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Research Article

Gas Chromatography Mass Spectroscopy (GC-MS) Analysis of Bioactive Compounds from *Albizia gumifera* Leaf Extract: A Sacred Indigenous Medicinal Plant among the Akamba People of Lower Eastern Kenya

Idi Nuhu[1,4], Mercy Githua[1], Roshila Moodley[2], Ming Q Guo[3], Harami M Adamu[4], Patrick G Kareru[1]

[1]Department of Chemistry, Faculty of Mathematics and Physical sciences, Jomo Kenyatta University of Agriculture and Technology Kenya.

[2]School of Chemistry and Physics Private Bag X01, Scottsville, University Road West ville, Durban South Africa.

[3]Wuhan Botanical garden, Chinese Academy of Sciences , China.

[4]Deparment of Chemistry, Abubakar Tafawa Balewa University Bauchi, Nigeria.

Corresponding author: nuhuidi20@gmail.com

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ABSTRACT

Gas chromatography-mass spectroscopy (GC-MS) is one of the most reliable analytical technique widely used to identify different bioactive molecules. In this study, *Albizia gumifera* was identified and selected. The leaf was extracted using solvents of different polarity, and the result showed that *A. gumifera* ethanol and *A. gumifera* water extract had a high percentage yield of 21.22 g and 11.14% while, *A. gumifera* hexane and *A. gumifera* dichloromethane showed the least recovery of 1.92 g and 5.34%, respectively. The extracts were further subjected to phytochemical screening and the result revealed the presence of alkaloid, anthroquinone, phenol, saponin, coumarin flavonoid and essential oil present in all the extracts. The extracts were further subjected to gas chromatography-mass spectroscopic analysis. The result revealed the presence of 44 bioactive molecules with a unique fragmentation pattern of keto-enol tautomerism and resonance stabilization through the formation of a trophylium ion. From the results, it can be concluded that *A. gumifera* is a unique medicinal plant with a special medicinal potential, which can further be utilized in the synthesis of drug lead compound and as a nutraceutical product.

Keywords: Bioactive, Chromatography, Fingerprints, Medicinal plants, Metabolites, Spectroscopy.

INTRODUCTION

Since the time immemorial, all people across the globe have used plants to treat different ailments.[1-3] The World Health Organization (WHO) has reported that 95% of people in developing nations depend on plant for their basic healthcare needs.[4] The indigenous knowledge on the use of plant is one of the determining factor that helps in defining the cultural identities and provides link with the previous knowledge of their use.[5] Different species of these plants are found mainly in the tropical rain forest and conserved botanical gardens. Most of them possess a wide range of biological activity.[6] It is generally believed that any plant's medicinal and therapeutic activity is due to the presences of the active bio-molecules commonly referred to as "Phytochemicals".[7,8] As a result of the challenge faced by low-income earners in accessing modern medicine, arising from the high cost and lack of access to all the modern healthcare infrastructures, the rural community has continued to rely on medicinal plants for their basic healthcare needs.[9,10] About 420,500 species of higher plants are occurring in nature, with less than 3% having been screened for various bio-actives. At least 17,500 compounds have been isolated from different medicinal plants.

Furthermore, medicinal plants, either in pure or isolated form, have provided a number of unlimited revival for the drug lead compounds. Due to the unlimited availability of their chemical bio-diversity, the knowledge of the phytochemical constituents of the plant would further be valuable in the development of all folkloric remedies.[11] Kenya is a nation that is rich in floral bio-diversity and its natural resources are endowed with promising chemotherapeutic potential that are yet untapped.[12] It is also a destination for traditional system of medicine. The herbal recipes are either prepared from a single plant or combined using different plants (synergistic). Polar and non-polar solvents are the main ingredients for plant extract preparation.[13] The Akamba tribes live in the lower eastern part of Kenya. They are mostly farmers, which has influenced the area's Agricultural activity. The county of Machakos is 4500 to 6700 m above the sea level. The county is rich in agricultural activity and the people depend mostly on herbs for their basic health care needs. Most of the herbal recipes are not backed by much scientific knowledge on their efficacy and safety consumption. The community use alcohol and water in the preparation of the recipes. In the current study, there is the use of polar and non-polar solvents which is the best option because most of

Table 1: The plant name, voucher specimen and part of the plant used for the study

S. No.	Plant species name	Family name	Voucher specimen number	Part used
1	*Albizia gummifera* (J.F.Gmel.) C.A.Sm.	Fabaceae	IN/AG/JKUAT/004/2020	leaf

the metabolites are soluble in both polar and non-polar solvents.[14] *A.gumifera* belongs to the class of Fabaceae.[15] The plant grows both in wetland and upland forest. It is one of the unique sacred plants that have been used in Africa's tropical region in the treatment of different ailments.[16] The plant have been reported to possess antimicrobial,[17] cytotoxicity,[18] and antiplasmodial activitie.[19] The plant has been widely used in managing malaria, skin disorder and stomach pain.[20,21] The medicinal potency of the plant *A. gumifera* results from the presence of a spermine alkaloid, saponin and terpene.[20-24] Despite the widespread of *A.gumifera* in managing different ailments, its metabolites are not well characterized.

Gas chromatography-mass spectroscopy (GC-MS) is a systematic spectroscopic technique that have been used in the identification of bioactive molecules. The unknown bio-actives are determined from the organic mixture by spectra of the known with that of the fingerprint spectra from the National Institute of Science and Technology (NIST) database library.[25] GC-MS has two advantages over other spectroscopic techniques. One, the capillary column has good separation precision and can produce a high-quality chemical fingerprint that can be used to interpret bio-active molecules. Secondly, the spectral database on the information of the chemical composition of the plants extract which would be important in the structural elucidation and in the synthesis of novel drug candidate molecules and nutraceutical products.

EXPERIMENTAL

Collection and Preparation of the Plant Samples

Fresh leaves of *A. gumifera* were collected from Machakos county of the lower eastern Kenya in January and February 2020. The plants were identified by a plant taxonomist at the Department of Botany, Jomo Kenyatta University of Agriculture and Technology, Kenya. The voucher specimen were deposited at the university herbarium for reference purpose. Table 1 contains the details of the plant.

