toxic [1]. Therefore, this has raised serious concern for toxicologists to evaluate the safety and potential toxicity of various bioactive compounds isolated and purified from plant extracts used in drug formulation and development. Today, different reliable, scientific *in vitro* and *in vivo* tests are available to evaluate the toxicity of herbal compounds used in drug development. One of such model is brine shrimps lethality test, which has been recognized as a vital technique for assessment of toxicity [2,3]. This technique has been employed for bioassay-guide fractionation of active cytotoxic and antitumor agents [4,5]. *Blighia sapida* belongs to family Sapindaceae. The fruits, which are basically yellow, usually split open into three cream-colored arils attached to black and shining seeds [6]. Locally, it is called "Isin" in Yoruba, "Gwanja kusa" in Hausa and "Okpu" in Igbo [7]. Folklorically, the aqueous extract of the plant had been reported as parasites expellant. Different parts of the plant have been used in the treatment and management of various diseases [8] including diabetes [9].

The present study evaluated the cytotoxicity potential of the plant extract using Brine shrimps (*Artemia salina*) and screened for the bioactive compounds in the ethyl acetate fraction (EAF) using gas chromatography-mass spectrometry (GC-MS) technique.

METHODS

Plant sample

Fresh *B. sapida* stem-bark was collected during rainy season, from Sekona-Ede Road (Latitude: 7° 39 N Longitude 4° 27 E Elevation 291 m), Osun State, Nigeria. The plant material was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The specimen copy was deposited at the Herbarium and specimen voucher number 17623 collected.

Preparation of ethanol extract (EE) of plant sample

Fresh stem-bark of *B. sapida* was cut into tiny pieces after removing the dead cells, shade dried, and ground into powder by electrical grinding Machine at the Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The powdered material (1 kg) was macerated in 70% (v/v) for 72 h and separated to obtain filtrates, according to Handa *et al*. [10]. The filtrates were sieved by means of white cotton gauze, followed using filter paper (Whatman No. 1) and concentrated with rotary evaporator (Edman High Vacuum Pump) at 40°C to yield a residue termed EE. The resulting extract was weighed, labeled and stored in the desiccator until needed for further analysis.

Fractionation of EE

The EE was partitioned using solvents of varying polarities as reported by Adekola *et al*. [11]. Typically, extract (30 g) was suspended in distilled water (200 ml) allowed until totally dissolved, shaken thoroughly and followed by filtration with filter paper (Whatman No. 1). The filtrate was partitioned sequentially with 400 ml each of ethyl acetate and butanol. The mixture was thoroughly shaken, allowed to separate into layers and separated. The fractions of the different solvents were separately concentrated in a rotary evaporator (Edman High Vacuum Pump), while the volume of the aqueous fraction (AQF) was only reduced before finally lyophilized. The fractions were weighed, labeled, and kept in desiccators until required for further analysis. A total of three fractions, namely, EAF, butanol fraction, and AQF were obtained.

Phytochemical screening

The EE of *B. sapida* along with fractions were screened for phytochemical constituents according to the methods described by Trease *et al*., Edeoga *et al*., Sofowora, and Prashant *et al*. [12-15].

Brine shrimps lethality test

The assay method for brine shrimps lethality test was carried out according to Solis *et al*. [16] as reported by Potduang *et al*. [17]. The hatching of brine

shrimps (*A. salina*) was carried out in sterile sea water formulated from commercial sea salt (Aqua Marine, Thailand) 40g/l supplemented with 6 mg/l dried yeast. Twenty nauplii were counted under a hand magnifying lens and placed in each vial containing 4.5 ml of brine solution. In each experiment, different volume of extract/fractions was added to 4.5 ml of brine solution to give different concentrations (0.00, 20, 40, 60, 80, and 100 µg/ml) and maintained at room temperature for 24 h under light. All the surviving larvae were counted. The experiment was conducted along with control (vehicle treated), of the test substances (in triplicates) per dose with thymol served as the standard reference. The LC_{50} of the extract and fractions was calculated using Probit analysis [18].

