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Research Paper

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Phytochemical Components of the DCM Fraction of the Methanol Stem Extract of *Pycnanthus Angolensis.*

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Abstract
Background: *Pcynanthus angolensis is a widely used medicinal plant belonging to the family Myristicaceace and it is commonly known as African nutmeg or false nutmeg and is native to the forest zones of West Central Africa. it is used extensively in traditional medicine to treat various ailments including cognitive impairment, inflammation, microbial infection, diabetes, wounds and as an antidote to poisoning.*
Objectives: *This study was undertaken to identify the phytochemical components of DCM fraction of the stem bark of the plant using Gas Chromatography-Mass Spectrometry.*
Materials and Methods: *The chemical constituents of the dichloromethane (DCM) fraction of the stem bark of was investigated using Gas chromatography-mass spectrometry (GC-MS). Analysis of mass spectrum GC-MS was conducted using the database of a reference library, that of National Institute Standard and Technique (NIST) which has more than 62,000 patterns.*
Results: *The result of the GC-MS analysis of the DCM fraction of Pycnanthus angolensis stem bark extract show 13 expected compounds. These include 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester and Hexadecanoic acid, methyl ester.*
Conclusion*: The study suggests that Pcynanthus angolensis contains pharmacologically important bioactive compounds. The presence of these bioactive compounds justifies the use of the plant for various ailments traditionally.*
Keywords: *Pcynanthus angolensis, dichloromethane, Gas chromatography-mass spectrometry.*

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I. Introduction

The discovery of several important medicinal drugs in current use and of many more potential drugs is associated with the studies of traditional medicine (Phyllistin and James, 2000). The pharmaceutical industry has over time used the traditional uses of plants as one of the most successful criteria in finding new therapeutic agents for the various fields of biomedicine (Wiart, 2006). Traditional uses of plants have led to the development of some very important medicinal drugs and these include: vinblastine and vincristine from *Catharanthus roseus* (the periwinkle) used for treating acute lymphoma, acute leukaemias etc. reserpine from *Rauwolfia serpentina* (Indian snake root) used for treating hypertension; aspirin from *Salix purpurea* (willow) used for treating inflammation, pain and thrombosis and quinine from *Cinchona pubescens* (cinchona) used for treating malaria (Sofidiya and Awolesi, 2015).

Traditional medicine is a very useful testing ground for the efficacy and application of herbs used in the treatment and prevention of various diseases of man and animals. It is also a veritable source for the development of new plant derived drugs. Several parts of plants such as leaves stem fruits root flower and seeds are being used in the management and prevention of diseases (Achel *et al.,* 2012). Plants are also useful to man as source of food and raw material for industries.

Though plants are not adequately documented they are still a veritable source of pharmaceuticals and therapeutics agents. Various plant families of angiosperms, gymnosperms, pteridophytes, bryophytes and thallophytes are a rich source of medicinal plants.

Pycnanthus angolensis (Welw) Warb., Myristicaceae, belongs to the order Magnonliflorae and is popularly known as 'African nutmeg' or 'false nutmeg'. Magnonliflorae is a super-order which is made up of 19 genera and 380 species of trees and shrubs. The natural habitant of the trees is lowland rain forest which are located in Asia (four genera), Africa and Madagascar (nine genera) and America (six genera). Some of the characteristics of the Myristicaceae include straight trunks, blood-like sap exudates, few leaves and nutmeg-like fruits. Myristicaceae is among the most primitive of the Angiosperms with simple and alternate stipules, and dark green leathery leaves with tiny male or female petal-less flowers on different trees (Mohammed and Imad, 2013). The plant is widely used in ethnomedicine in treating hyperglycaemia, sterility in women, as an antimicrobia agent, analgesic, anthelmintic, antidote for poisoning, anti-bleeding agent, anti-inflammatory and as a pain soothing agent (Kareem *et al.,* 2015).

The major chemical constituents of *Pycnanthus angolensis* include; Fatty acids, Steroids, Cerobrosides (Pycnangloside), Allantoin, Lignans, Plastiquinones and Ubiquinones, Glyceryl-1,3-ditetradecanoate, Terpenes and Sesquiterepenes (Imad and Muhanned, 2014c).