Extraction

The plant samples were air dried at room temperature at (25°C) for two weeks as recommended by Sofowora[26] after which it were ground to a uniform particle size powder. The solvents used for the extraction include hexane, ethylacetate dichloromethane, ethanol and water. The extraction was done by soaking 250 g of each powdered plant sample starting with the solvent of least polarity in a 1000 mL and was left to soak for 48 to 72 hours in an orbital shaker and was filtered using the Whatman filter paper. The extract was concentrated using a rotary evaporator in the water bath at 45°C. The percentage and recovery yield of each extract were calculated. This was repeated exhaustively with other solvents. The solvents used were of high performance liquid chromatography analytical grade and were purchased from British Drug House and Sigma Aldrich.

Preliminary Phytochemical Screening

The phytochemical screening of each plant extract were performed using the standard procedure for the determination of the presence of alkaloid, saponin, anthroquinone, flavonol and phenol, essential oil, coumarin and terpene.[27-31]

GC-MS analysis

Chromatography condition

Plant extract were all diluted in suitable solvent. The extracts were filtered using 0.45 µm nylon syringe filters and transferred into auto-sampler vials for GC-MS analysis. Shimadzu QP 2010-SE GC-MS coupled to an autosampler was used for the analysis. Ultra-pure Helium (He) was used as the carrier gas at a flow rate of 1-mL/minute. A BPX5 non-polar column, 30 m; 0.25 mm ID; 0.25 µm film thickness was used for separation. The Gas chromatography (GC) was programmed as follows; 50℃ (1 minute); 5℃/min to 250°C (9 minutes). The total run time was 50 minutes. Only 1-µL of the sample was injected. The injection was done at 200℃ in split mode, with split ratio set to 10:1. The interface temperature was set at 250℃. The EI ion source was set at 200℃. Mass analysis was done in full scan mode, 50 to 600 m/z.

Identification of compounds using GC-MS

The identification of the metabolite and the interpenetration of GC-MS spectrum were conducted on the library database of the National Institute of Science and Technology (NIST), having more than 75,000 patterns. The spectrum of each extract from the crude fingerprint matched that of the National Institutes of Science and Technology (NIST) Ver 3.1 mass library database. International union of pure and applied chemistry (IUPAC) name, molecular weight, peak number and concentration of each compound were all established**.** Detected peaks were matched against the National Institutes of Science and Technology 2014 mass spectra library database for possible identification.[32]

RESULTS

Extraction and percentage yield

The result of the extraction of the leaf of *A. gumifera* extract using a different solvent is presented in (Table 2). The results showed that *A. gumifera* ethanol extract (AGE) had the highest recovery and percentage yield of 42.54 g and 21.22%, While *A. gumifera* hexane extract had the least recovery and percentage yield with 3.87 g and 1.92% (Table 2). All the extracts obtained were grey and oily in texture excepts that polar extract *A. gumifera* ethanol extract and *A. gumifera* water extract (AGE & AGW), which are brown in color and pastry in texture.

Table 2: Summary of the result of extraction and the physical parameters of *A. gummifera*

Extracts	*Recovery (g)*	*(%)Yield*	*Color*	*Texture*
AGH	3.87	1.92	Grey	Oily
AG Ethyl	12.45	6..23	Grey	Oily
AG DCM	10.68	5.34	Brown	Solid
AGE	42.54	21.22	Brown	Pastry
AGW	22.39	11.14	Brown	Pastry

Keys : AGH- *A. gumifer*a hexane extract AGEthyl-*A.gumifer*a ethylacetate extract AGDCM-*A. gumifera* dichloromethane extract AGE- *A.gumifera* ethanol extract AGW-*A. gumifera* water extract

Table 3: The summary of phytochemical screening of *A. gumifera* extract using a different solvent of extraction

Extracts/ Phytochemical	Alkaloids flavonoid phenol saponin terpenes anthroquinones essential oil coumarins
AGH	++ ++ -- ++ - - + + - - + +
AGEthyl	++ - - ++ + + + + + + + + + +
AGDCM	-- ++ + + + + - - + + + + + +
AGE	++ + + + + + + + + + + + + + +
AGW	++ ++ + + + + + + + + + + + +

Keys : ++ - Present-- Absent AGH- *A. gumifer*a hexane extract AGEthyl-*A.gumifer*a ethylacetate extract AGDCM- *A. gumifera* dichloromethane extract AGE- *A.gumifera* ethanol extract AGW-*A.gumifera* water extract

Phytochemical Screening

Table 3 shows the summary of the phytochemical screening of the leaf of *A. gumifera* using the different solvents of polarity index. The result were confirmed by the color change and frothing, which shows the presence of different phytocompound in each of the solvent extracts in different quantities.[32]

GC-MS

The result of the spectral analysis of GC-MS are presented in Figs 1 to 8.

Fragmentation pattern of all the extract of *A. gumifera*

The gas chromatography-mass spectroscopy analysis results of all the plant extracts are presented in Table 4, showing a total of 44 bio-active compounds were identified. The results revealed the analysis of the relative concentration of the different bio-active molecules based on their retention time. All the spectra obtained are the fingerprint of the actual molecule and this was identified by matching them with the possible spectrum and that of the National Institute of Science and Technology library database. Table 4 contain the *A. gumifera* hexane extract. Twelve (12) bio-active compounds was identified and prominent among them are ; 1,3,5 cyclohepta triene, n-hexadecanoic acid methyl ester, methylpenta-1,1 diol, docasa 2,6,10,14 ,18 penta-22-al, di-iso octylphthalate, 2,9 dimethyl decane, tri-isopropyl methoxysilane, tert-butyl dimethylsilyl acetate, n-methoxy-n-methyl acetamide, methyl -9-cis- octadecadi enoate and 2- hexadecen-1-ol 3,7,11, 15 tetra methyl. For ethyl acetate and dichloromethane extract (AGEthyl &AGDCM). A total of[19] bio-active compound were identified (Table 5,6). Prominent among them includes; 2- butyl -2-hydroxy -n-2,3 phenylpropenylidino hexanohydrazide, trans 1,4 cyclohexane, diol trimethyl silyl ether,

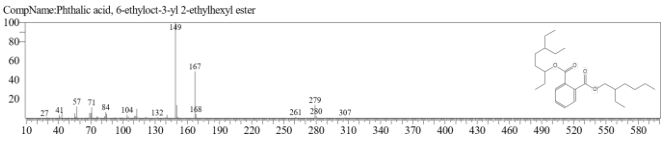


Fig. 1: GC-MS spectrum of bis- 6-methyl heptyl phthalate from ethylacetate extract of *A. gumifera* [AGEthyl]