GC-MS

A Hewlett Packard Agilent GC coupled with Hewlett Packard mass spectrophotometer was used to analyze the EAF of *B. sapida* with a view to identifying its bioactive principles. Interpretation of mass spectra from the GC–MS analysis was conducted using standard database (NIST 11) and literature. Precisely, GC coupled with mass spectrophotometer (GC–MS) analysis was carried out on Hewlett Packard Agilent GC (Model 19091J–413:3516.156884, USA) fitted with flame ionization detector and Hewlett Packard mass spectrophotometer (5975C series injector). The injector, transfer line and ion source temperature were maintained between 300°C and 150°C. The GC separation was performed with a capillary column–Agilent J HP–5MS (length; 30 m×250µm; film thickness 0.25µm) treated with 5% phenyl methyl silox. The carrier gas was helium (99.999% purity) operated at a constant flow rate of 1.504 ml/min. Sample was dissolved in acetone at split less injection (split ratio of 30:1; split flow of 45.12 ml/min) of aliquot sample of EAF (1 µl), the primary GC oven pressure was maintained at 11.604 psi and the temperature kept constant at 35°C for 5 min before being raised by 4°C /min to 150°C for 2 min. Temperature of the secondary oven was also held isothermally at 35°C for 5 min then raised by 20°C/min to 260°C for 5 min. The slow fan was disabled throughout the period of analysis. The total run time was 46 min at a flow rate of 1.5 mL/min. The detector of the MS was operated in scan mode between 50 and 750 amu and the ion source run at 70 eV. A scan interval of 5 min and fragment from 50 to 600 Da was maintained. The test was run in triplicate. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass spectrum solution software provided by Agilent was used to control the system and acquire the mass spectra. The compounds were identified by comparing the mass spectra (peak) obtained with those of the standard mass spectra obtained from the National Institute of Standards and Technology (NIST) 11 (NIST) library or database.

Data analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test using the software GraphPad Prism (version 3). The means were compared by significance difference at $p<0.05$. Values were expressed as mean ± standard error of mean (SEM).

RESULTS AND DISCUSSION

In this study, *B. sapida* stem bark was screened for phytoconstituents, cytotoxicity was investigated with brine shrimp lethality assay using *A. salina* and active compounds from EAF identified using GC-MS.

Phytochemicals

Phytochemical tests revealed the presence of major classes of secondary metabolites in the EE and fractions of *B. sapida,* as shown in Table 1. In general, plants serve as origins of various phytochemicals, which are responsible for their diverse biological and pharmacological activities. These phytochemicals, commonly known as secondary metabolites, have been reported in the treatment of different disorders such as cancer, cardiovascular, and neurodegenerative diseases [19]. The phytochemical tests revealed the presence of alkaloids, phenolic, cardiac glycoside, tannins, flavonoids, and terpenoids in the EE, ethyl acetate, butanol, and AQF of *B. sapida*. The study by Emmanuel *et al*. [20] reported the presence of phytochemicals such as phenols, alkaloids, tannins, saponins, flavonoids phlobatannins, cardiac glycosides, anthraquinones, terpenoids, and reducing sugar in the seed and seed oil of *B. sapida*. Furthermore, Veronica *et al*. [21] observed the phytochemicals such as saponins, glycosides, and tannins and phenolic contents in the aril part of *B. sapida*. These secondary metabolites have been recognized to be responsible for various biological activities such as anti-diarrheal activity [22], hypoglycemic effect [23], antioxidant benefits [21,24], and inhibition of lipid peroxidation in diabetes [24] exhibited by the plant. These secondary metabolites have been reported for the antioxidant property displayed by medicinal plants such as *Terminalia bellirica* Roxb [25].

