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PHYTOCHEMICAL SCREENING AND GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS OF METHANOLIC EXTRACT OF *Mucuna bracteata* SEEDS

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ABSTRACT

Plant and plant-based medicaments are the basis of many modern pharmaceuticals which are use for diverse ailments. *Mucuna bracteata* is a velvet beans plant, a tropical legume mostly found in Africa, tropical Asia and India which are widely naturalized and cultivated. It is a climbing shrub with long vines, its seed pod are covered in loose hairs. The plant is known for its medicinal properties. The aims of the study were to screen for phytochemicals and characterized the bioactive chemicals constituents of methanolic extract of *Mucuna bracteata* seedplant using Gas Chromatography-Mass Spectroscopy and Infared spectroscopy. The dried seeds of the plant were pulverized and extracted with methanol by using soxhlet extractor for 12 hours at constant temperature of 60^{o}C. The crude extracts were then concentrated using rotary evaporator. Column chromatography and thin layer chromatography were carried out on the crude extract and the fractions respectively. The preliminary phytochemical screening was performed on the crude extract and the result revealed the presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, anthraquinones and saponins. GC-MS and IR spectra data of the methanol fraction reveal the presence of many organic compounds which are mostly fatty acid, esters, alcohols, amino acid, alkanes, and amines. The compounds identified are: hexadecanoic acid, hexadecanoic acid methyl ester, 9-octadecanoic acid (oleic acid), tetradecanoic acid ethyl ester (myristic acid), hexadecanoic acid ethyl ester, dodecanoic acid ethyl ester, dodecane, octadecanoic acid (stearic acid), 9, 12, 15-octadecanoic acid, 9-octadecanoic acid methyl ester (recenolic acid methyl ester)

Keyword: *Mucuna bracteata;* Phytochemicals; GC-MS Analysis

INTRODUCTION

Mucuna bracteata is a velvet beans plant, a tropical legume mostly found in Africa, tropical Asia and India which are widely naturalized and cultivated (Wikipedia 2012). It is called cowitch or cowhage in English, kararah in Hausa, eroemnie in Doemak Qua'an Pan Local Government Area of Plateau state and kworenge in Tangale (Gombe state) Nigeria. Velvet beans plant is a climbing shrub with long vines that can reach 15 cm in length. The plant bears white lavender or purple flower, its seed pod are about 10 cm long and are covered in loose orange hairs that's cause severe itch when come in contact with both human and animal skin due to serotonin on its surface. For this reason, many villagers plant the shrub around their houses or farms to prevent snake or pests (humans, cow and goat).

The plant is almost completely covered with furry hairs when young, but it is almost free of hairs if matured (Lucia *et. al*, 2012). The seeds are shiny black or brown drift. The leaves are trip innate, ovate, reverse ovate or rhombus shape. All part of *Mucuna* plant possess medicinal properties (Lucia *et. al*, 2012). The main medicinal effect of *Mucuna bracteata* came from the seed but the pod and its hairs and other plant part can also be used in herbal preparation. *Mucuna bracteata* have many traditional uses these include: the seed are use as anti- venom for snake bite, and also use as antioxidants (Natarajan *et. al*, 2012) it increase libido in both men and women, prevent or lower the effect of poisonous snake bite. Use to treat Parkinson's diseases, infertility, depression and stress due to the L-dopa found in the seed. The hair on the *mucuna bracteata* pod are used to treat several species of parasitic worms (Lucia *et. al*, 2012)

MATERIALS AND METHODS

Sampling

The seeds of *Mucuna bracteata* were obtained from Mazza Mountain in Jos North Local Government Area Plateau State of Nigeria. It was identified by the Department of Horticulture, Federal College of Forestry Jos. The samples were cleaned and milled into powder using mortar and pestle which were then sieved with siever of 2 mm in size to get fine power sample and stored in a plastic container for the analysis. The smaller the particle size the lager the surface area and the more the extraction efficiency.

Extraction of Crude Sample

Soxhlet extractor was used to extract the crude sample. 100g of the sample was weighed using analytical weighing balance; the sample was tight in muslin cloth and placed in a thimble. The extraction was carried out with methanol as a solvent for 12 hours with constants temperature of 60^{o}C to obtained maximum extraction of the component. The extract obtained were concentrated using rotary evaporator, which were then dried on water bath at low temperature and stored in an air tight container (Azwanida, 2015)

Phytochemical Screening

The crude extracts were screened for the presence of phytochemicals: saponins, tannins, cardiac glycosides, anthraquinones, flavonoids, alkaloids, terpenes and steroids.