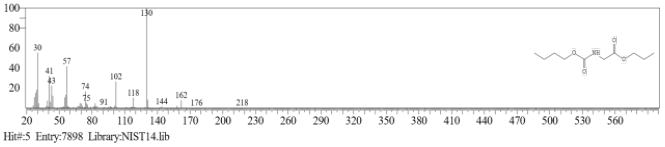


Fig. 2: GC-MS spectrum of of N-butoxy carboxyl propyl ester from ethylacetate extract of *A. gumifera* [AGEthyl]

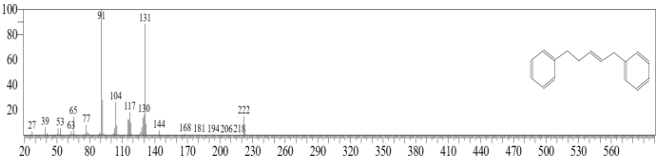


Fig 3: GC-MS spectrum of 3E-5-phenyl-3-pentenyl benzene from ethyl acetate extract of *A. gumifera* [AGEthyl]

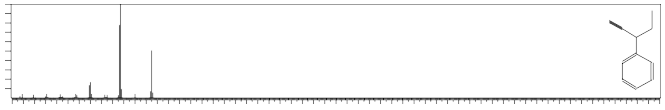


Fig. 4: GC-MS spectrum of 3-phenyllbutylnitrile from dichloromethane extract of *A. gumifera* [AGDCM]

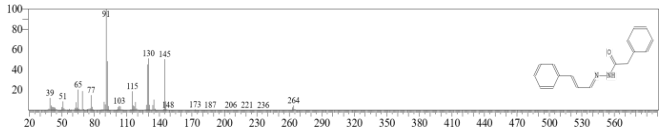


Fig. 5: GC-MS spectrum of 3-phenyl propenal alpha-toluyl-hydrazone from ethyl acetate extract of *A. gumifera* [AGEthyl]

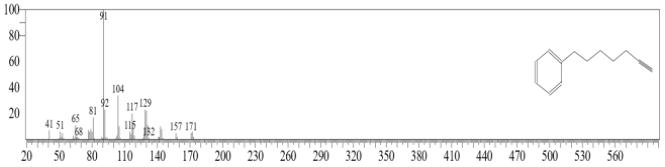


Fig 6: GCMS spectrum of 6-heptenyl benzene from dichloromethane extract of *A. gumifera* [AGDCM]

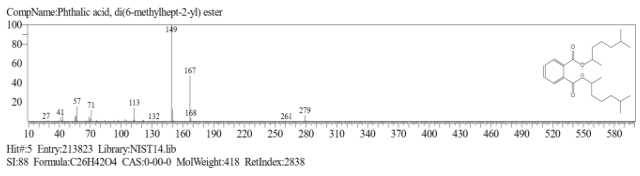


Fig. 7: GCMS spectrum of 6-ethyloctyl-3-yl-2-methylpropyl ester from ethyl acetate extract of *A. gumifera* [AGEthyl]

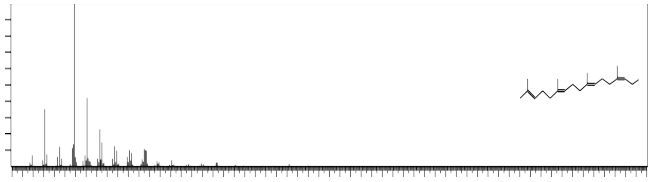
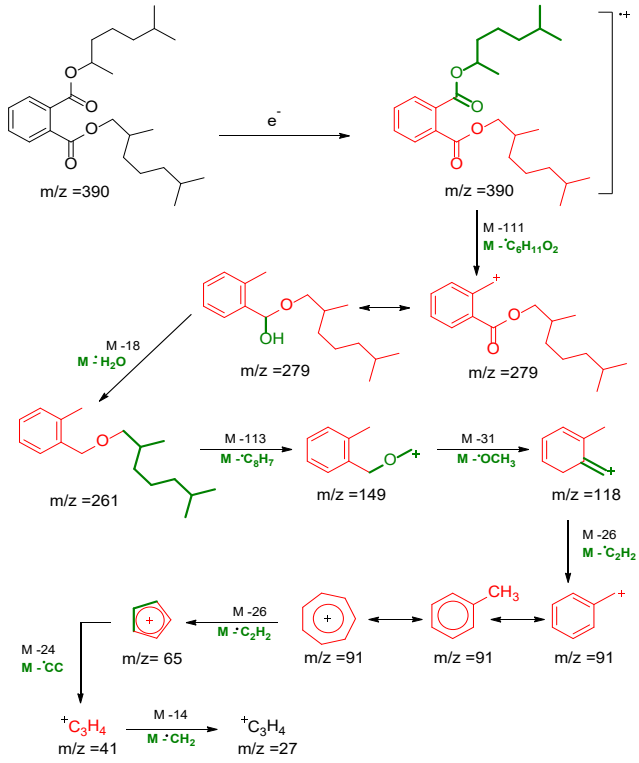


Fig 8: GC-MS spectrum of hexadeca 2,6,10,14,18 tetra-en-1-ol. from ethyla acetate extract of *A. gumifera* [AGEthyl]