Brine shrimp lethality

Brine shrimp lethality activities of the extract and its fractions were expressed in LC_{50} (µg/ml) with AQF having the least value (17.73 µg/ml), followed by EAF (50.90 µg/ml), as shown in Table 2. Brine shrimp lethality test is a simple bioassay for testing plant extracts bioactivity which commonly correlates with activities against rapidly dividing cells such malarial parasites and tumor. The aqueous and EAF s of *B. sapida* revealed potency against brine shrimp larvae which was evidenced by their LC_{50} values with aqueous showing the least value, corresponding to highest potency. This could be related to its traditional uses in the treatment and management of different ailments including malaria. Sonibare *et al.* [26] evaluated brine shrimp lethality/cytotoxicity of *B. sapida* leaves extract and observed LC_{50} of 114.9 µg/ml compared to 63.57 µg/ml obtained in the stem bark extract of the same plant in this study. Furthermore, Olufade *et al.* [27] reported weak lethality in shoot extract of *B. sapida*. All these findings support the result of this study by establishing the fact that different parts of *B. sapida* possess certain degree of lethality/cytotoxicity. The result corroborates the work of Barakaeli and Mhuji [28] on another plant, in which *Mentha piperita* ethyl acetate leaf and *M. piperita* methanol leaf showed potency against brine shrimp larvae. In support of variously reported cytotoxic activities of different medicinal plants [29], cytotoxicity/ lethality of EE of *B. sapida* stem bark showed the presence of distinct phytochemicals which could be responsible for numbers of pharmacological and biological properties.

Bioactive compounds present in EAF

GC interfaced with MS (GC-MS) is an established technique for reliable identification of volatile bioactive principles in plants [30], used in the detection of drugs or poisons in the biological specimens of suspects, victims, or deceased [31,32]. The bioactive constituents in triphala have been studied by Apata *et al*. [33] using GC-MS. The EAF of *B. sapida* showed 13 peaks in the GC-MS chromatogram (Table 3) which were identified according to their retention time. These compounds contain hydrocarbons of both straight chain and aromatics of different nature such as organoSilicone, phenol, conjugated phenol, palmitic acid, ester, and fatty acid. Hexadecanoic acid was identified as most prominent compound with peak area (10.13%). The phytoconstituents with large

Table 1: Phytochemical constituents of *Blighia sapida* stem-bark

Constituents	Alkaloid	Phenolics	Cardiac glycoside	Tannins	Flavonoids	Terpenoids
Ethanol extract	+	+	+	+	+	+
EAF	−	+	+	+	−	+
n-BF	+	+	−	−	+	−
AqF	+	+	−	−	+	+

+: Present, −: Absent, EAF: Ethyl acetate fraction, n-BF: Butanol fraction, AqF: Aqueous fraction

peak areas have been reported to be responsible for the majority of activities possess by medicinal plants [34]. The reported biological activities of the bioactive compounds identified in the EAF of the plant extract are presented in Table 4.

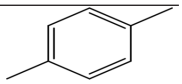
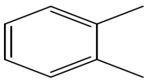
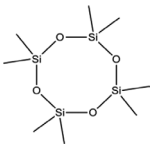
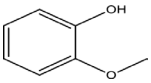
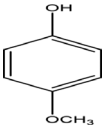
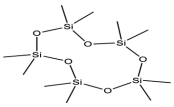
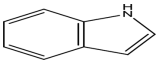
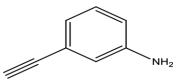
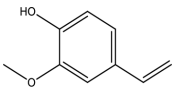
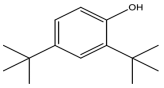
Table 2: Brine shrimp bioassay results of ethanol extract and fractions of *Blighia sapida*

Sample	LC_{50} (µg/ml)
Ethanol extract	63.57±0.63
Ethyl acetate	50.90±0.33
Butanol	72.86±0.58
Aqueous	17.73±0.83
Thymol	62.09±0.17

Each value represented the mean±S.E.M, (n=3)