Test for Saponins

About 0.5g of the extract was dissolved in 0.5ml of distilled water in a test tube and shaking well. The formation of frothing which persisted on warming indicated the presence of saponins (wall *et. al*, 1954)

Test for Tannins (reduction test)

About 0.5g of the extracts was stirred with 10ml of distilled water and filtered. Then few drops of 1% ferric chloride solution were added to the filtrate. The formation of a blue-black or blue-green presence of tannins (Trease and Evans, 2002)

Test for Cardiac Glycoside (Keller Killiani test)

About 0.1g of the extracts was dissolved in 1.0ml of glacial acid containing one drop of ferric chloride solution. 1.0ml of concentrated sulphuric acid was added gently by the side of the test tube. A brown ring form at the interphase indicate the presence of deoxy sugar characteristic of cardianolides (Trease and Evans, 1989)

Test for Flovanoids

About 0.5g of the extract was completely detanned with acetone on a water bath. The

mixture was filtered while still hot, then cooled and used for the lead acetate test for flavonoid (segelman *et. al,* 1979) Lead acetate solution was added to 5ml of the detaned extract. A yellow colouration indicates the presence of flavonoid.

Test for Alkaloids

About 0.5g of the extract was stirred with 3ml of 1% aqueous hydrochloric acid on steam bath and filtered. 1ml of the filtrate was treated with few drops of mayer's reagent. The formation of turbidity or precipitate indicates the presence of alkaloids. (Trease and Evans, 1989)

Test for Terpenes (salkowski test)

About 1g of extract was dissolved in 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added. A reddish brown colour at the interface indicates the presence of terpenoids (Sofowora, 1982).

Test for anthraquinone

0.5g of the extract was dissolved and shaken in 10ml of benzene and then filtered. 5ml of 10% ammonia solution was added to the filtrate and then shaken. Appearance of pink red or violet colour in the ammonia lower phase indicates the presence of anthraquinone (Sofowara, 1993)

Column Chromatography Analysis

0.5g of crude extract was dissolved in a 2ml of hexane and methanol, respectively. The column was packed in n-hexane. 12g of the adsorbent (silica gel 60-120 mesh) was poured in 100 ml of the mobile phase and the slurry was used to pack the column ensuring no air bubbles was trapped while packing (Kwaji *et. al*, 2018) The column was allowed to settle evenly. The sample was applied on top of the column and allowed to run through the column. A suitable eluant flow was maintained through the column during the complete period of the separation by gravitational feed. The effluent was collected in small fractions of 10 ml each.

Thin Layer Chromatography Analysis

A silica gel pre-coated thin layer chromatographic plate (20x20 cm) was cut into slides of 4x10 cm. The plate was spotted with the fractions obtained from the column chromatography using capillary tube and allowed to dry. The plate was then transferred into the developing tank already saturated with vapour of the mobile phase with varying polarity as methanol:ethylacetate (1:2), n-hexane:methanol (2:2), methanol:acetone (3:1) and many more. The spotted TLC plates were run in the developing tank. The mobile phase was allowed to rise to about 3/4 of the plate, which was then removed and was marked. The plate was allowed to dry and the separated components were viewed under Ultraviolet light where their position was circled for the determination of their retention factor (Kwaji *et. al*, 2018)

Analysis of Fractions

The fractions obtained from the crude extract were pulled together according to their TLC profile and were analyzed by the IR and GC-MS spectroscopy at National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna state of Nigeria.

RESULTS

Phytochemicals

The result in table 1 below shows that, the seed of *Mucuna bracteata* contain saponin, alkaloids, tannins, flavonoids, cardiac glycoside and steroids however, anthraquinone and terpenes were absent.

Table 1: Phytochemical Constituent of Methanol Extract

Phytochemicals	Present study results	Reported results Renata *et.al*(2015)
Saponins	+	+
Alkaloids	+	-
Tannins	+	-
anthraquinones	-	
flavonoids	+	+
Cardiac glycoside	+	
Steroids	+	+
Terpenes	-	

Key: - = not detected, + = present

Fourier Transform – Infrared Spectroscopy Result

Table 2, 3 and 4 below shows the functional groups possibly present in the plant seed. The frequency of absorptions at around 858, 2935, 3877, indicates C-H bond from aromatics, alkyl and methyl or methylene respectively. Absorption at around 1518 and 1680 shows the present of C=C and C=O bond from aromatics or alkene or amide. A broad peak at around 3360 shows the present of O-H bond from alcohol or carboxylic functional group. N-H bond from amide or amines shows peak at around 3742.

