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Studies of the medicinal plant *Pausinystalia yohimbe* ethanol leaf extract phytocomponents by GCMS analysis

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ABSTRACT:

An *in vitro* phytochemical study of ethanolic leaf extract of *Pausinystalia yohimbe* was done using Gas Chromatography-Mass Spectrometry to identify the presence or absence of erectile compounds. The chromatogram of the leaf of *Pausinystalia yohimbe* showed eight peaks indicating the presence of eight phytocompounds. The most abundant compound was Linoleic acid with a [Retention Time: 22.689; Peak Area: 52.99% ; Molecular Formula: $C_{18}H_{32}O_2$] followed by n-Hexadecanoic acid with [Retention Time:19.91; Peak Area: 15.87% ; Molecular Formula: $C_{16}H_{32}O_2$] followed by 2-Methylene-11-hexadecanoic acid with [Retention Time: 21.774; Peak Area: 7.57% ; Molecular Formula of $C_{17}H_{28}O_2$]. Other components identified were 2,4-Dimethy-1,3-dioxane, n-Hexadecanoic acid, methyl ester, 11-phenyundecanoic acid, Octadecanoic acid and 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one. The bioactivities of *Pausinystalia yohimbe* leaf extract were anti-carcinogenic, lipid metabolism regulation, antioxidation, anti-atherosclerotic and antifungal from the GCMS result. Therefore the leaf of *Pausinystalia yohimbe* may be having bioactivities different from the bark which is known to contain yohimbine for erectile disfunction.

Keywords: GCMS, *Pausinystalia yohimbe*, Linoleic acid, n-Hexadecanoic acid; 2, 4- Dimethyl-1,3- dioxane.

INTRODUCTION

The use of natural product for the treatment of diseases has increased for the last four decades. Medicinal plants have played important roles in the world health care system. It is said that only about 2% of all the plants on earth have been subjected to pharmacological investigations. The rationale for the utilization of medicinal plants has rested largely on long term clinical experience with little or no specific data on their efficacy and safety [1]. With increase in the use of herbs for medicine, a scientific investigation of these plants is very important based on the need to validate their unscientific usage [2].
There are insufficient information on the phytochemical components in *Pausinystalia yohimbe* and their structural relationship to bioactivity. This research is therefore necessary to provide this information in order to close the gap in knowledge about the phytochemical content of the plant leaf. The scientific information provided in this research will play a role in ethno-medicinal applications of the plant and assist future development of phytopharmacology.
Pausinystalia yohimbe also known as *Corynanthe yohimbe* has a common name Yocon. The Hausa tribe in Nigeria calls it *Dankamaru. Pausinystalia yohimbe* (family: Rubiaceae) is a valuable tree native to the Gulf of Guinea and distributed in evergreen closed canopy forests from southern Nigeria to Congolese Mayombe, Gabon, Rabublic of Congo and Cameroon [3].

Pausinystalia yohimbe is an evergreen tree belonging to family Rubiaceae. The plant is native to South, West and Central Africa where it is commonly found in the forest and jungles of Cameroon, Congo, Gabon, Nigeria and Equatorial Guinea [4]. *Pausinystalia yohimbe* usually grows up to 30m with a spread of 8m. The erect stems branch extensively, with ovate or elliptical leaves. The seed have very short viability, which declines rapidly in dry and warm conditions [5]. The bark extract has also been used traditionally as tonic for the management of exhaustion, chest pain, skin disorders, epilepsy and inflammations [4].There are several alkaloids in *Pausinystalia yohimbe* but it is the Yohinbine which is an indole alkaloid similar and related to ibogaine and mitragyine and is present in the bark of this species between 2 and 15% [6]. Yohimbine, the active principle found in nature from the bark of *Pausinystalia yohimbe* tree and is used for treatment of impotence [7]. It acts by blocking alpha-2 adrenoreceptors

