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GC-MS analysis of alkaloid-rich fraction of *Zanthoxylum Zanthoxyloides* leaf

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Abstract
The need to scientifically proof the traditional claim of the medicinal uses of plant materials in combating diseases has led to investigations into the medicinal potencies of some plants. *Zanthoxylum zanthoxyloides* stem has been traditionally used to treat diabetes, dental, malarial and other pathogenic infections. Ethnobotanical investigations have suggested that *Z. zanthoxyloides* possesses active phytochemical constituents which are responsible for its observed bioactivity. In this study we attempted to identify and characterize the active compounds in the leaf of the plant, using GC-MS analysis. The methanolic extract of the plant's leaf was partitioned, and GC-MS analysis was done on the crude alkaloid fraction. The main compounds identified were 2-pentanone 4-hydroxyl-4-methyl (32.96 %), 2-acetoxyisobutyryl chloride (32.96 %), α-hydroxyisobutyric acid acetate (32.96 %). These compounds have been shown to possess antimicrobial, antifungal, antibacterial, antioxidant and hypocholesterolemic properties respectively. The presence of these compounds in the plant corroborates its use in the traditional treatment of several pathogenic and metabolic diseases.

Keywords: active compounds, bioactivity, antifungal, antioxidant, antibacterial, hypocholesterolemic, antimicrobial

Introduction

Plants have been used as sources of traditional medicines and pharmaceutical preparations for man and other animals. According to a survey by the United Nations Commission for Trade and Development (UNCTAD, 1974) [15], more than 33 % of modern drugs and medicinal products are derived from plants.

The use of plants in traditional medicine predates the introduction of antibiotics and other modern drugs into the African continent. Africans have been able to cure a lot of diseases by using concoctions made from different plant materials, and these have been passed from generation to generation.

Plants are capable of synthesizing an overwhelming variety of low molecular-weight compounds. At present, over a 100,000 such compounds have been isolated from higher plants (Verpoorte and Memelink, 2002) [16]. The biosynthesis of secondary metabolites varies among plant species even in different organs of plants (Khan *et al.*, 2010) [11].

Z. zanthoxyloides is a very popular plant in Nigeria commonly used as chewing stick and had been used in the treatment of malaria, diabetes, sickle cell anaemia and tooth cavities. The plant belongs to the citrus family that is *Rutacea*. It is a common component of the rain forest vegetation. It occurs more abundantly in the savannah, tropical vegetation of south-western Nigeria and dry forest vegetation, coastal dunes extending to Niger state. A major characteristic is that the trunks, branches, branchlets, leaf stalks and inflorescence axes of all these species are covered by prickles or what others describe as spines (Waterman, 1986) [17]. The leaflets and inflorescences of these species contain volatile oils which bring forth sweet smells / odours and fragrances.

In general, it occurs at low altitude, it is called orin ata in Yoruba (chewing stick), and fasahuari in hausa. *Z. zanthoxyloides* contains substances that not only kill the malaria parasite, but also the mosquitoes that transmit the disease. It has been shown to exhibit antibacterial activity against *Streptococcus pyrogen*. It has also been shown that some of its species possess pharmacological properties of interest including anti-tumor, anti-leukemic, antimicrobial (de Moura *et al.*, 1997; Nissanka *et al.*, 2001) [5, 13], antibacterial (de Abreu *et al.*, 2003) [4], anti-HIV (Cheng *et al.*, 2005) [1], antimalarial (Jullian *et al.*, 2006) [10], trypanocides (Ferreira *et al.*, 2007) [6] [Guendéhou *et al.*, 2018] [7]. The aim of this research was to identify the phytochemicals in the plant through GC-MS analysis, to elucidate structure-function relationships.

Materials and Methods

Plant material

The aerial parts of Z. zanthoxyloide were collected from the Fulani farmstead in Ugbe Akoko on the 24th of April, 2017 at 6.45am during the raining season. The plant was identified and authenticated by the taxonomy section, Department of plant science and Biotechnology, Adekunle Ajasin University, Akungba Akoko.