Table 2: FT-IR Spectra data of Fraction 1 of Methanol Extract

Frequency of Absorption cm^{-1}	Bonds/Vibrations	Remarks
858.35	C-H bending	Aromatics
1055.10	S=O Stretching	Sulfones
1518.03	C=C Stretching	Aromatic
1666.55	C=C Stretching, C=O	Alkene, Amide
2935.76	C-H stretching	Alkyl
3360.11	O-H Stretching	Alcohol and Carboxylic acid
3742.03	N-H Stretching	Amide,Amines
3877.05	C-H stretching	Methyl, Methylenes

Table 3: FT-IR Spectra data of Fraction 3 of Methanol Extract

Frequency of Absorption cm^{-1}	Bonds/Vibrations	Remarks
864.42	C-H bending	Aromatics
1051.24	S=O Stretching	Sulfones
1518.03	C=C Stretching	Aromatic
1666.55	C=C Stretching	Alkene
2933.83	C-H Stretching	Alkyl
3358.18	O-H Stretching	Alcohols and Carboxylic
3878.98	C-H stretching	Methyl, Methylene

Table 4: FT-IR Spectra data of Fraction 2 of Methanol Extract

Frequency of Absorption cm^{-1}	Bonds/Vibrations	Remarks
1236.41	C-H Bending	Methyl, Methylenes
1521.89	C=C Stretching	Aromatic
1680.05	C=C Stretching, C=O Stretching	Akene, Amide
2922.25	C-H Stretching	Alkyl
3358.18	O-H Stretching	Alcohols, Carboxylic
3610.86	N-H Stretching	Amide, Amine
3880.91	C-H stretching	Methyl, Methylenes

GC-MS Result

The data base of NARICT which has more identified patterns was used for the interpretation of the mass spectrum. The fragmentation pattern spectra of the unknown compounds were compared with those of the known components stored in the NARICT library. The base peak, fragmentation pattern and molecular weight of each component were compared with the library, the one that matches the unknown spectra were used. And for those that's were not found in the library the fragmentation base on mass to charge ratio was calculated to ascertain the molecular name and the molecular formula. Hence the compounds below in table 5, 6 and 7 were obtained.

Table 5: Identified Compounds in Fraction 1 of Methanol Extract. (GC-MS Spectra)

M/Formular	Names of Compound	Reported Activity of the Compound
C_9H_{12}	1, 2, 4-trimethyl benzene	
$C_9H_{21}N$	2-propylhexamine	
$C_{18}H_{18}$	2-methylheptane	
$C_{10}H_{27}N$	1-aminooctene	
C_9H_{18}	Nonene	
$C_8H_{19}N$	2-ethyl-1-en-heptamine	
$C_7H_{17}N$	1-heptamine	
$C_7H_{15}NO$	Heptanamide	
$C_{16}H_{32}O_2$	Hexadecanoic acid	Antioxidant, Hypercholesterolemic, Lubricant, Pesticide, Hemolytic, 5-Alpha reductase inihibitor, flavor, Antiandrogenic. (Omotoso et al.,2014)
$C_{18}H_{34}O_2$	9-Octadecenoic acid (Oleic acid)	
$C_{18}H_{36}O_2$	Octadecanoic acid	Flavourant (Omotoso et al.,2014)
$C_{13}H_{18}N_2O_3$	9-Eicosanmide	

Table 6: Identified Compounds in Fraction 2 of Methanol Extract (GC-MS Spectra)

M/Formular	Names of Compound	Reported Activity of the Compound
C_9H_{20}	n-Nonane	
$C_{10}H_{22}$	n-Decane	
$C_{11}H_{24}$	n-Undecane	
$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)	Antifungal, Antioxidant, Pesticide Hypercholesterolemic, (Hema et al., 2011)
$C_{16}H_{32}O_2$	Hexadecanoic acid	Antioxidant, Hypercholesterolemic, Lubricant, Pesticide, Hemolytic, 5-Alpha reductase inihibitor, flavor, Antiandrogenic. (Omotoso et al.,2014)
$C_{19}H_{34}O_2$	9, 12-Octadecadienoic acid, methyl ester	Anti-imflamatory, Hypercholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Antihistamine, Antieczemic, Anti-acne, 5-Alpha reductase inihibitor, Antiandrogenic (Omotoso et al.,2014)
$C_{18}H_{36}O_2$	9-heptadecanoic acid, methyl ester	
$C_{17}H_{35}NO$	Heptadecanamide	
$C_{18}H_{34}O_2$	9-Octadecenoic acid (Oleic acid)	
$C_{18}H_{36}O_2$	Octadecanoic acid	Flavourant (Omotoso et al.,2014)
C_4H_6	2-Butyl-en	
$C_{19}H_{38}O_4$	Hexadecanoic acid, 2, 3-dihydroxylpropyl ester	
$C_{19}H_{36}O_3$	12-hydroxyl, 9 Octadecenoicacid, methyl ester (ricinoleic acid methyl ester)	