II. Materials and Methods

2.1. Collection and identification of Plant material

Fresh stem bark of *Pycnanthus angolensis* collected from the Opa area of of Ile-Ife. The specimen was identified by the IFE Herbarium curator: Mr. Ibhanesebhor Gabriel. The specimen was deposited at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, with the voucher number: 17635.

2.2. Extraction procedure

After collection the stem bark was further cut into smaller pieces and dried in a shade at room temperature for three weeks. The air dried material (2.3k g) was grinded into powder using a milling machine and extracted thrice by maceration with 80% methanol at room temperature for 48 hr. The combined methanol extract was filtered with double-layered muslin cloth and concentrated on a water bath at 40◦C to yield a blackish-brown solid (2.8%, w/w). The extract thus obtained was further fractionated using N- hexane, Dichloromethane (DCM) and ethyl acetate. The DCM fraction was subsequently subjected to the GC-MS analysis.

2.3. GC-MS analysis

The GC-MS analysis of the DCM fraction of the stem bark extract of *Pcynanthus angolensis* was carried out using Agilent Technologies 7890A GC system, with fused capillary silica tubing column (Polysiloxanes) 30m × 0.25m. 1 µl of the DCM extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min (Hamza *et al.*, 2015; Yanping *et al.*, 2010), the sample was injected in split less mode. The carrier gas was Helium at a flow rate of 1ml/min. Oven temperature was programmed from 80°C with an increment of 10°C/min to 240°C and this temperature was held for 6 minutes, the injector temperature was 250°C. The mass spectrometric detector (MSD) was Agilent Technologies 5975C with Triple-Axis Detector-Software adopted to handle mass spectra. The ionization voltage was 70 eV and mass spectral scan range was set at 45-500(MHZ).

2.4. Detection of components

Analysis of mass spectrum GC-MS was conducted using the database of a reference library, that of National Institute Standard and Technique (NIST) which has more than 62,000 patterns. The fragmentation pattern of the mass spectrum of the unknown component was compared (head to tail) with those stored in the NIST Library. This way the name, molecular weight, molecular formula, structure of the components in the DCM fraction of the extract were ascertained (Ingole *et al.,* 2016; Akpuaka *et al.*, 2013).