Preparation of plant extract

After collection and identification of the plant, the leaves were washed thoroughly with tap water in order to remove the dust and soil particles. Then the leaves were air dried in the laboratory to prevent ultraviolet rays from altering the chemical constituents (Ncube *et al.*, 2008; Das *et al.*, 2010) [12, 2]. They were later pulverized (ground into powder form) to produce a homogenous sample.

To 800 g of the sample, 4 litres of methanol was added, stirred and allowed to stand for 72 hrs. The mixture was then sieved with a clean muslin cloth. After filtration, the solvent was removed under reduced pressure using a rotary evaporator at 40 ^{0}C, to minimize any thermal degradation of the alkaloids. Alkaloid-rich extract was obtained using the procedure described by Hadi and Bremner (2001) [9]. The crude alkaloid mixture was then separated from neutral and acidic and water-soluble materials, by initial extraction with aqueous acetic acid, followed by dichloromethane partitioning and then alkanation of the aqueous solution, before further dichloromethane partitioning.

GC-MS analyses.

The alkaloid-rich fraction obtained from the leaf of *Z. zanthoxyloides* was analyzed separately by GC-MS using a HP-5MS capillary column (30 m ×250 µm, i.d., 0.25 µm film thickness) in an Agilent 5975 series MDS gas chromatograph (Agilent Technologies, 7890 A GC system) coupled with mass spectrometer. The carrier gas was helium with a constant flow rate of 3 mL/min. The oven temperature was initially kept at 40 ºC for 2 min then ramped at 25 ºC/min to 90 ºC. The temperature was gradually increased from 15 ºC/min to 170 ºC and held isothermally for 2 min. An amount of 8µl of the sample was injected in the split mode of 10:1. The relative % amount of each component was calculated by comparing its average peak area to the total areas. The software adopted to handle mass spectra and chromatograms was Chemstation obtained by EI at 70 eV over the scan range m/z 50-550. The compounds were identified by comparison of their mass spectra with those of the National Institute Standard and Technology (NIST) mass spectral library having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

The GC-MS chromatogram of the alkaloid-rich fraction showed twelve peaks which indicated the presence of twelve phytochemical constituents