Volatile compounds, 13 belonging to hydrocarbons of both straight chain and aromatic with different natures, were identified from EAF of the extract through GC-MS analysis. Interpretation of each of the mass spectra from GC-MS analysis was conducted using standard database. These compounds have been identified from many other medicinal plants and reported to serve as both plant defense and pharmacological [35]. The fragmentation patterns of the mass spectra were compared with standard compounds in the NIST. A total number of 13 active compounds were identified from EAF of *B. sapida* based on retention time, peak area, names, and structural formula. The highest peak represents the most prominent compounds with a peak area of 10.13% while the lowest peak represents the least prominent compounds with a peak area of 0.40%. The group of compounds detected were; p-xylene, o-xylene, Octamethyl-cyclotetrasiloxane, 2-methoxy phenol, Mequinol, Decamethyl- cyclopentasiloxane, indole, m-Aminophenylacetylene,

Table 3: List of bioactive compounds identified by the GC-MS analysis of the ethyl acetate fraction of *Blighia sapida*

S/No	Retention time	Peak area (%)	NIST matching (%)	Name of compound	Molecular formula	Molecular weight	Structural formula
1.	5.480	3.10	97	p-xylene	C_8H_{10}	106.1650	
			95	o-xylene	C_8H_{10}	106.16	
2.	11.190	5.13	91	Octamethyl-cyclotetrasiloxane	$C_8H_{24}O_4Si_4$	296.61	
3.	14.364	3.84	95	2-methoxy phenol	$CH_3OC_6H_4OH$	124.14	
			91	Mequinol	$C_7H_8O_2$	124.14	
4.	17.184	0.65	91	Decamethyl-cyclopentasiloxane	$C_{10}H_{30}O_5Si_5$	370.77	
5.	21.834	0.47	94	Indole	C_8H_7N	117.15	
			91	m-Aminophenylacetylene	C_8H_7N	117.15	
6.	22.423	2.86	90	2-Methoxyl-4-vinylphenol	$C_9H_{10}O_2$	150.17	
7.	28.881	0.44	93	2, 4-bis (1,1-dimethylethyl) phenol	$C_{14}H_{22}O$	206.32	
8.	38.825	10.13	99	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	
9.	40.035	2.02	99	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	
				Pentadecanoic acid	$C_{15}H_{30}O_2$	242.40	

NIST: National Institute of Standards and Technology, GC-MS: Gas chromatography-mass spectrometry

Table 4: Reported bioactivity of phytocomponents identified in ethyl acetate fraction of *Blighia sapida* by GC-MS

Compound name	Biological activity	Nature of the compound
Xylene	Laboratory chemical, used in paints and coating [41]	
Octamethyl-cyclotetrasiloxane	Antimicrobial, Antiseptic, Hair Conditioning Agent, Skin- Conditioning Agent-Emollient; Solvent [42]	Organosilicone compound
2-methoxy phenol	Anticancer [43]	phenol
Mequinol	Anticancer [43]	phenol
Decamethyl- cyclopentasiloxane	Antimicrobial, Antiseptic, Hair Conditioning Agent, Skin- Conditioning Agent-Emollient; Solvent [42]	Organosilicone compound
Indole	No Activity Reported	
m-Aminophenylacetylene	No Activity Reported	
2-Methoxyl-4-vinylphenol	Antioxidant, Cytotoxic [43]	Conjugated phenol
2, 4-bis (1,1-dimethylethyl) phenol	Antimicrobial, Anesthetic, Antioxidant, Antiseptic, Cancer preventive, Pesticide, Fungicide [42]	Phenolic compound
n-Hexadecanoic acid	Antifungal, Antioxidant, hypocholesterolemic, nematicide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor, potent antimicrobial agent, antimalarial and antifungal [44]	Palmitic acid
Octadecanoic acid	Non-cytotoxic [45] Antioxidant, antiviral, anticancer, anti-acne and cosolvent [46]	Fatty acid
Pentadecanoic acid	Antioxidant [46]	Palmitic acid