causing increase in cholinergic activity; also inhibits monoamine oxidase that catabilizes norepinephrine and 5-Hydroxytryptamine (Serotonin), without increasing testosterone level [8].
The most popular use *Pausinystalia yohimbe* has been as an aphrodisiac and to improve erection [9,7]. Pharmacologically Yohimbine is contraindicated in people with high blood pressure, heart problems, anxiety and panic attacks, all these worsen with adrenergic activity [10]. Yohimbine has been associated with side effects like kidney disease, peptic ulcer, pregnancy as well as breast feeding mother, should not use yohimbine [11].
This research is to find out the phytocompounds in the ethanolic leaf extract of *Pausinystalia yohimbe* by GCMS and to note any erectile (or otherwise) effect thereafter. Therefore we will present our findings as preliminary study and recommend an *in vivo* study to corroborate the bioactivities on the leaf with bark of *Pausinystalia yohimbe.*

2. MATERIAL AND METHODS

2.1 Plant Materials

Fresh leaves of *Pausinystalia yohimbe* was harvested at Ohafia town in Abia State, Nigeria. The plant leaves were identified by Prof M C Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

2.2 Preparation of Plant Extract

The plant material of *Pausinystalia yohimbe* was collected from wild, shade dried for 10 days and pulverized to powder using mechanical grinder. The plant extract was prepared using Soxhlet method described by [12]. Thirty five grams (35 g) of powdered sample was introduced into the extraction chamber of the Soxhlet extractor using methanol as solvent. Temperature was maintained at 70^{o} C throughout the extraction period of 48 hrs. At the end of the extraction period, the extract was concentrated using oven at 35^{o} C to obtain dried extract which was sent for GCMS analysis.

2.3 GCMS analysis of *Pausinystalia yohimbe*

The characterization of the Phytochemicals in *Pausinystalia yohimbe* was done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5).The initial column temperature was 80^{o}C for 1min, and then increased linearly at 70^{o}C min^{-1} to 220^{o}C, held for 3 min followed by linear increased temperature 10^{o}C min^{-1} to 290^{o}C for 10 min. The temperature of the injection port was 290^{o}C and the GC-MS interface was maintained at 290^{o}C .The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min^{-1}. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS.

2.4 Identification of Phytocompoments in *Pausinystalia yohimbe*

GC-MS Chromatogram of *Pausinystalia yohimbe* revealed eight peaks showing that eight different compounds were present. Identity of the active components in the extract was done by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored in the database of National Institute of Standards and Technology (NIST) and also with published literature, NIST08.LIB [13], WILEY8.LIB [14], PESTEI-3.LIB and FA-ME.LIB library sources were used for matching the identified components from the plant material. The name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.

3. RESULTS AND DISCUSSION

3.1 Results

GCMS chromatogram of the ethanolic extract of *Pausinystalia yohimbe* (Figure 1) showed eight peaks which indicated the presence of eight phytochemicals constituents.