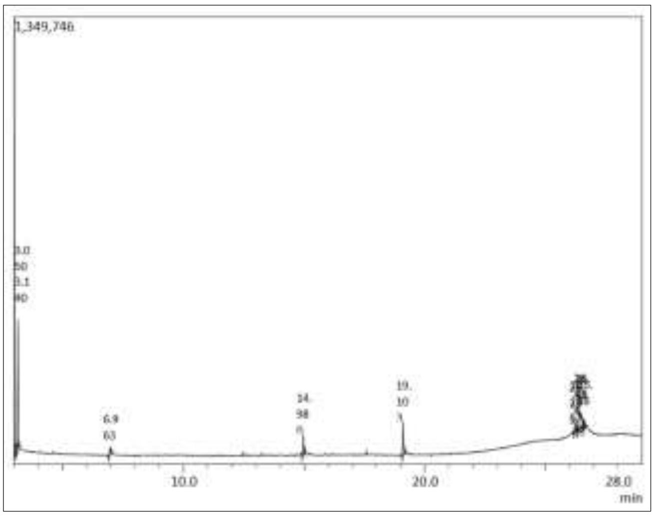
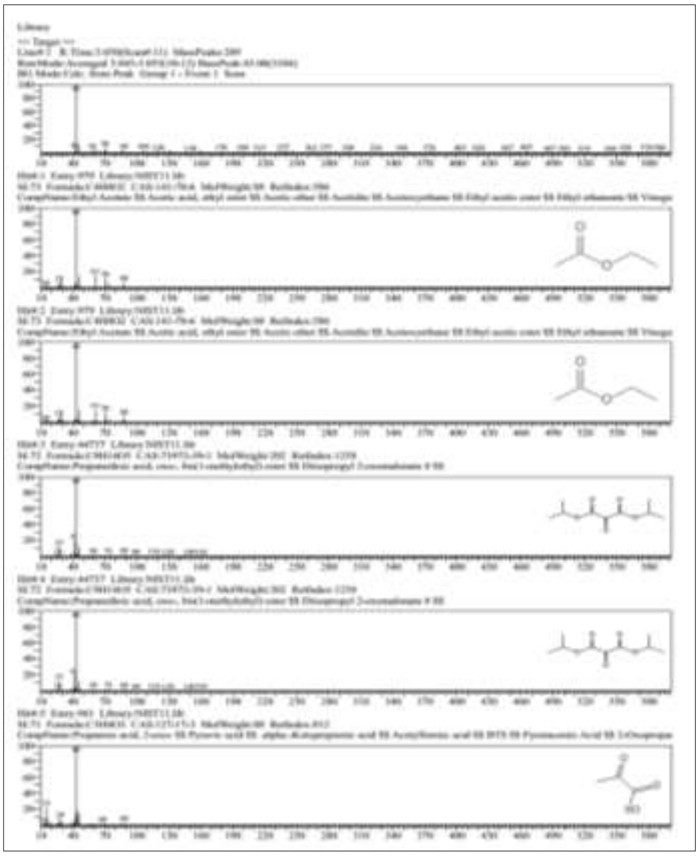
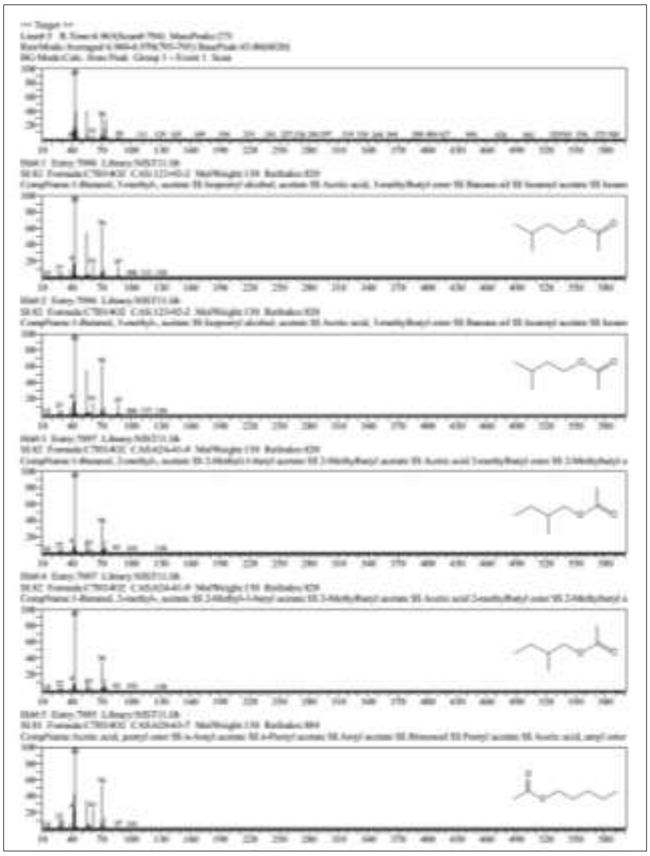