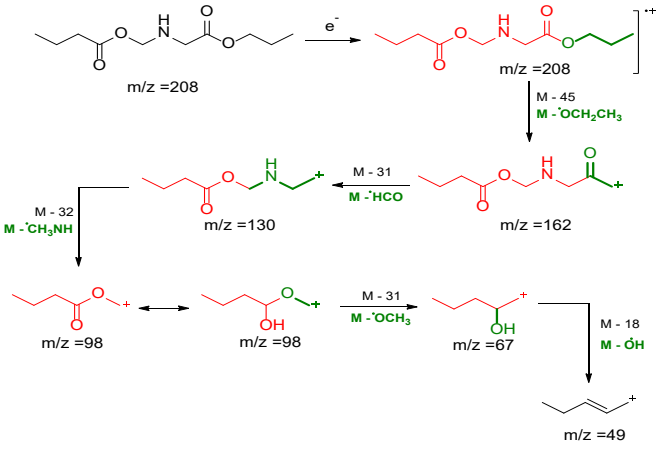
3-phenyl propenal alpha- toluyl hydrazone, 6-heptenyl benzene, (3E)-5- phenyl-3-pentenylbezene, 1-O-toluyl prop-2-e al, 4 (2-tert butyl-5-oxo-1,3 dioxan -4-yl) butyl formamide, n-butoxy carbonyl ester, 1-methoxy-5-trimethylsilyoxyhexane, 2-propenoic tetra decylester, bis 6- methyl heptyl) phthalate, bis [2-ethylhexyl], 2- Benzene dicarboxylate, 6-Ethyloctyl-3-yl-2-methyl ester, dis [6-methylhepta-yl] ether. In the polar extract of *A. gumifera* ethanol and *A.gumifera* water extract (AGE & AGW) (Table 7,8). A total of[14] different bio-active compounds were identified. These comprise of; 5- methyl -2-isopropyl cyclohexane -1-ol, 4- trimethylsilyl)] oxy-cis-cyclohexanol,2-hydroxy-2-methylbutanedioic, 3-o-methylhex-2-ulose, cis-2-hexe-1oltrimethylsilylether, methyl-2-hydroxyoctadeca 9,12,15, tri-enoate, [2E, 6E] 3,7,11, trimethyl 2,6,10 dodecane triene-1-ol, 3-methylthio thiopene, di ethyl 2,2 ethane, 1,2 diylbis(oxy) diacetate, 2-bromo tetradecane, 4-hexadecyl cyclodecane, 4-hexadecyl cycloheyl-3-nitrobenzene sulphonyl chloride and 3,5 difluoro-phenyl tetra-decyl ester. The results of the fragmentation pattern of the identified bio-active compounds are presented in Schemes 1 to 8.

Scheme 1 shows the summary of the fragmentation pattern of Bis-(6- methyl heptyl phthalate ester from *A. gumifera* ethylacetate extract (AGEthyl). Prominent peaks were observed at m/z = 390, 279, 261, 167, 149, 132, 113, 71, 57, 41 & 27 respectively. The radical cation was formed at m/z = 390. The loss of the radical ion and the (m-*$C_6H_{11}O_2$) account for the peak at 279. At the m/z = 279 there was an observable stabilization of the molecule through the formation of the resonance, this lead to the formation of the keto-enol tautomerism. The loss of the water (m-H_2O) and octyl radical ion (m-*C_8H_7) account for the peaks at m/z = 261and m/z = 149. The m/z = 149 is the base peak of the reaction. The loss of the methoxide radical ion (m-*OCH_3) and the ethylene molecule (m-C_2H_2) account for the peaks at m/z = 118 and m/z = 91. The formation of the m/z = 91 stabilizes through the resonance to form the trophylium ion the most stable molecule. The final loss of the fragment of ethylene molecule (m-C_2H_2) and the formation of the methyl radical ion (m-*CH_3) account for the final peaks at m/z = 65, m/z = 41 and m/z = 27, respectively.

Scheme 2 shows the summary of the fragmentation pattern of (N-butoxy carbonyl propyl ester) from *A. gumifera* ethyl acetate extract (AGEthyl). Prominent among the peaks obtained were m/z = 208, 162, 130, 118, 98, 67 & 49, respectively. The radical cations were formed at m/z = 218 as the result of the radiation source ionization led to the radical cation's generation. The loss of ethoxy radical (m-*OCH_2CH_3) accounts for the formation of the fragment ions at m/z = 162. The loss of the (m-*CHO) and (m-CH_3NH) account for the peaks at m/z = 130 and m/z = 98, respectively. The m/z = 130 is the reaction's base peak; at m/z = 98, the molecule was stabilized



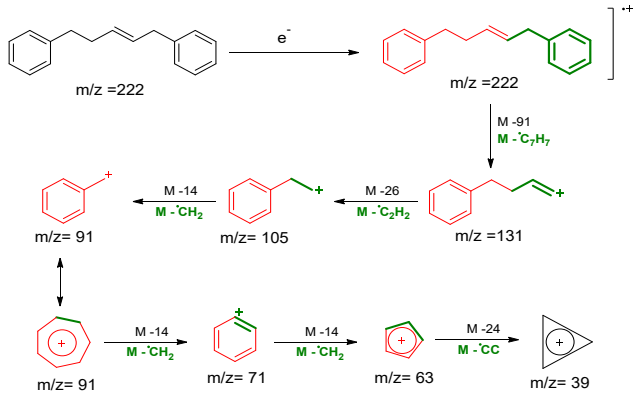
Scheme 1: Fragmentation pattern of Bis-6- methyl heptyl phthalate ester from ethylacetate extract of *A. gumifera* (AGEthyl)



Scheme 2: Fragmentation pattern of N-butoxy carbonyl propyl ester from ethyl acetate extract of *A. gumfera* (AGEthyl)

through the resonance and led to the formation of the Keto-enol tautomerism. The loss of the hydroxyl radical (m-*OH) and the methoxide radical ion (m-*OCH_3) account for the peaks at m/z = 67 and m/z = 49. The final loss of the methyl radical ion (m-*CH_3) accounts for the final peak at m/z = 34, respectively.

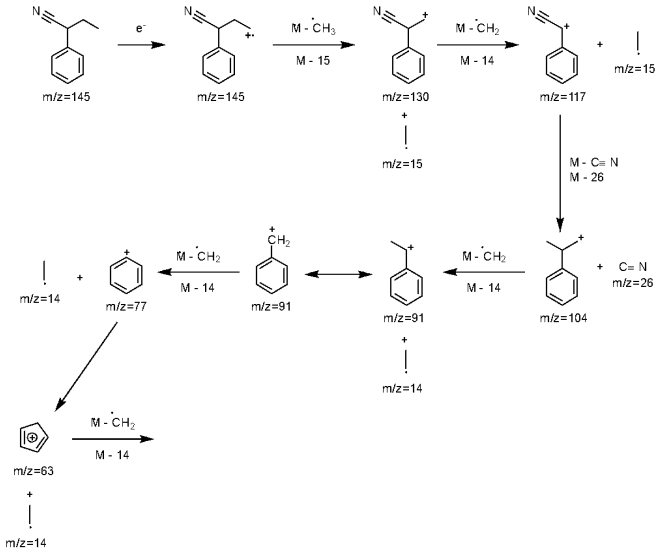
Scheme 3 shows the summary of the fragmentation pattern of (3E)-5-phenyl-3-pentenyl benzene from *A.gumifera* extract (AGEthyl). Prominent Peaks were observed at m/z = 222, 194, 181, 168, 144, 131, 130, 117, 104, 91, 77, 65, 53, 39 & 27. At the m/z = 222 the radical cation was formed. The loss of the methylbenzyl radical (m-*C_7H_7) and methylene molecule (m-C_2H_2) account for the