DISCUSSION

The research has shown that *Mucuna bracteata* seed plant contains various phytochemicals which are secondary metabolites that have the potential for treatment of some disease conditions. Phytochemical investigation revealed the presence of saponin, alkaloid, tannin; flavonoid, cardiac glycoside and steroid as shown in Table 1. This agrees with the observation of Renata *et. al,* (2015) who found steroid, saponin and flavonoid from plant of *Mucuna bracteata* grown in northern Brazil but there were no alkaloids and tannins. In another report by Manalisha and Chadra (2002) who evaluate the phytochemicals of *Mucuna bracteata* extract, observed the absence of saponin, weak presence of resin and presence of tannins, alkaloids, flavonoids, steroids, phenols and glycoside. This suggests that, phytochemicals of plant can vary depending on several factors such as climate, habitat, soil, and nutrient, time of harvest and physiological age of the plant. (Farnsworth & Soejarto, 1991) *Mucuna* plant is widely used as aphrodisiac and stimulant of testosterone biosynthesis. It may be due to high concentration of steroids in the seed, since steroid stimulates the production of anabolic androgenic hormone. Flavonoids

comprise a large group of polyphenolic compounds responsible for a variety of pharmacological activities related to anti-oxidants activity (Natarajan *et. al*, 2012).

Table 7: Identified Compounds in Fraction 3 of Methanol Extract (GC-MS Spectra)

M/Formular	Names of Compound	Activity of the Compound
$C_{17}H_{34}O_2$	Hexadecanoic acid methyl ester	Antioxidant, Hypercholesterolemic, Lubricant, Pesticide, Hemolytic, 5-Alpha reductase inihibitor, flavor, Antiandrogenic. (Omotoso et al. ,2014)
$C_{16}H_{32}O_2$	Hexadecanoic acid	Antioxidant, Hypercholesterolemic, Lubricant, Pesticide, Hemolytic, 5-Alpha reductase inihibitor, flavor, Antiandrogenic. (Omotoso et al., 2014)
$C_{19}H_{34}O_2$	9, 12-Octadecadienoic acid methyl ester (linoleic acid)	Anti-imflamatory, Hypercholesterolemic, cancer preventive, Hepatoprotective, Nematicide, Antihistamine, Antieczemic, Anti-acne, 5-Alpha reductase inihibitor, Antiandrogenic. (Omotoso et al., 2014)
$C_{18}H_{34}O_2$	9-Octadecenoic acid (Oleic acid)	
$C_{18}H_{36}O_2$	Octadecanoic acid (Stearic acid)	Flavourant (Omotoso et al., 2014)
$C_{18}H_{30}O_2$	9, 12, 15-Octadecatrienoic acid	
$C_{24}H_{38}O_4$	2-hexylethylThalate or 1, 2-Benzenedicarboxylic, dioctyl ester	Antimicrobial, Antifouling (Devi and Muthu,2014) Anti-viral activity
$C_{20}H_{42}O$	Eicosanol	

The Gas Chromatography- Mass Spectroscopy (GC-MS) result of methanol crude fractions 1, 2 and 3 as shown in Table 5, 6 and 7 respectively contain hexadecanoic acid methyl ester, hexadecanoic acid, 9, 12-octadecanoic acid methyl ester, 9-octadecanoic acid, methyl ester, 9-heptadecenamide, 9-octadecanoic acid, Eicosanoic acid. 2-hexylethylThalate or 1, 2-Benzenedicarboxylic, 9, 12, 15-octadecatrienoic acid (Omega 3) Compound such as alkane, dodecanoic acid ehtyl ester, tetradecanoic acid ethyl ester (myristic acid), hexadecanoic acid methyl ester(palmitic acis methyl ester), hexadecanoic acid, 9-heptadecenamide were also identified. In all the fractions fatty acid, amines, alkanes, alkenes, alcohols and amide were present. And this agrees with the result from the evaluation of nutritional, phytochemical composition and microbiological quality of *Mucuna bracteata* grown in northeastern Brazil by Renata *et. al,* (2015) shows that's the seed flour and extract contained fatty acid such as myristic, palmitic, oleic and linoleic acid but differs with the one reported by Joseph and Sankaran, (2018) from India thus, different species (*Mucuna prurien*) which reveals the presence of 5 major compounds namely: pentadeconoic acid, 14-methyl-methylester, dodecanoic

acid, z,z-methylester, 9,12-octadecadienoic acid and 2-myristynoylglycinamide.