Fig 1

Components detected in extract of *B* GC-MS





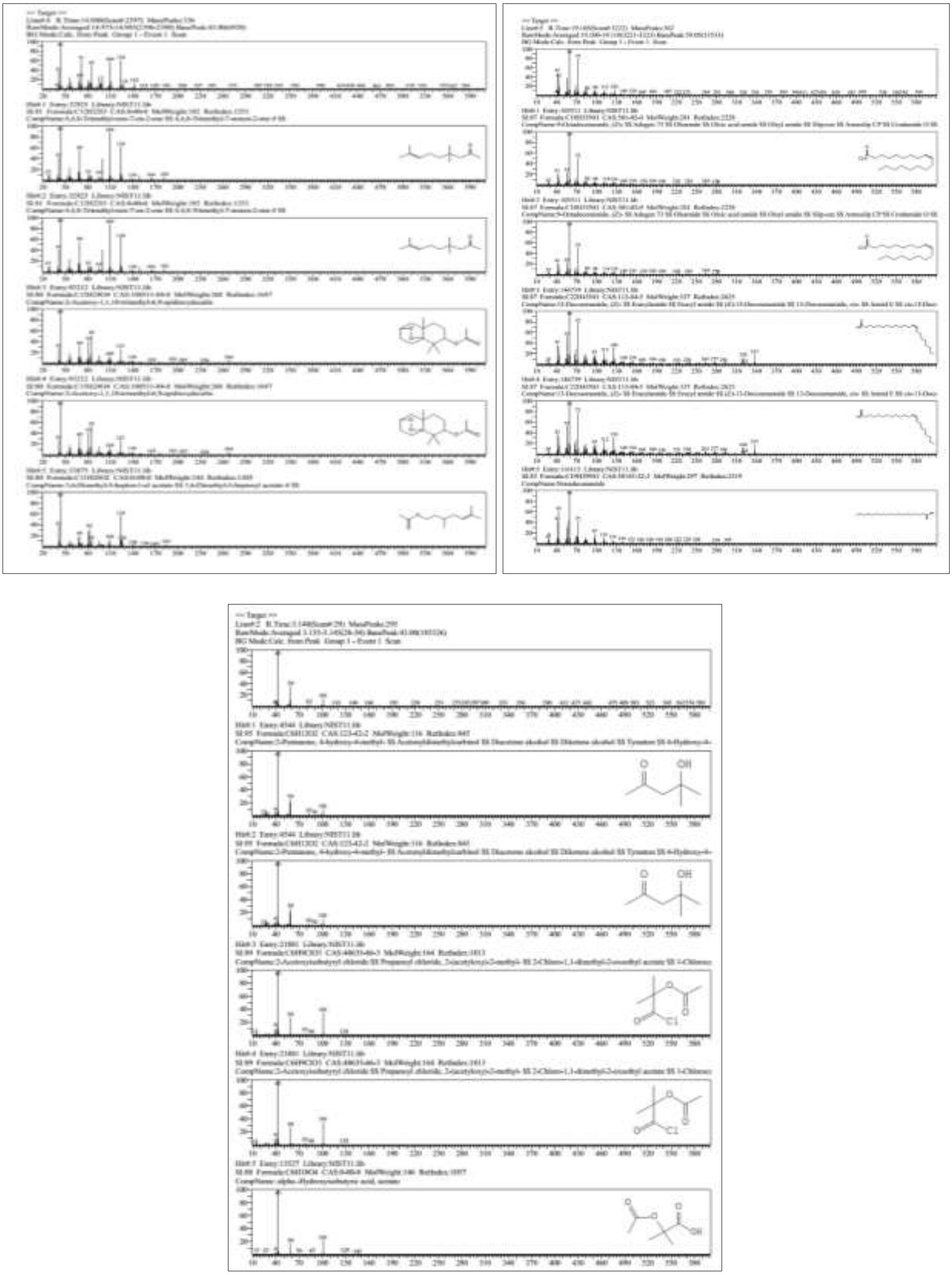


Fig 2: shows the mass spectra of alkaloid-rich extract of *Z. zanthoxyloides.*

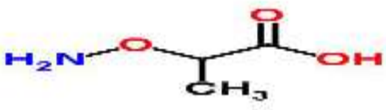
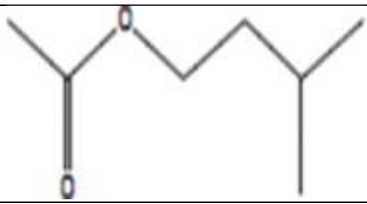
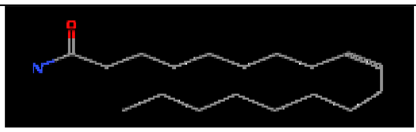
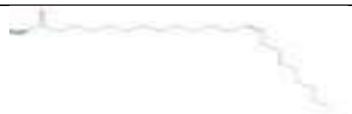
The active principles with their retention time (RT) and concentration (peak area %) are presented below: the molecular weight, formula, structure and bioactivities of phytochemicals that contributed to the medicinal activity of *Z. zanthoxyloides* are also shown below. Among the twelve compounds identified, the main abundant compounds were 2-pentanone 4-hydroxyl-4-methyl, 2-Acetoxyisobutyryl chloride, Alpha-hydroxyisobutyric acid acetate with percentage peak of 32.96 each and with bioactivities ranging from antimicrobial, hypocholestrolemic and antioxidants activities.