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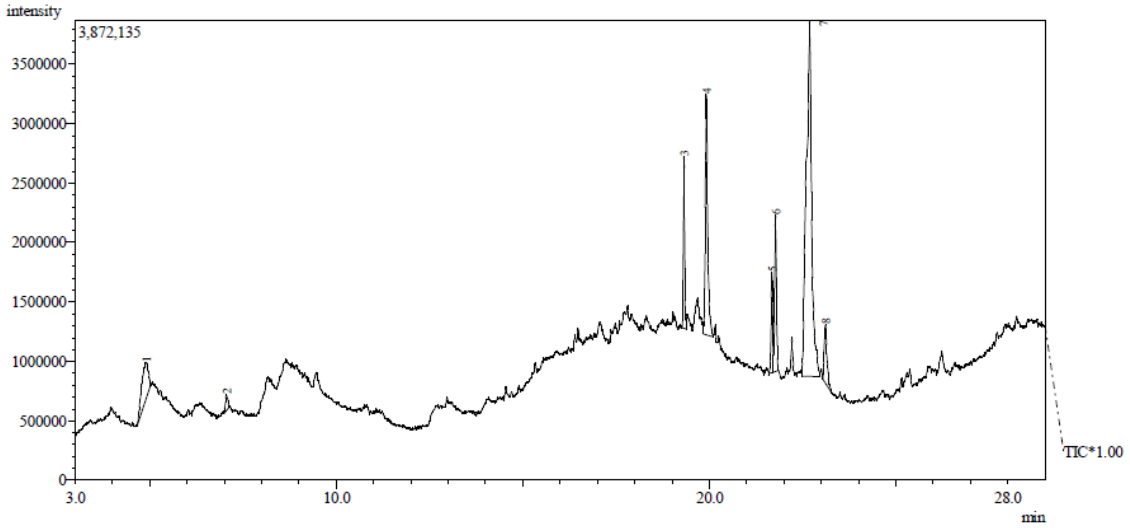
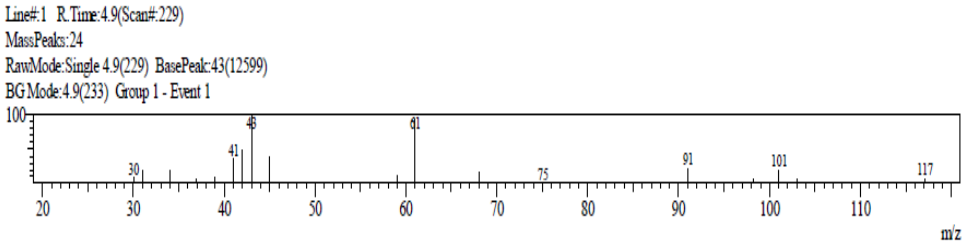
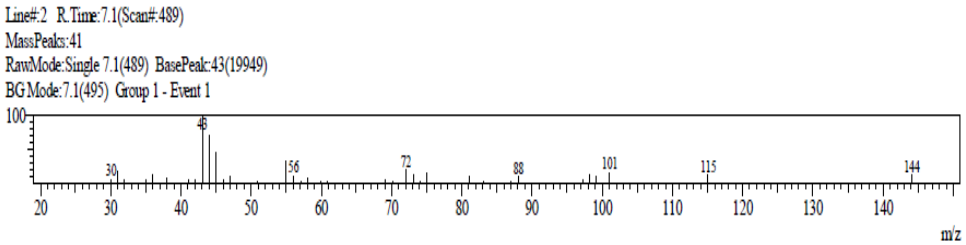
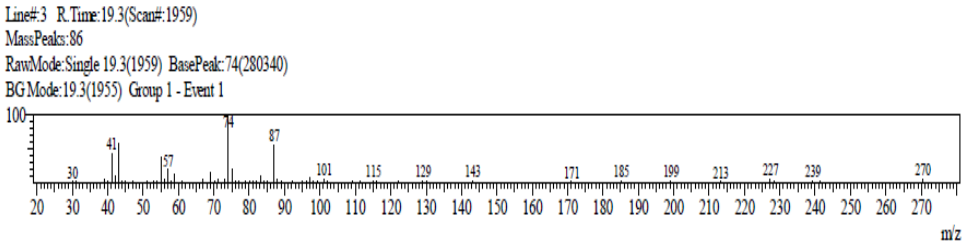