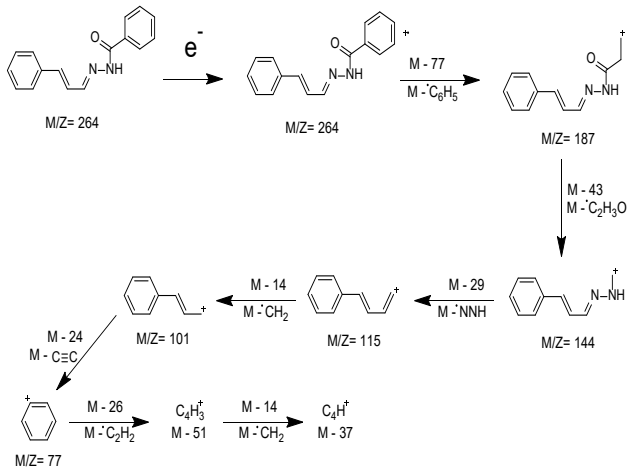
Scheme 3: Fragmentation pattern of 3E-5-Phenyl-3-petenylbenzene from ethyl acetate extract of *A. gumifera* (AGEthyl)

prominent peak at m/z = 131 and m/z = 105. The loss of methylene (m-CH_2) account for the final peaks at m/z = 91, m/z = 71m/z = 63 and m/z = 39, respectively. Scheme 4 shows the summary of the fragmentation pattern of 3- Phenyl butynitrile from *A.gumifera* dichloromethane (AGDCM) extract. Peaks were observed at m/z = 145, 130,117, 104, 91, 77, 63 and 14 respectively. The prominent peaks were obtained at m/z = 145, radical ion was formed at the same peak. The loss of neutral molecules (m-CH_3), (m-C≡N) and (m-CH_2) account for the observable peaks at m/z = 117, 104 and m/z = 91, respectively. The molecule obtained at m/z = 91 was stabilized through the formation of resonance. The final loss of (m-CH_2) molecules account for the peaks at m/z = 77 and m/z = 63, respectively.

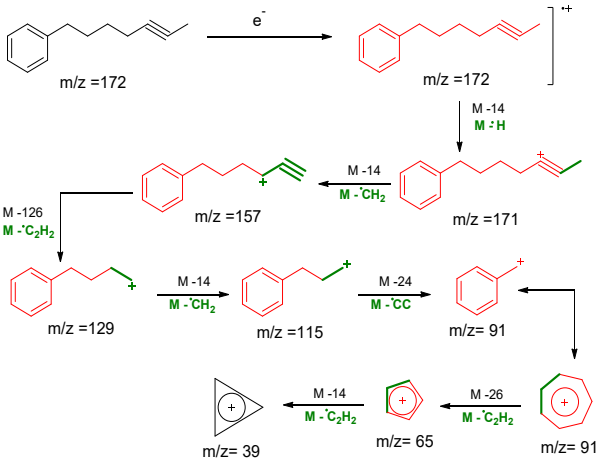
Scheme 5 shows the summary of the fragmentation pattern of (3-phenyl-2- propenyl alpha toluyl hydrazone) from *A. gumifera* ethylacetate extract (AGEthyl). Prominent peaks were observed at m/z = 264, 187, 145, 115, 101, 77, 51 & 37, respectively. The radical cation was formed at the m/z = 264. The subsequent loss of the fragment's ions of the methyl benzyl radical ion (m-*C_7H_7) account for the peak at m/z = 187, the final loss of ethoxide ion (m-*C_2H_3O) and secondary amine molecule (m-NNH) account for the peak at m/z = 144 and m/z = 115 respectively. The m/z = 115 is the base peak of the reaction. The final loss of the fragment's ions ethylene molecule and the methyl radical ion account for the final peak at m/z = 77, m/z = 61, and m/z = 37, respectively. Scheme 6 shows the summary of the fragmentation pattern of (6-heptenylbenzene) from *A. gumifera* ethylacetate extract (AGEthyl). Prominent peaks were observed at m/z = 172, 171,157, 129, 115, 91, 66 and 39. The radical cation was formed at m/z = 172. The loss of hydrogen radical ion (m-•H), methyl radical ion (m-•CH_3) account for the fragment ions at m/z = 171 and m/z = 157. A resonance was formed at the m/z = 91. The molecules formed was stabilized through resonance and it lead to the formation of the trophylium ion.The subsequent loss of the ethylene molecule (m-C_2H_2), methylene and ethylene molecule (m-C = C) account for the peaks at m/z = 157, m/z = 129 and m/z = 155. The loss of (m-C = C) and (m-C_2H_2) account for the various peaks at m/z = 91, m/z = 65 and m/z = 39, respectively. Scheme 7 shows the summary of the fragmentation pattern of (6-ethyloct-3-ylethylhexyester) from *A. gumifera* ethyl acetate extract. Prominent peaks were observed at m/z = 347, 276, 249, 149, 132, 115, 91, 71, 66, 41 and 27. The



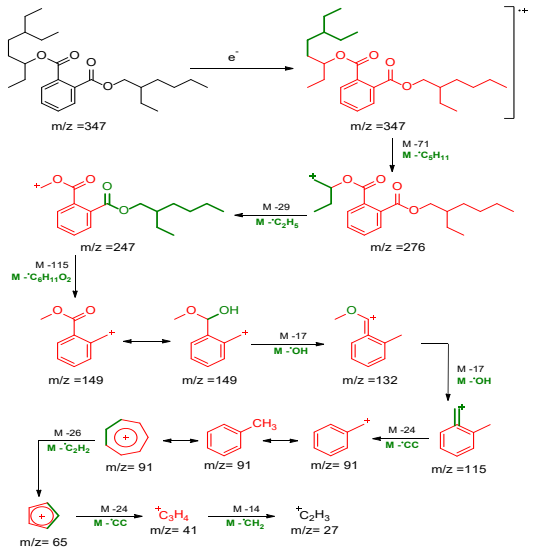
Scheme 4: Fragmentation pattern of 3-Phenyl butyl nitrile from dichloromethane extract of *A. gumifera* (AGDCM)