CONCLUSION

The seed of *Mucuna bracteata* was found to be very effective for the treatment of many diseases such as heart related disease, fungal or viral or bacterial infection, cancer etc. as the seed contains fatty acids such as 9-octadecanoic acid (Omega 9), 9, 12-octadecadienoic acid (Omega 6), 9, 12, 15-octadecatrienoic acid (Omega 3), their corresponding esters, tetradecanoic acid methyl ester, 1,2-bezenedicarboxylic, dioctyl ester, hexadecanoic acid, hexadecanoic acid methyl ester, nonadecan-1-ol, amide and amines which are known for their antioxidant, anticancer, antiviral, antibacterial, anti-inflammatory, pesticide, hypercholesterolemic, antihistamine activities etc. also the esters present may contribute to the anti snake venom property of the seed.

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REFERENCES

Azwanida NN (2015) A Review on the Extraction Methods Use in Medicinal Plants, Principle of Strength and Limitation, *Medicinal and Aromatic Plants* Vol 4(3) Page 1-6.

Devi J. A, Muthu A. K (2015) GC-MS Analysis of Phytocomponent in the Ethanolic Extracts from whole Plant of *Lactuca runcinata*. *Asian Journal of Pharmaceutical and Clinical Research* Vol 8(1) Page 202-205.

Farnworth N. R. and Soejarto D. D. (1991) Global Important of Medicinal Plant in: Flavonoids from Aerial Part of *Hiptage benghalensis.International Journal of Life Science* 2(3).

Joseph Swithin Fernando and Sankaran Saveetha (2018) Analysis of Chemical Present in the Seed of *Mucuna Pruriens* by GC-Ms, 14th*World Congress on Taxicology and Pharmacology* Vol 4 Page 65.

Kwaji A., H. M. Adamu, I. Y. Chindo, Atiko. R. (2018) Isolation, Characterization and Biological Properties of Betulin from *Entala Africana Guill* and Perr. (Mimosaceae) *Journal of Applied and Advanced Research* Vol. 312 Page 138.

Lucia R. L., Alessio C., Roberts G., Claudia S., Givseppe V. (2012). Journal *of Traditional and Complementary Medicine* Vol. 2(4) Page 331-339.

Manalisha D. and Chandra K. J. (2002). Preliminary Phytochemical Analysis and Acute Oral Toxicity Study of *Mucuna* linn in albino mice. *International Research Journal of Pharmacy* Vol. 3(2) Page 181-183.

Natarajan K, Narayanan N, and Ravichandran N. (2012) Review on *Mucuna* the Wonder Plant *International Journal of Pharmaceutical Science* Vol. 17(1) Page 86-93.

Omotoso A. E., Eseyin O. O. and Suleiman M. (2014) Phytochemical Analysis of *Cnidoscolus aconitifolius* (Euphobiaceae) Leaf with

Spectrometric Techniques. *Nigerian Journal of Pharmaceutical and Applied Science Research* Vol. 3(1) Page 38-49.

Renataleite T., Alexandre Sergio S., Anarejina N. C, Alexandre R. P and Schuler J. S (2015) Nutritional Composition, Phytochemical and Microbiological Quality of the Legume *Mucuna prurient*. *African Journal of Biotechnology* Vol 4 (8) Page 676-682.

Sofowora A. (2008) Medicinal Plant and Traditional Medicine in Africa (spectrum book limited) 3rd edition page 205-214.

Sofowora A. (1993) Medicinal Plants and Traditional Medicine in Africa 2nd edition, Sunshine house Ibadan Nigeria. Spectrum book ltd; Screening Plant for Bioactive Agent Page 134-156.

Trease G. E. and Evans W. C. (2002) Textbook of Pharmacognosy, 15th edition London: Saunders publishers, Page 42-44.

Trease G. E. and Evans W. C. (1989) Textbook of Pharmacognosy, Bialliere Tinall Ltd. London

Wall M. E., Krider M. M., krewson C. F., Eddy C. F., Wilaman J. J., Cordell D. S. and Gentry H. S. (1954). Steroids Sapogenins XIII, Supplementary Table of Data for Sapogenins VII. *Agric Research Service Circle* Vol. 363 Page17.

Wikipedia 2012.