Figure 1: Shows the chromatogram of *Pausinystalia yohimbe*,







DOI: 10.18535/ijsrm/v4i5.03

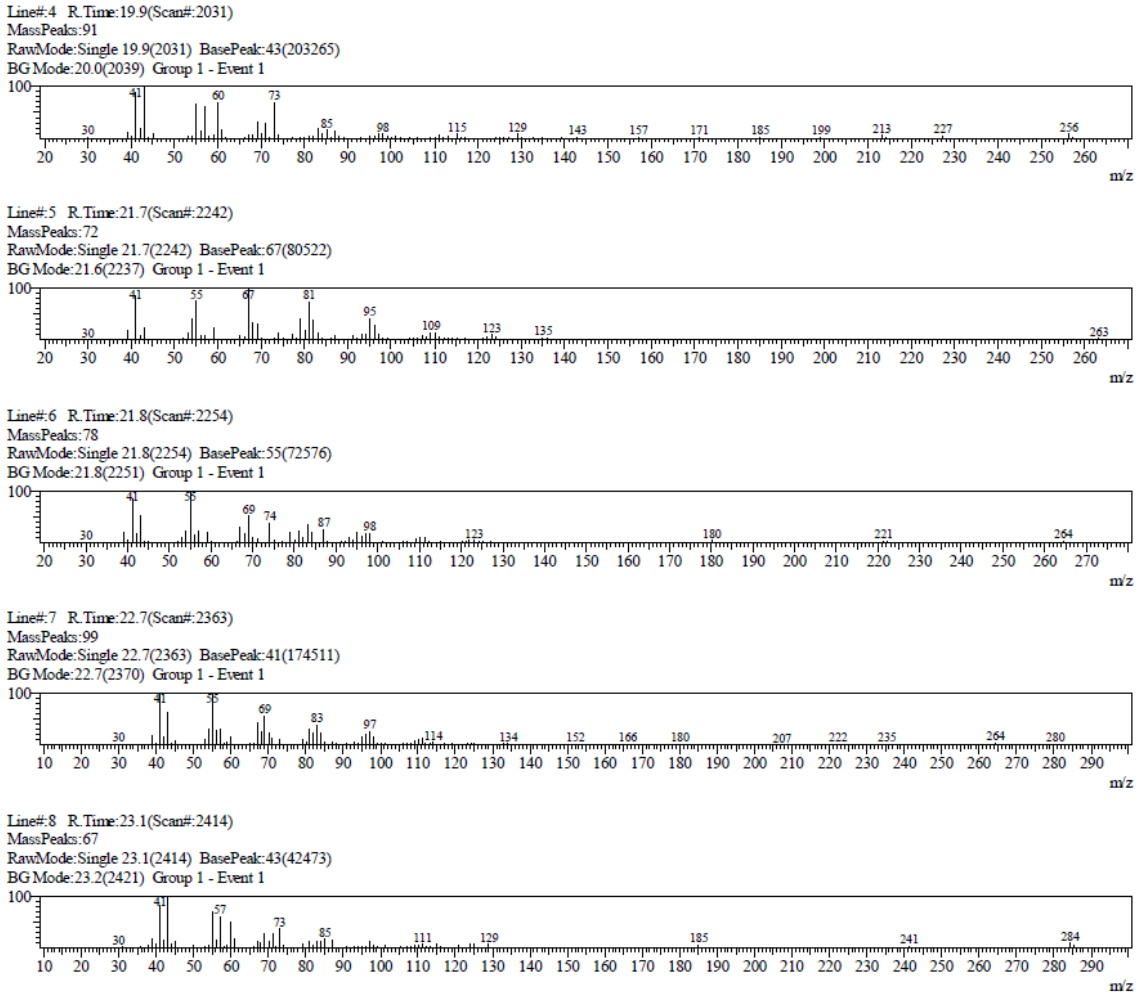
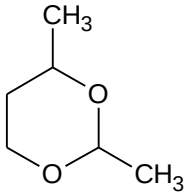
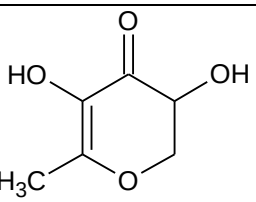
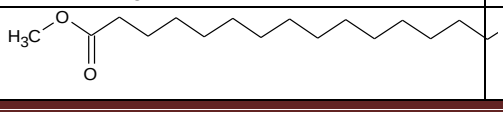
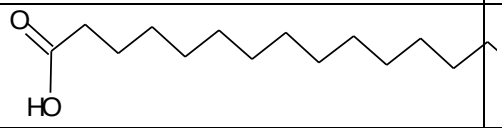
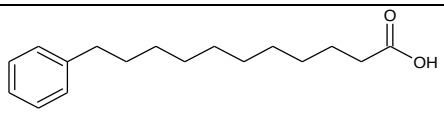
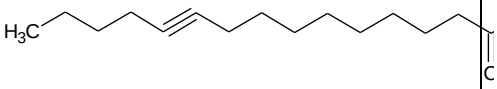
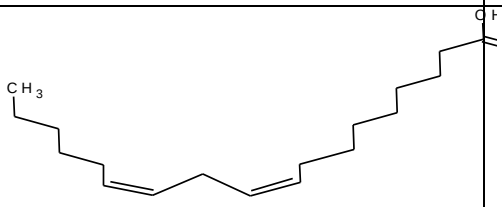
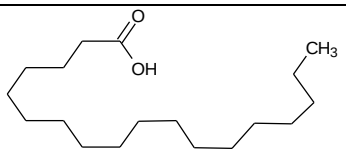