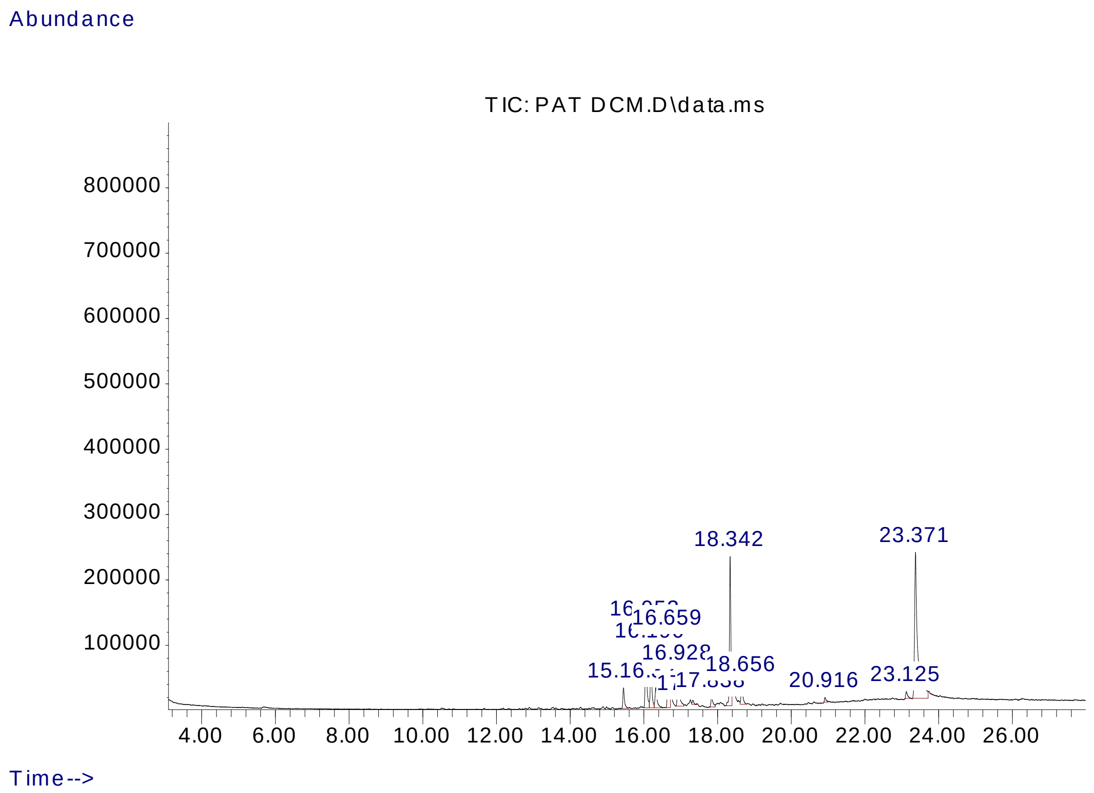

Figure 1: GC-MS Chromatogram of the DCM fraction of the stem bark extract of *Pcynanthus angolensi*

Table1: Phytocomponents identified in the DCM fraction of the stem bark extract of *Pcynanthus angolensis*

PK No	RT (min)	Compound	Mol. Formula	Mol. Mass	Area %	Nist Matching (%)
1	15.446	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	2.67	90
2	16.053	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	C16H22O4	278	10.10	90
3	16.190	Hexadecanoic acid, methyl ester	C17H34O2	270	7.18	99
4	16.322	Phthalic acid, isobutyl nonyl ester	C28H46O4 446		3.35	90
5	16.928	Dibutyl phthalate	C16H22O4	278	5.70	90
6	17.838	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	C20H30O4		1.65	86
7	18.342	9-Octadecenoic acid, methyl ester	C19H36O2	296.49	17.57	99
8	18.656	Methyl stearate	C19H38O2		3.15	98
9	20.916	Eicosanoic acid, methyl ester	C21H42O2	326.549	0.61	38
10	23.125	Docosanoic acid, methyl ester	C23H46O2	354.619	0.99	46
11	23.371	Bis(2-ethylhexyl) phthalate	C24H38O4	390.23	37.21	90

Table 2: Reported biological activities of the identified phytocomponents in the DCM fraction of the stem bark extract of *Pcynanthus angolensis*

S/No	Compound Name	Biological Activity
1	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Antifungal, Antibacterial, Antiviral and Antioxidant activities (Hema *et al.*, 2011).
2	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	Antimicrobial and Antifouling (Idan *et al.*, 2015).
3	Hexadecanoic acid, methyl ester	Anti-oxidant, hypocholesterolemic, antiandrogenic, hemolytic-5-α reductase inhibitor, anti-inflammatory (Elija *et al.*, 2012; Syeda *et al.*, 2011).
4	Phthalic acid, isobutyl nonyl ester	Preventing many diseases such as anti-hypertension, cholesterol (Balachandran *et al.*, 2012).
5	Dibutyl phthalate	Antimicrobial, antifungal, anti-malarial, antiviral and antioxidant activities (Bagavathi and Ramasamy, 2012).

6	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	Antifungal, anti retroviral, anti tumor,anti diabetic anti cancer,antioxtioxidant, anti scabies anti inflammatory, potent antimicrobial agent (Andréa *et al.*, 2012; Arcadi *et al.*, 1998; Sathyaprabha *et al.*, 2010).
7	9-Octadecenoic acid, methyl ester	Antioxidant activity, Anticarcinogenic,-exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligand, dermatitigenic flavor (Syeda *et al.*, 2011; Andréa *et al.*, 2012).
8	Methyl stearate	Anti-diarrheal. cytotoxic and antiproliferative activity (Cox and Balick, 2018).
9	Eicosanoic acid, methyl ester	Anti-inflammatory, anti-oxidant, antiarthritic, anti-coronary.
10	Docosanoic acid, methyl ester	NA
11	Bis(2-ethylhexyl) phthalate	Antifungal, Antibacterial, Anti tumor activities (Lad, 2006).

NA—No activity.