GC-MS: Gas chromatography-mass spectrometry, biological activity

2-Methoxyl-4-vinylphenol, 2, 4-bis (1, 1-dimethylethyl) phenol, n-Hexadecanoic acid, Octadecanoic acid, and Pentadecanoic acid one of which is prominent compound. Different biological activities have been reported for many of these identified active compounds ranging from estrogenic, anti-inflammatory, antioxidant, antibacterial, analgesic, antihistaminic, antimicrobial, hypocholesterolemic, nematicide, pesticide, cytotoxic, insecticidal, antitumor, anticancer, and antifungal activities [36-38]. The prominent phytoconstituent in *B. sapida* EAF is n-hexadecanoic acid with peak area of 10.1% as identified in this study, has been reported for various activities such an insecticide, a surfactant and saturated fatty acid, it is hypocholesterolemic and antioxidant [39]. Emmanuel *et al.* [20] reported the presence of n-hexadecanoic acid among other fatty acids in the seed oil of *B. sapida* using GC-MS and concluded that the seeds may serve as a source of therapeutic agents and industrial oil. Furthermore, Hesham *et al.* [40] have proved that n-hexadecanoic acid in hydro extract of *Vitex negundo* Linn possessed better antioxidant property.

CONCLUSION

The EE and the fractions of *B. sapida* stem-bark exhibited lethality against brine shrimp as such, it can be concluded that the extract and its fractions contained active constituents supporting its medicinal values. The study revealed 13 active constituents, of which one is prominent in EAF of the extract by GC-MS analysis and phytochemical screening also detected various secondary metabolites. The identified compounds with various biological activities indicate the medicinal value and wide ethno-medicinal use of the plant. Hence, extract of *B. Sapida* stem-bark could be suggested for use in the synthesis of drugs with potential new mechanism of action to combat the menace of drug resistance.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors declare that we have no conflicts of interest.

AUTHOR'S CONTRIBUTIONS

M. B. Adekola, J. O. Areola, and O. O. Babalola – Designed and carried out the work.

M. B. Adekola, O. E. Apalowo, and A. F. Adesina – Extracted the plant

M. B. Adekola, J. T. Apata, and O. V. Oriyomi– Analyzed the results and prepared the manuscript.

ACKNOWLEDGMENT

The authors appreciate the efforts of Professor O. O. Oyedapo, Department of Biochemistry and Molecular Biology, Faculty of Science, Obafemi Awolowo University.