Figure 2: Shows the mass spectra of the eight phytocompounds identified by GCMS analysis in *Pausinystalia yohimbe*,

Table1: Shows the names, retention time, peak area percentage, molecular weight, molecular formula and bioactivity of compounds identified in *Pausinystalia yohimbe*, by GCMS.

S.No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Molecular structure	Bioactivity
1	2,4-Dimethyl-1,3-dioxane	4.907	7.33	116.15	$C_6H_{12}O_2$		peroxisome proliferator activated receptor
2	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	7.054	1.11	144.12	$C_6H_8O_4$		S-adenosyl-homocysteine hydrolase
3	n-Hexadecanoic acid methyl	19.319	6.01	270.45	$C_{17}H_{34}O_2$		Mild antioxidant and anti-atherosclerotic

	ester or Palmitic acid, methyl ester						activity [15].
4	n-Hexadecanoic acid or Palmitic acid	19.919	15.87	256.42	$C_{16}H_{32}O_2$		Mild antioxidant and anti-atherosclerotic activity [15].
5	11-Phenylundecanoic acid	21.675	4.62	262.38	$C_{17}H_{26}O_2$		androgen receptor
6	2-Methylene-11-hexadecynoic acid	21.774	7.57	264.40	$C_{17}H_{28}O_2$		Antifungal agent [16].
7	Linoleic acid	22.689	52.99	280.44	$C_{18}H_{32}O_2$		Anti-carcinogenic, lipid metabolism regulation, anti-inflammatory, anti-obese and antioxidant activities [17]
8	Octadecanoic acid or Stearic acid	23.112	4.52	284.47	$C_{18}H_{36}O_2$		Antiinflammatory, Antiandrogenic Cancer preventive,Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor,anemiagenic insectifuge, flavor [18]

3.2 Discussion

The chromatogram of *Pausinystalia yohimbe* showed 8 peaks which indicated the presence of 8 commands in the sample.
The compound 2, 4- dimethyl -1, 3-dioxane with a retention time of 4.90 and a peak area percentage of 7.33% had peroxisome proliferator activated receptor activity. Peroxisomes play an important role in B-oxidation leading to the formation of Acetyl CoA and hydrogen peroxide which is broken down by Catalase [19].
The peroxisome system facilitates the oxidation of very long chain fatty acids example C_{20} and C_{22}.
Peroxisomes shorten the side chain of cholesterol in the bile acid formation and also take part in the synthesis of etherglycerolipids [20].