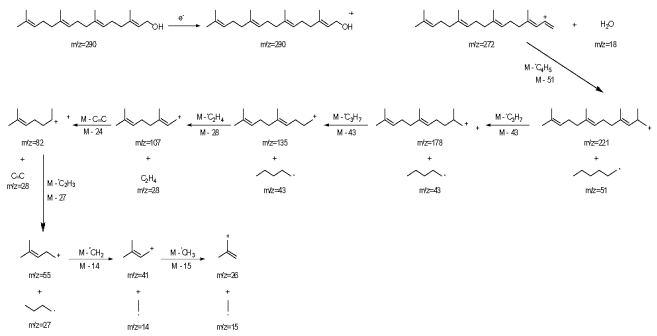
Scheme 5: Fragmentation pattern of 3- phenylpropenal alpha toluyl hydrazone from ethylacetate extract of *A. gumifera* (AGEthyl)



Scheme 6: Fragmentation pattern of 6-hepteylbenzene from dichloromethane extract of *A. gumifera* (AGDCM)



Scheme 7: Fragmentation pattern of 6-ethyl octyl-3-yl-2-methyl propyl ester from ethylacetate extract of *A. gumifera* (AGEthyl)



Scheme 8: Fragmentation pattern of Hexadeca 2,6,10,14,18 tetra-en-1-ol from ethylacetate extract of *A. gumifera* (AGEthyl)

radical cation was formed at m/z = 347. The loss of pentyl radical ion (m– $*C_5H_{11}$) and ethyl radical ion (m-$*C_2H_5$) account for the peaks at m/z = 276 and m/z = 247. The loss of fragment ions (m–$*C_5H_{11}O_2$) accounts for the m/z = 149 which is the base peak of the reaction. The stabilization of the molecule by the resonance leads to the keto-enol tautomerism. The subsequent loss of the hydroxyl radical ion (m-*OH) and ethylene molecule (m– C_2H_2) account for the peaks at m/z = 115 and m/z = 91. At m/z = 91 radical cation to form the most stable ion (trophylium ion). The loss of fragment ions of ethylene molecule (m-C_2H_2) and methyl radical ion (m -$^{\bullet}CH_2$) account for the final peaks at m/z = 65, m/z = 41 and m/z = 27 respectively. Scheme 8 shows the summary of the fragmentation of pattern of (Hexadeca 2, 6, 10, 14 tetraen-1-ol) from *A. gumifera* ethyl acetate extract. Prominent peaks were observed at m/z = 290, 272, 221, 178, 135, 107, 82, 55, 41 and 25. The radical cation ion as formed at m/z = 290 after the irradiation from the electromagnetic spectrum source. Further loss of (m-C_4H_5), (m-H_2O), (m-$C_3H_7)_2$ account for the peaks at m/z = 272, 221, 178 and 135, respectively. The final loss of (m-C_2H_4), (m-C≡C-H]), (m-CH_2) and (m-CH_3) account for final peaks at m/z = 105, m/z = 82, m/z = 55, m/z = 41 and m/z = 25 respectively.

DISCUSSION

A. gumifera, an important plant widely used in African ethnomedicine [33], were analyzed for the presence of different phytochemical compositions. The Akamba people prepare the herbal medicine from *A.gumifera* using alcohol and water, both polar solvent.[34] In the current study, both polar and non-polar solvent were used in the extraction to ensure the extraction of the non-polar metabolite[35] for a more complete phytochemical analysis of the plant. *A. gumifera* ethanol extract result revealed the high recovery and percentage yield of 42.54 g and 21.22%, while *Albizia gumifera* hexane extract revealed the least percentage yield of 3.87 g and 1.92%, respectively, which is in congruent with the findings of Troung *et al.*[36] Our findings in this study revealed that the efficiency of the extraction method depends solely on the following factors; solvent used for the extraction, temperature and the solubility of the metabolite.[37] To unravel the medicinal potential of *A. gumifera* the results of the phytochemical screening have revealed that the different bio-active were extracted from *A. gumifera* using the different solvent polarity and are responsible for their unique medicinal potential. This result showed that the crude extract of *A. gumifera* contain all the screen phytocompound, which is congruent with the findings of Oluruntola *et al.*[38] However, the higher extraction yield was observed in ethanol extract of A. gumifera with 42.54 g and 21.22% and compared with the percentage yield from *A. gumifera* hexane extract of 3.89 g and 1.92%, respectively. is at variance with the findings of Troung *et al.*[39] Ethylacetate extract of *A. gumifera* despite non-polar solvents had 12.45 g and 6.23%. The result of the phytochemical screening revealed a unique bio-active compound with a unique medicinal potential. Agu and Thomas[40] have reported that alkaloid have a defense mechanism through which plants have strong effects on all pests.. Alkaloids are also reported to be used in the treatment of cardiovascular and in kidney diseases.[41] Other medicinal alkaloid includes; malaria, cancer,[42] and antihyperglycemic activity.[43] Saponin present in the leaf extract of *A gumifera* indicate that the plant can be used to prepare cough syrup.[44] Flavonoid have been reported to possess antioxidant, antigenicity, antitumor and antidiarrheal activity.[45,46] Phenol have been reported to possess antioxidant properties due to large number of hydroxyl groups.[47] The gas chromatography-mass spectroscopic of the bio-active compound of *A. gumifera* revealed the different bio-active of unique medicinal potential. The ethyl acetate extract of *A. gumifera* despite being the least polar solvent extract, revealed the presence of the following bio-active compounds; 2-phenyl propenal toluyl hydrazone, 6- heptenyl benzene, 3E-5-phenyl-pentenylbenzene, n-butoxy carbonyl propyl ester, Bis -6-Methylheptylphthalate, 6-ethyloctyl-3-yl, 2-ethyl indane, 2-methyl ester, 2-phenylbutynitrile. The choice of ethylacetate solvent extracts were based on the fact that ethyl acetate is the least polar extracts in comparison to ethanol and water which are common universal solvent used by the local in the preparation of the recipe. *A. gumifera* ethyl acetate the results had shown that ethylacetate is a suitable extraction solvent as most of the metabolites are not only soluble in the polar solvent but also non-polar solvent.