Figure 2: Mass spectrum of Bis (2-ethylhexyl) phthalate

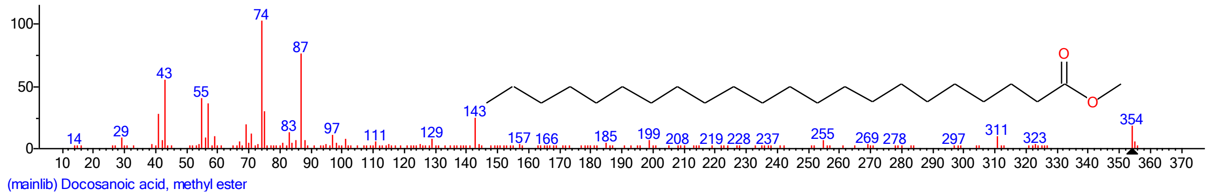

Figure 3: Mass spectrum of Docosanoic acid, methyl ester

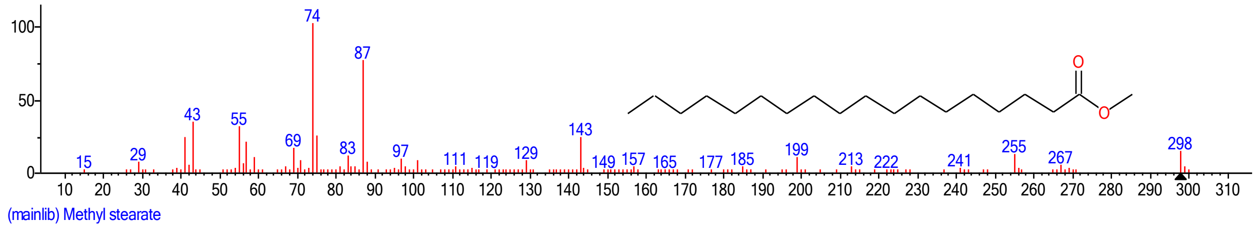

Figure 4: Mass spectrum of Methyl stearate

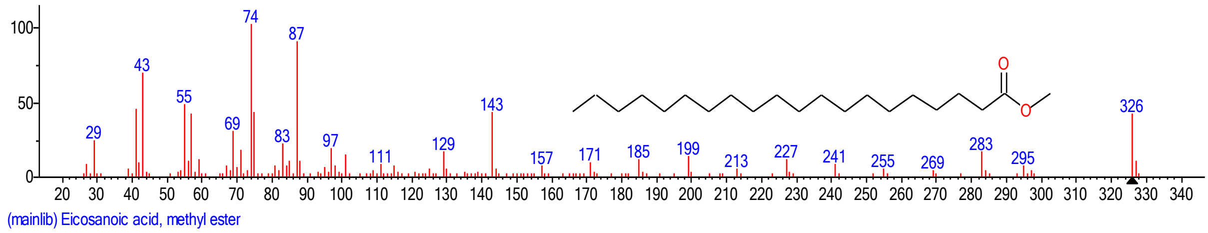

Figure 5: Mass spectrum of Eicosanoic acid methyl ester

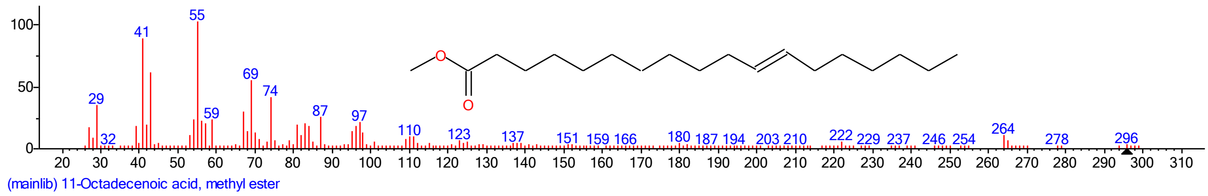

Figure 6: Mass spectrum of 11-Octadecenoic acid, methyl ester

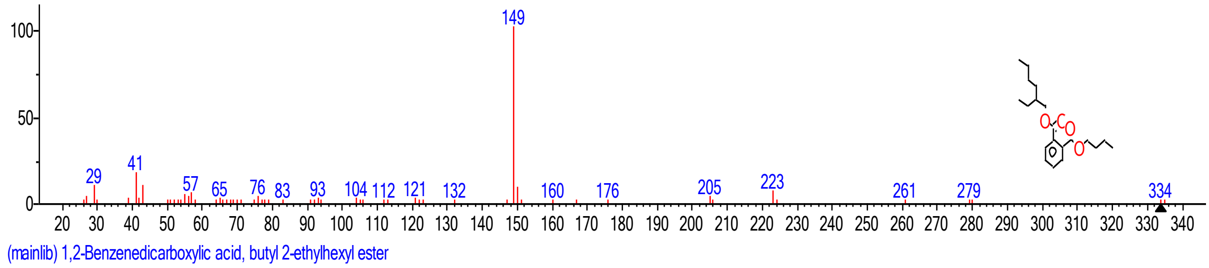

Figure 7: Mass spectrum of 1, 2-Bezenedicarboxylic acid, butyl 2-ethylhexyl ester

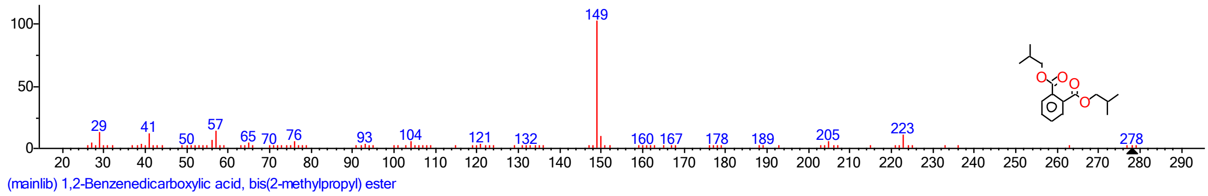

Figure 8: Mass spectrum of 1, 2-Bezenedicarboxylic acid, bis(2-methylpropyl) ester