The compound 3, 5-dihydroxy – 6-methyl-2, 3- dihydro 4H – pyran- 4 –one with a retention time of 7.054 and a peak area percentage of 1.11% had S-adenosyl homocysteine hydrolase activity.
B-adensoyl homocysteine hydrolase is an enzyme that hydrohyzes S-adenosyl homocysteine into adenosine and homocysteine [21].
The compound n-Hexadecanoic acid methylester or palmitic acid, had a retention time of 19.319 and a peak area percentage of 6.01% and n-Hexadecanoic acid or palmitic acid with retention time of 19.919 and a peak area percentage of 15.87% showed a mild antioxidant and anti-atherosderolic activity [15].
Antioxidants are substances that prevent or reduce damage caused by reactive oxygen species or reactive nitrogen species. They are used as dietary supplements in animals for aiding in the treatment of cancer, enhancing immune function and reducing treatment toxicity [22].

Atherosclerosis is a risk factor of hypertension which can predispose to coronary heart disease, heart failure, stroke, peripheral artery disease and death [23].The risk for coronary artery disease and stroke depends to a great extent on the risk factors such as obesity, smoking and elevated cholesterol levels [23]

Therefore the compound linoleic acid which has a retention time of 22.689 and the highest concentration of 52.99% (peak area percentage) also has antioxidant activity along with other activities like anti-carcinogenic, lipid metabolism regulation, anti-inflammatory and anti-obesity activities [17]

Together with the compound Octadecanoic acid or stearic acid at retention time of 23.113 and peak area percentage of 4.52% also had anti inflammatory activity, antiandrogenic, cancer prevention, hypocholesterlemic, 5-Alpha reductase inhibitor, (Gopalakrishnan and vadivel, 2011).

The compound 11 – phenylundecanoic acid with a retention time of 21.675 and a peak area percentage of 4.62% had androgen receptor activity.

This compound can be said to produce an alkaloid Yohimbine which is an $\propto_2$ adrenegic receptor antagonist used to reverse sedation produced by $\propto_2$ adrenogenic receptor agonists such as xylazine [24].

An alkaloid is one of a large group of small organic compounds mainly derived from amino acids and contains nitrogen found in plants. They are water soluble, usually bitter in taste and are characterized by powerful physiological activity [25].

The compound 2-methylene-11-hexadecynoic acid with a retention time of 21.774 and a peak area percentage of 7.57% had antifungal agent [16]

The target site of the two most important families of antifungal agents is the cytoplasmic membranes of yeasts or molds.

Fungal membranes differ from human cell membranes in that they contain the sterol ergosterol instead of cholesterol. The polyene family of antifungal compounds (eg, Amphotericin B, Nystatin) preferentially binds to ergosterol and forms holes in the cytoplasmic membranes causing leakage of the fungal cell contents and eventually, lysis of the cell [26]. The imidazole class of drugs (eg fluconazole, itraconazole, voriconazole, posaconazole) inhibits the synthesis of ergosterol, thereby damaging the integrity of the fungal cytoplasmic membrane. Both types of drugs bind to a certain extent to the cholesterol component of host cell membranes and elicit a variety of toxic side effect in treated patients [27]. Medically speaking, many Phenols and their derivatives exhibit topically antifungal properties. There agents are believed to interfere with the cell membrane function of fungi. They are used in treatment of athlete's foot, jock itch and ringworms. Tolnefitate is the active ingredient in Tinactin, Odor Eaters and Desenex [28]. Yohimbe contain 2-methylene-11-hexadecynoic ($C_{17}H_{28}O_2$) though not a phenol, it has antifungal effect [16] shows that compounds from leaf of *Pausinystalia*.*yohimbe* can serve as antifungal agent.

4. CONCLUSION

From the result of the GCMS analysis of the ethanoic leaf extract of *Pausinystalia yohimbe* contain phytocompounds that could be used to treat or control ailments like inflammation, cancer, and skin infections.

That from the molecular formula, structure that the leaf extract has majority saturated fatty acids that can be used to produce essential oil.

That eight phytocompounds were present in the ethanoic leaf extract using GCMS analysis which can be further be researched on further investigate their medicinal properties.

The ethanoic extract of *Pausinystalia yohimbe* may not cause erection of penis because the GCMS analysis did not reveal the presence of yohimbine, indole alkaloids or its related compounds.

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