Hence, the choice of the GC-MS over other spectroscopic techniques in the analysis of the bio-active compounds, were based on the nature of the expected phytocompound of the analysis

Table 4: Summary of identified bioactive compounds from hexane extract of *A. gumifera* [AGH]

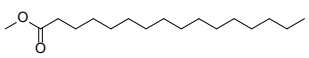
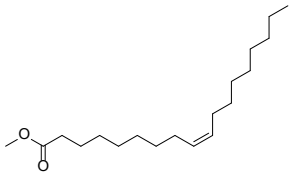
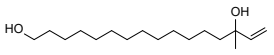
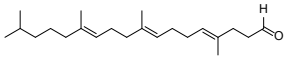
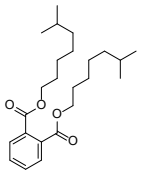
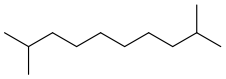
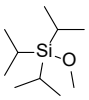
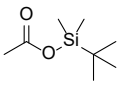
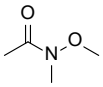
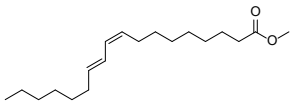
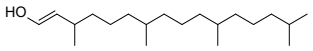
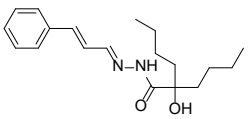
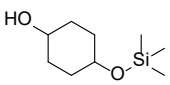
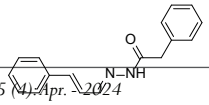
S/N	*R_t (minutes)*	*IUPAC name*	*MW ($gmol^{-1}$)*	*MF*	*Chemical structure*	*Biological activity*
1.	3.67	1,3,5 cyclo-heptatriene	92	C_7H_8		Anti-septic [54]
2.	33.47	N-Hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$		Anti-oxidant[55] Anti-microbial[56]
3.	37.01	Methyl cis-9- octadecenoate	296	$C_{19}H_{36}O_2$		Anti-microbial [56]
4.	37.18	13- Methyl Penta di-14-ene-1- 1,13 diol	256	$C_{16}H_{32}O_2$		Anti-oxidant [55] Anti-microbial [56]
5.	41.34	Docosa 2,6,10, 14 ,18 Penta-22-al	384	$C_{27}H_{44}O$		Anti-oxidant[55]
6.	45.71	Di-iso octyl Phthalate	390	$C_{24}H_{38}O_4$		Anti-malarial[57]
7.	12.05	2,9 dimethyl decane	170	$C_{12}H_{26}$		Anti-emulsifier[58] Anesthetic[59]
8.	14.94	Tri-isopropyl methoxy silane	188	$C_{10}H_{24}OSi$		Anti-coagulant[60]
9.	16.89	Tert-Butyl dimethyl silyl acetate	174	$C_8H_{18}O_2Si$		Anti-microbial[56]
10.	18.79	N-Methoxy N-Methyl acetamide	103	$C_{19}H_{34}O_2$		Anti-malarial[62]
11.	36.92	Methyl-9-Cis 11 trans Octa decadio enoate	294	$C_{19}H_{34}O_2$		Anti-malarial[62] Anti-microbial[56]
12.	37.19	2- Hexadecen-1-ol 3,7,11,15 tetra methyl	296	$C_{20}H_{34}O_2$		Antioxidant[55] Anti-septic[54]

Table 5: Summary of bio-active compounds identified from ethyl acetate extract of *A. gumifera* [AGEthyl]

12.	36.27	2- butyl-2- hydroxy N2-(3-Phenylpropenylidino) Hexanohydrazide	316	$C_{19}H_{20}$ OSi		Anti-malarial[62] Anti-microbial[56]
13..	32.14	Trans-1,4 Cyclo hexane diol trimethylsilyl ether	188	$C_{17}H_{16}N_2O$		Anti-microbial[56]
14.	40.44	3-Phenyl propenal alpha-toluyl hydrazone	264	$C_{17}H_{16}N_2O$		Anti-malarial[62] Anti-microbial[56]

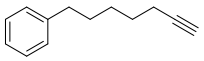
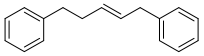
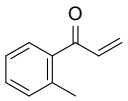
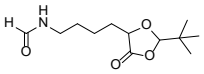
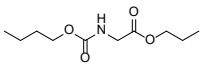
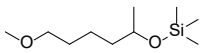
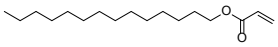
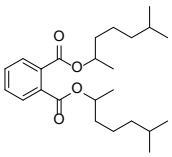
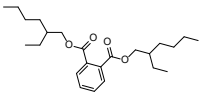
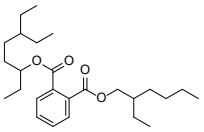
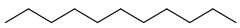
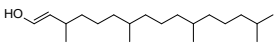
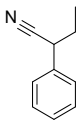
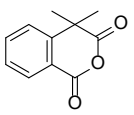
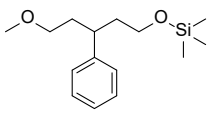
15.	40.44	6- Heptenyl benzene	172	$C_{13}H_{16}$		Anti-emulsifiers [58] Anesthetic[59]
16.	40.44	(3E)-5- Phenyl-3-) Pentenyl benzene	222	$C_{17}H_{18}$		Anesthetic[59]
17.	40.44	1-O-Toluyl pro-2-en-1- one	146	$C_{10}H_{10}O$		Anti-inflammatory[64]
18.	44.04	4-(2-tert butyl-5-oxo-1,3 dioxan-4-yl) butyl formamide	243	$C_{12}H_{21}NO_4$		Anti-malarial[62]
19.	44.04	N-butoxy carbonyl Propyl ester	217	$C_{10}H_{19}NO_4$		Anti-malarial[62] Anti-microbial[56]
20.	44.48	1-Methoxy-5-Trimethyl silyloxy hexane	204	$C_{10}H_{24}O_2Si$		Anesthetic[62]
21.	40.58	2-Propenoic tetra decyl ester	268	$C_{17}H_{32}O_2$		Antioxidant[55] Antimalarial[62]
22.	45.72	Bis(6-Methylheptyl) Phthalate	390	$C_{24}H_{38}O_4$		Antimalarial[62]
23..	45.72	Bis (2-ethyl hexyl) 1,2 benzene dicarboxylate	390	$C_{24}H_{38}O_4$		Antimicrobial[56]
24.	45.72	6-EthylOctyl-3-yl-2- Methyl ester	418	$C_{26}H_{42}O_4$		Antimalarial[62] Antimicrobial[56]
25.	45.71	Di (6-Methyl hept-2-yl) Ester	390	$C_{24}H_{38}O_4$		Antimalarial [62] Antimicrobial[56]