Compounds Detected by GCMS

Table 1

S/N	RT	COMPOUNDS	M.FORMULAR	MW	PEAK AREA%
1	3.050	Ethyl Acetate	$C_4H_8O_2$	88	4.46
2	3.050	Propanedioic acid	$C_9H_{14}O_5$	202	4.46
3	3.140	2-pentanone 4-hydroxyl-4-methyl	$C_6H_{12}O_2$	116	32.96
4	3.140	2-Acetoxyisobutyryl chloride	$C_6H_9ClO_3$	164	32.96
5	3.140	Alpha-hydroxyisobutyric acid acetate	$C_6H_{10}O_4$	146	32.96
6	6.963	1-Butanol,3-methyl-acetate	$C_7H_{14}O_2$	130	3.58
7	14.980	4,4,8,-Trimethyl-non-7-en-2-one	$C_{12}H_{22}O$	182	5.80
8	14.980	2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin	$C_{15}H_{24}O_4$	268	5.80
9	14.980	3,6-Dimethyl-5-hepten-1-ol acetate	$C_{11}H_{20}O_2$	184	5.80
10	19.103	9-Octadecenamide	$C_{18}H_{35}NO$	281	11.93
11	19.103	13-Docosenamide	$C_{22}H_{43}NO$	337	11.93
12	19.103	Nonadecanamide	$C_{19}H_{39}NO$	297	11.93

Table 2

S/No	Name of Compound	Nature of Compound	Structure	Biological Activity
1	Ethyl Acetate			Antibacterial
2	Propanedioic acid	Fatty acid		Anti-cancer
3	2-Pentanone 4-hydroxyl-4-methyl	Ketone		Antimicrobial, Flavor
4	2-Acetoxyisobutyryl chloride			Antibacterial and antifungal
5	alpha.-Hydroxyisobutyric acid, acetate			Antioxidant, hypocholesterolemic
6	1-Butanol, 3-methyl-, acetate	Fatty Acid		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic,5-Alpha reductase inhibitor, Anti-inflammatory
7	4,4,8-Trimethyl-non-7-en-2-one	Fatty Acid		Flavour
8	2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin			Antioxidant, hypercholesterolemia, cancer-preventive, cosmetic
9	9-Octadecenamide	Fatty Acid		Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic
10	13-Docosenamide	Alkyl amides		Antibacterial and antifungal
11	Nonadecanamide	Hydrocarbon		Antimicrobial, Antifungal.

Reference: Dr Duke and NIST Phytochemical and Ethnobotanical databases

Discussion

The GC-MS analysis revealed that the alkaloid-rich fraction of Zanthoxyllum zanthoxyloides composed mainly of ketones, fatty and organic acids. These compounds are responsible for various pharmacological actions possessed by the plant such as hepatoprotective, antioxidant, wound healing, and antimicrobial activities. From the GC-MS profiles of alkaloid-rich extract of Z. zanthoxyloides obtained in this work, 12 different active compounds were identified. The qualitative GC-MS profiles showed the compounds; 2-pentanone-4-hydroxyl-4-methyl, 2-acetoxyisobutyryl chloride and alpha. -hydroxyisobutyric acid acetate to have the highest peak and 1-butanol-3-methyl-acetate, ethyl acetate and propanedoic acid had the lowest peak.

2-pentanone-4-hydroxyl-4-methyl is known to be an antimicrobial and a flavoring agent which support the ethnomedical use of the plant in wound healing and in treatment of tooth problems. This agrees with the work of Phuong *et al.* (2018) [14]. 2-Acetoxyisobutyryl chloride is also known to possess potent antibacterial and antifungal properties which further give credence to the traditional use of the plant in the treatment of bacterial and fungal infections. Alpha-hydroxyisobutyric acid acetate possesses antioxidant and hypocholesterolemic activities. These properties appear to give credence to the use of the plant in the traditional treatment metabolic and pathogenic diseases like diabetes, malaria and toothache.