REFERENCES

1. Mohamed B, Fatima ZM, Mostafa E, Abdelkhaleq L, Hassane M, Driss L. *et al*. Toxic effects of some medicinal plants used in Moroccan traditional medicine. Moroccan J Biol 2006;2:21-30.
2. Mayorga P, Pérez KR, Cruz SM, Cáceres A. Comparison of bioassays using the *Anostracan crustaceans*, *Artemia salina* and *Thamnocephalus platyurus* for plant extract toxicity screening. Braz J Pharm Sci 2010;20:897-903.
3. Veni T, Pushpanathan T. Comparison of the *Artemia salina* and *Artemia fransiscana* Bioassays for toxicity of Indian medicinal plants. J Coast Life Med 2014;2:453-7.
4. Ahmed Y, Sohrab H, Al-Reza SM, Shahidulla-Tareq F, Hasan CM, Sattar MA. Antimicrobial and cytotoxic constituents from leaves of *Sapium baccatum*. Food Chem Toxicol 2010;48:549-52.
5. Ramachandran S, Vamsikrishna M, Gowthami KV, Heera B, Dhanaraju MD. Assessment of cytotoxic activity of agave cantula using brine shrimp (*Artemia salina*) lethality assay. Asian J Sci Res 2011;4:90-4.
6. Morton JF. Ackee. In: Morton F, Miami FL, editors. Fruits of Warm Climates. Vermont: Echo Point Books & Media; 1987. p. 269-71.
7. Owolabi OA, James DB, Ibrahim AB, Folorunsho OF, Bwalla I, Akanta F. Changes in lipid profile of aqueous and ethanolic extract of *Blighia sapida* in rats. Asian J Med Sci 2010;4:177-80.
8. Kean EA, Hare EA. Gamma glutamyl transpeptidase of the ackee plant *Blighia sapid*a. Phytochemistry 1980;19:199-204.
9. Gbolade AA. Inventory of anti-diabetic plants in selected districts of Lagos state, Nigeria. J Ethnopharmacol 2009;121:135-9.
10. Handa SS, Khanuja SP, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants, No. 66 Italy: United Nations: Industrial Development Organisation and the International Centre for Science and High Technology; 2008.
11. Adekola MB, Areola JO, Omisore NO, Asaolu FT, Ogunleye SG, Apalowo OE, *et al*. Sub-chronic toxicity study of ethanol stem-bark extract of *Blighia sapida* (*Sapindaceae*) in Wistar rats. Heliyon 2020;6:e02801.
12. Trease GE, Evans WC. In: Tyler VE, Brady LR, Robbers JE, editors. Pharmacognosy. 15th ed. London: Sounders and Co.; 2002. p. 229-46.
13. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005;4:685-8.
14. Sofowora A. Phytochemical screening: Medicinal plant and traditional practice in Africa. 2nd ed. Ibadan: Spectrum Books Limited; 2008. p. 150-3.
15. Prashant T, Bimlesh K, Mamdeep K, Gurpreet K, Harleen K. Phytochemical screening and extraction: A review. Int Pharm Sci 2011;1:1.

16. Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med 1993;59:250-2.
17. Potduang B, Chongsinroeg C, Benmart Y, Giwanin R, Supatanakul W, Tapanich S. Biological activities of *Schefflera leucantha*. Afr J Tradit Complement Altern Med 2007;4:157-64.
18. Finney DJ. Probit Analysis. 3rd ed. Cambridge England: Cambridge University press; 1971. p. 269-82.
19. Mooza A, Nora A, Shah AK. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) fiori leaves. Asian Pac J Trop Biomed 2014;4:964-70.
20. Emmanuel CO, Helmina OA, Egwim CE. Phytochemical constituents of seeds of ripe and unripe *Blighia sapida* (Koenig) and physicochemical properties of the seed oil. Int J Pharm Sci Invent 2014;3:31-40.
21. Veronica MD, Jacob KA, Sussana C, Sarah A. Ackee (*Blighia sapida*) fruit arils: Nutritional, phytochemicals and antioxidant properties. Int J Nutr Food Sci 2014;3:534-7.
22. Antwi S, Martey ON, Donkor K, Nii-Ayitey Okine LK. Anti-diarrhoeal activity of *Blighia sapida* (*Sapindaceae*) in rats and mice. J Pharmacol Toxicol 2009;4:117-25.
23. Saidu AN, Mann A, Onuegbu CD. Phytochemical screening and hypoglycemic effect of aqueous *Blighia sapida* root bark extract on normoglycemic albino rats. Br J Pharm Res 2012;2:89-97.
24. Amira PO, Oloyede HO. Antioxidant activity of aqueous extract of *Blighia sapida* stem bark in alloxan-induced diabetic rats. Glob J Med Res 2017;17:2249-4618.
25. Gupta R, Singh RL, Dwivedi N. *In vitro* antioxidant activity and GC-MS analysis of the ethanolic extract of *Terminelia bellirica* roxb (Baheda). Int J Pharm Pharm Sci 2016;8:275-82.
26. Sonibare MA, Oloyede GK, Adaramola TF. Antioxidant and cytotoxicity evaluations of two species of *Blighia* providing clues to species diversity. Electron J Environ Agric Food Chem 2011;10:2960-71.
27. Olufade II, Asimi T, Owonibi KS, Olaoluwa TD, Adegbola VM, Owolabi AS. Lethality investigation of toxicity of extracts from natural plants: A case study of *Blighia Sapida* leaves. Int Conf Sci Eng Environ Technol 2018;3:190-3.
28. Barakaeli AN, Mhuji K. Brine shrimp lethality assay of selected medicinal plants in Tanzania. J Complement Altern Med Res 2018;5:1-6.
29. Krishnaraju AV, Rao VN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (brine shrimp test). Int J Appl Sci Eng 2006;2:115-25.
30. Kumar A, Kumari PS, Somasundaram T. Gas-chromatography-mass spectrum (GC-MS) analysis of bioactive component of the methanol extract of halophyte, *Sesuvium portulacastrum* L. Int J Adv Pharm Biol Chem 2014;3:766-72.
31. David S, Penton Z, Fulton G, Kitson G. Gas Chromatography and Mass Spectrometry: A Practical Guide. United States: Academic Press; 2011.
32. Apata JT, Ogunleye SG, Ogunbiyi OJ, Babalola OO. GC-MS analysis and phytochemical screening of n-hexane fraction constituents from the leaf of *Clerodendrum volubile* P. Beauv. Int J BioSci Technol 2017;10:80-8.
33. Amala VE, Jeyraj M. Determination of antibacterial, antifungal, bioactive constituents of triphala by FT-IR and GC-MS analysis. Int J Pharm Pharm Sci 2014;6:123-6.
34. Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Res 2010;4:191-5.
35. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 42nd ed. India: Nirali Prakashan; 2008.
36. Pramod K, Devala R, Lakshmayya G, Ramachandra S. GC-MS analysis and antiulcer activity of ethanol extract of *Momordica tuberosa* cogn (CUCURBITACEAE) in rats. J Appl Pharmacol 2011;4:359-69.
37. Hussein HM, Hameed IH, Ibraheem OA. Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of *Adiantum capillus*-veneris using GC-MS and FT-IR spectroscopy. Int J Pharmacogn Phytochem Res 2016;8:369-85.
38. Wei LS, Wee W, Siong JY, Syamsumir DF. Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of *Peperomia pellucida* leaf extract. Acta Med Iran 2011;49:670-4.
39. Hesham HA, Abdurahman HN, Rosli MY. GC-MS analysis of bioactive constituents of Hibiscus flower. Aust J Basic Appl Sci 2017;11:91-7.
40. Fatema S, Ubale MB, Farooqui M, Arif PM. Analysis of biological activity and gas chromatography-mass spectrometry study of conventional extraction of *Vitex negundo* Linn. Leaves. Asian J Pharm Clin Res 2019;12:1-4.
41. Sumia F, Ramesh C, Saheel Q. GC-MS Analysis of the polyherbal mixture. J Pharm 2019;9:30-3.
42. Mary PF, Giri RS. GC-MS analysis of bioactive compounds of *Achyranthes aspera.* World J Pharm Res 2018;7:1045-56.
43. Goraksh JH, Keshav KD, Raghunath DP, Tukaram RG, Narendra DP. Phytochemical studies on *Nerium oleander* L. using GC-MS. Int J Pharmacogn Phytochem Res 2017;9:885-91.
44. Rajeswari J, Rani S. Gc-Ms analysis of whole plant of *Leptadenia reticulata*. Int J PharmTech Res 2014;6:2043-50.
45. Salmiah I, Farid AJ, Amir HT, Mohsen Z, Kamyar S, Zamberi S, *et al*. Chemical composition and antibacterial and cytotoxic activities of *Allium hirtifolium* Boiss. BioMed Res Int 2013;1:696835.
46. Odo IF, Lawrence US, Victor NO, Parker EJ, Innocent UO. FTIR and GC-MS spectroscopic analysis of methanol and chloroform extracts of *Brenania brieyi* root bark. Am J Res Commun 2017;5:44-54.