Table 6: Summary of identified bio-active compound from dichloromethane extract of *A. gummifera* [AGDCM]

26.	12.04	3,6 dimethyl decane	184	$C_{13}H_{28}$		Anesthetic [59]
27.	12.04	Hende decane	156	$C_{11}H_{24}$		Anesthetic [59]
28..	14.94	2-Phenylbutynitrile	142	$C_{10}H_{11}N$		Antimalarial [62]
29.	14.14	Benz[c] Pyran 1,3 dione	110	$C_{11}H_{10}O_3$		Antimalarial [62]
30.	14.94	Ethyl [5- Methoxy-3-Phenyl Pentyl] Oxy dimethyl Silane	280	$C_{16}H_{28}O_2Si$		Antimalarial [62]

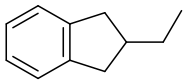
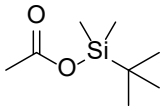
31.	14.94	2-Ethyl indane	146	$C_{11}H_{14}$		Anasthetic [59]
32.	16.91	Tertbutyl dimethyl Silyl acetate	174	$C_8H_{18}O_2Si$		Antimalarial [62]

Table 7: Summary of identified bio-active compound from ethanol extract of *A. gummifera* [AGE]

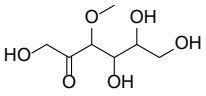
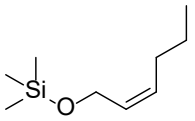
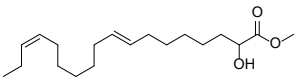
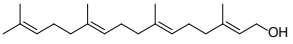
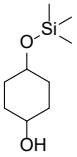
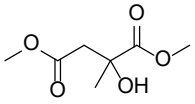
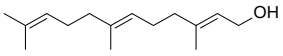
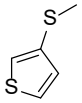
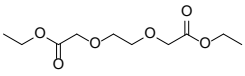
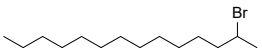
33.	37.19	5- Methyl-2-Isopropyl Cyclohexan -1-ol	156	$C_{10}H_{20}O$		Antioxidant [55] Antimalarial [62] Antimicrobial [56]
34.	44.04	4-(Trimethyl Silyl) Oxy Cis cyclohexanol	188	$C_9H_{20}OSi$		Antioxidant [55] Antimalarial [62] Antimicrobial [56]
35.	44.49	2- hydroxy-2- Methyl butane dioic	176	$C_7H_{12}O_5$		Antimicrobial [56] Antioxidant [55]
36.	29.04	3-0-Methyl hex-2- ulose	194	$C_7H_{14}O_6$		Antioxidant [55] Antimicrobial [56]
37	36.27	Cis-2- hexen-1-ol trimethyl silyl ether	172	$C_9H_{20}Osi$		Antimalarial [62]
38.	37.05	Methyl-2-Hydroxy Octa deca9,12,15 Tri- enoate	308	$C_{19}H_{32}O_3$		Antimalarial [62] Antiinflammatory [64]

Table 8: Summary of identified bio-active compounds from water extract of *A. gummifera* [AGW]

39.	41.33	(2E,6E) 3,7,11 Tri methyl 2,6,10 dodecane triene -1-ol	222	$C_{15}H_{26}O$		Antioxidant [55] Antimalarial [62]
40.	44.04	3- Methylthio Thiopene	130	$C_5H_6S_2$		Antimalarial [62]
41.	44.48	Diethyl ,2, 2 ethane 1,2 diyl bis (Oxy) diacetate	234	$C_{10}H_{10}O_6$		Antimalarial [62]
42	47.08	2-Bromotetra decane	276	$C_{14}H_{29}Br$		Anasthetic [59]
43.	47.08	4-Hexadecylcyclohexy) -3-nitrobenzene sulphonyl chloride	445	$C_{22}H_{36}FNO_5S$		Antimalarial [62]
44.	49.83	3,5 difluoro-phenyl tetra decyl ester	398	$C_{22}H_{32}F_2O_4$		Antimalarial [62] Antiinflammatory [64]

such as the long chain hydrocarbons, fatty acids, esters, alkanoic, alkanoates, essential oils which are based on reported literatures. Gas chromatography mass spectroscopy is a suitable spectroscopic tool for the analysis.[48] On the other hand, the nature of the Gas chromatography-mass spectroscopic techniques such as the capillary column which is a good separation precision and the spectral database which could be used in analysing the chemical information of the bio-actives by comparing the fingerprints with that of the information on the database of the GC-MS. Hexadecanoic acid acts as an antioxidant, pesticide, hamolytic and alpha reductase.[49] Octadecanoic acid have been reported to possess antimicrobial properties.[50] Alkanes act by interfering with the cell membrane.[50] Fatty acid like n- hydrocarbon is hexacosane. Hexacosane have previously been reported to possess antimicrobial activity.[51] Tetra octadecanoic acid has antifungal and antibacterial properties.[52] Phenols act as antibacterial agents.[53] These findings validate the use of this plant in traditional medicine. However, it has laid a foundation for further synthesis of the plant metabolite in pharmaceutical and nutraceutical products.

CONCLUSION

It can be concluded that the identified bio-active compounds from the ethyl acetate extract of *A. gumifera coupled* with the unique fragmentation pattern involving keto-enol tautomerism, stabilization through resonance and free radical have validated the claim of the plant *A. gumifera* in traditional medicinal therapy and as well it can be utilized as the bedrock for the synthesis of novel drug lead compound and as a neutraceutical products.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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