Conclusion

The bioactive compounds possessed by Z. zanthoxyloides shows that it has medicinal potentials, and could be a potential precursor for drug production against life threatening diseases like malaria, toothache, diabetes and cardiovascular diseases. However, further research needs to be carried out to isolate, purify and characterize the individual bioactive compounds through nuclear magnetic resonance spectroscopy.

References

1. Cheng MJ, Lee KH, Tsai IL, Chen IS. Two new sesquiterpenoids and anti-HIV principles fromthe root bark of Zanthoxylum ailanthoides. Bioorganic & Medicinal Chemistry, 2005; 13:5915-5920.
2. Das K, Tiwan RKS, Shivasteva DK. Techniques for evaluation of medicinal plant products as anti-microbial agent: Current Methods and Future Trends. Journal of
3. Medicinal Plants Research, 2010; 4:104-111.
4. de Abreu W, Weber AD, Giacomelli SR, Simionatto E, Dalcol II, Dessoy ECM, *et al*. Composition and antibacterial activity of the essential oils from Zanthoxylum rhoifolium. Planta Medica, 2003; 69:773-775.
5. De Moura NF, Ribeiro HB, Machado EC, Ethur EM, Zanatta N, Morel AF, *et al*. Benzophenanthridine alkaloids from Zanthoxylum rhoifolium. Phytochemistry, 1997; 46:1443-1446.
6. Ferreira ME, Nakayama H, de Arias AR, Schinini A, de Bilbao NV, Serna E, *et al*. Effects of canthin-6-one alkaloids from Zanthoxylum chiloperone on Trypanosomacruzi-infected mice. Journal of Ethnopharmacology, 2007; 109:258-263.
7. Guendéhou F, Djossa BA, Kènou C, Assogbadjo CAE. Review of studies on Zanthoxylum zanthoxyloides (Lam): availability and ethnomedical, phytochemical,
8. pharmacological uses. Scholars Journal of Research in Agriculture and Biology. 2018; 3(3):244-254.
9. Hadi S, Bremner JB. Initial studies on alkaloids from Lombok medicinal plants. Molecules, 2001; 6:117-129.
10. Jullian V, Bourdy G, Georges S, Maurel S, Sauvain M. Validation of use of a traditional antimalarial remedy from French Guiana, Zanthoxylum rhoifolium Lam. Journal of Ethnopharmacology, 2006; 106:348-352.
11. Khan S, Al – Qurainy F, Ram M, Ahmad S, Abdin MZ. Phyllanthin biosynthesis in *Phyllanthus amarus*. Schum and Thonn, growing at different altitudes. Journal of Medicinal Plant Research. 2010; 4(1):041-048.
12. Ncube NS, Afolayan AJ, Okoh AT. Assessment techniques of anti-microbial properties of natural compounds of plant origin: current methods and future trends. Africa Journal of Biotechnology. 2008; 7(12):1797-1806.
13. Nissanka AP, Karunaratne V, Bandara BR, Kumar V, Nakanishi T, Nishi M, *et al*. Antimicrobial alkaloids from Zanthoxylum tetraspermum and caudatum. Phytochemistry, 2001; 56:857-861.
14. Phuong TV, Lam PVH, Diep CN. Bioactive compounds from marine streptomyces sp. by Gas Chromatrography-Mass Spectrometry. The Pharmaceutical and Chemical Journal. 2018; 5(1):196-203.
15. United Nations Commission on Trade and Development. (UNCTAD). Markets for selected medicinal plants and their derivatives. UNCTAD/GATT, Geneva, 1974, Pp 30- 32.
16. Verpoorte R, Memelink J. Engineering secondary metabolites production in plants. Current Opinion on Biotechnology, 2002; 13:181-187.
17. Waterman PG. A Phytochemist in the African rain forest. Phytochemistry. 1986; 25(1):3-17.