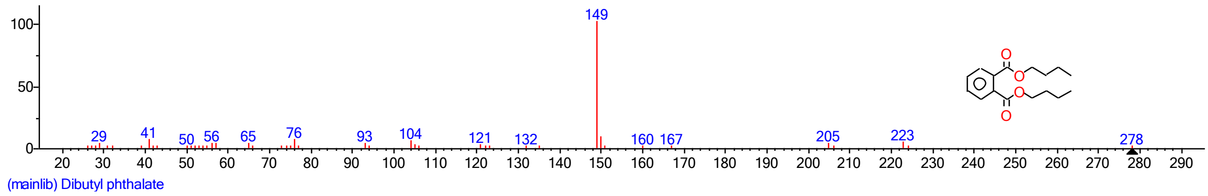

Figure 9: Mass spectrum of Dibutyl phthalate

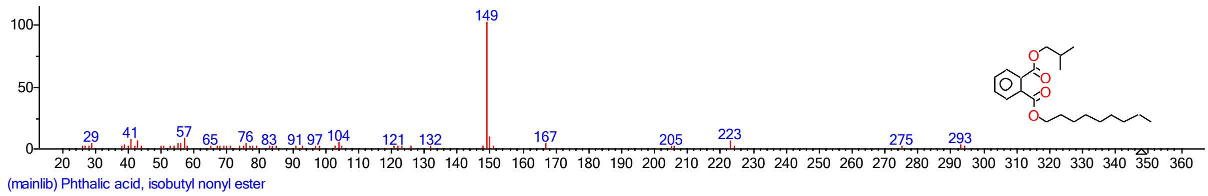

Figure 10: Mass spectrum of Phthalic acid, isobutyl nonyl ester

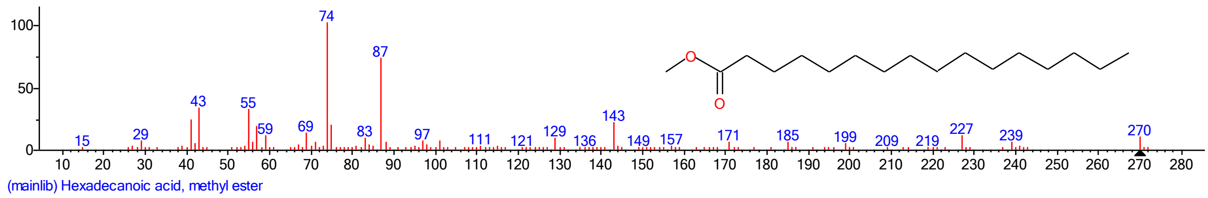

Figure 11: Mass spectrum of Hexadecanoic acid, methyl ester

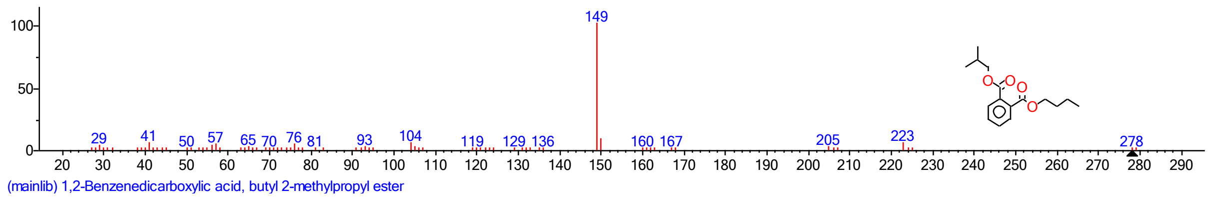

Figure 12: Mass spectrum of 1, 2-Bezenedicarboxylic acid, butyl 2-methylpropyl ester

III. Results and Discussion

GC-MS is used to analyze complex organic and biochemical mixtures (Skoog *et al.,* 2007) and it is a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), GC-MS analysis determines how many components and in what proportion they exist in a mixture. It also establishes the nature and chemical structure of these separated and quantified compounds.

Analysis of mass spectrum GC-MS was conducted using the database of a reference library, that of National Institute Standard and Technique (NIST), the fragmentation pattern of the mass spectrum of the unknown component was compared (head to tail) with those stored in the NIST Library (Ingole *et al.,* 2016; Akpuaka *et al.,* 2013).

The result of the GC-MS analysis of the DCM fraction of *Pycnanthus angolensis* stem bark extract show 13 expected compounds, the retention time, peak area, molecular formulae, molecular weight, and the biological activities of the identified components of the DCM fraction of the stem bark of *Pcynanthus angolensis* are shown in Table 1, and 2 above and they include 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester, Hexadecanoic acid, methyl ester, Phthalic acid, isobutyl nonyl ester, Dibutyl phthalate, 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester, 9-Octadecenoic acid, methyl ester, Methyl stearate, Eicosanoic acid methyl ester, Docosanoic acid, methyl ester, and Bis(2-ethylhexyl) phthalate.

The GC-MS Chromatogram of the DCM fraction of the stem bark extract of *Pcynanthus angolensis* is shown in figure 1 while the mass spectrum of each compound is as shown in figures. 2-12.

Some of the medicinal uses of the compounds found in the DCM fraction of the stem bark of the plant have been shown in Table 2. Studies have shown that compound like 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester, hexadecanoic acid methyl ester and Eicosanoic methyl ester exhibit both antioxidant and anti-inflammatory activities and protects cell membranes from free radicals (Elija *et al.,* 2012; Syeda *et al.,* 2011).

The literature of the biological activities of the components identified by the GC MS analysis suggests that the following compounds may be responsible for effects of the DCM fraction as seen in the present study; 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester, hexadecanoic acid methyl ester and Eicosanoic methyl ester. This is because they exhibit both antioxidant and anti-inflammatory activities, several other components have anti-oxidant activity. However as components of a plant extract usually act synergistically to produce their effects it may well be that the activity of the DCM fraction as seen in the present study could be due to a combination of effects of multiple components.

IV. Conclusion:

The formula and structure of the active molecules in the DCM of the plant have been produced by this study, also the biological activities of these active molecules corroborates the uses of the plant in traditional medicine.

References

[1]. Achel, D.G., Alcaraz, M., Kingsford-Adaboh, R., Nyarko, A.K. and Gomda, Y. (2012). A review of the medicinal properties and applications of Pycnanthus angolensis (welw) warb PharmacolofgyOnLine 2: 1-22
[2]. Akpuaka, A., Ekwenchi, M.M., Dashak, D.A. and Dildar, A. (2013) Biological Activities of Characterized Isolates of n-Hexane Extract of Azadirachta indica A. Juss (Neem) Leaves. New York Sci. J; 6(6):119-124.
[3]. Andréa M. do Nascimento, Raphael Conti, Izabel C. C. Turatti,Bruno C. avalcanti, Letícia V. Costa-Lotufo, Cláudia Pessoa, Manoel O. de Moraes, Viviane Manfrim, Juliano S. Toledo, Angela K. Cruz, Mônica T. Pupo. (2012) Bioactive extracts and chemical constituents of two endophytic strains of Fusarium oxysporum Brazilian Journal of Pharmacognosy. 22(6): 1276-128.
[4]. Arcadi, F. A., Costa, C., Imperatore, C., Marchese, A., Rapisarda, A., Salemi, M., Trimarchi, G. R, and Costa, G. (1998). Oral toxicity of bis (2-ethylhexyl) phthalate during pregnancy and suckling in the Long-Evans rat. Food Chem. Toxicology 36: 963–970.
[5]. Bagavathi, P.E., and Ramasamy, N. (2012). GCMS analysis of phytocomponents in the ethanol extract of Polygonum chinense L. Pharmacognosy Research. l 4: 11-14.
[6]. Balachandran, C., Lakshmi, R.S., Duraipandiyan, V. and Ignacimuthu, S. (2012). Antimicrobial activity of Streptomyces sp. (ERI-CPDA-1) isolated from oil contaminated soil from Chennai, India. Bioresource Technology, 129(35): 14-21
[7]. Cox P.A. and Balick MJ. (1994). The ethnobotanical approach to drug discovery. Sci. Am. 270 (6):82-87.
[8]. Cox P.A. and Balick MJ. (2018) Current outlook and future promise of ethnobotany in Nigeria: A review and personal observation. African j of Plant Sci. 12(4): 73-80.
[9]. Elija, K., Vaishali, B., Adsul, M.K., Deshpande, N.R. and Kashalkar, R.V. (2012). Antibacterial activity of Dibutyl Phthalate : A secondary metaboli isolated from Ipomoea carnea stem. Journal of Pharmacy Research, 5(1): 76-82
[10]. Hema, R., Kumaravel, S. and Alagusundaram. (2011). GC/MS Determination of Bioactive components of Murraya koenigii. Journal of American Science, 7(1): 57-63
[11]. Hamza, L.F., Sabreen, A.K. and Imad, H.H. (2015). Determination of metabolites products by Penicillium expansum and evaluating antimicrobial activity. J. Pharmacogn. Phytother. 7(9):194-220
[12]. Imad, H., Muhanned, A., Aamera, J. and Cheah, Y. (2014c). Analysis of eleven Y-chromosomal STR markers in middle and south of Iraq. Afr. J. Biotechnol. 13(38):3860-3871
[13]. Kareem, M. A., Hameed, I. H., Hussein, H. J. and Hamad, N. S. (2015). Identification of five newly described bioactive chemical compounds in methanolic extract of Mentha viridis by using gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy 7 (7): 107-125.
[14]. Lad W. (2006). Ethnomedicine. West Indian Medical Journal 55 (4): 215–16.

[15]. Mohammed, A. and Imad, H. (2013). Autosomal STR: From locus information to next generation sequencing technology. Research Journal of Biotechnoloy. 8.10: 92-105.
[16]. Phyllistin, A.B. and James, F.B. (2000). Tips for preventing food poisoning “Herbs” American No.1 Guide Natural health, 3rd edn. Publ. Averge. No.9 : 383-386.
[17]. Skoog, D., Holler. F. and Crouch, S. (2007). Principles of Instrumental Analysis. 6th Edition. Brooks/Cole Cengage Learning
[18]. Sofidiya, M.O. and Awolesi, A.O. (2015). Antinociceptive and antiulcer activities of Pycnanthus
[19]. angolensis. Revista Brasileira de Farmacognosia 25: 252–257
[20]. Syeda, F.A, Habib-Ur- Rehman, Choudahry, M.I. and Atta-Ur-Rahman. (2011). Gas Chromatography-Mass Spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bioassays of crude extract of Iris germanica. International Journal of Genetics and Molecular Biology, 3(7): 95-100.
[21]. Yanping, H., Suhua, H., Jincheng, W.U. and Shunquan, L. (2010). Identification of essential oils from the leaves of 11 species of Eriobotrya. 4379-4382.
[22]. Wiart C. Family Myristicaceae. In: Medicinal plants of the Asia- Pacific: Drugs for the future?: Singapore: World Scientific Publishing Co. Pte. Ltd, 2006; 27-31.