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Research Article

Phytochemical Screening and Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds Present in Stem Bark of *Picralima nitida* (stapf)

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Abstract

Over the years, traditional societies and ethnic nationalities have engaged plants with medicinal properties for the treatment of a range of diseases without any scientific knowledge of it inherent bioactive compounds that are responsible for its medicinal and pharmacological potentials. The aim of this study is to screen for the presence of phytochemical constituents and to identify the bioactive compounds domicile in the stem bark of *Picralima nitida* by the use of Gas Chromatography - Mass Spectrometry. The result of the quantitative investigation of the stem bark extract of *P. nitida* showed the presence of some phytochemical compounds such as saponins (3.22%), alkaloids (2.43%), flavonoids (6.05%) tannins (6.25%), oxalate (12.70%), phytate (2.87%), anthracene glycosides (2.14%) and cyanogenic glycosides (1.37%). Eleven (11) different bioactive compounds were recognized in the stem bark extract of *P. nitida* by Gas Chromatography - Mass Spectrometry analysis. The percentage of major bioactive compounds were vitamin E (69.31%), Cis-Myrtanol (5.57%), Octadecanoic acid methyl ester (4.52%), 11-Octadecenoic acid methyl ester (4.42%), 9-Methyl-2-phenyl-9H-imidazo (1,2-a) benzimidazole (3.70%), Pentadecanoic acid 14-methyl- methylester (3.21%) and 7,9-Dimethyl-6H-Indolo (2,3-b) quinoxaline (3.11%). From these findings, it could be concluded that *P. nitida* stem bark is rich in various bioactive compounds which possess antioxidant, laxative and other diverse medicinal properties. Therefore, it can be recommended as a plant of phytomedicinal value.

Keywords

Phytochemical Constituents, Gas Chromatography - Mass Spectrometry Analysis, *Picralima nitida*, Bio-Active Compounds, Aqueous Extract

1. Introduction

The focus of the pharmacological Biochemist for more than 10 years has been the, accessibility of several natural products from plants. In Nigeria, several plants are exploited as herbal medicine for the management of diseases. Majority of the rural residents in underdeveloped nations nearly exclusively treat all illnesses using traditional medicine. The World Health Organization (WHO) projected that, sixty-five to eighty percent of the people living in these nations primarily receive their medical care from herbal remedies [1]. Many nations, like Nigeria, are seeing a dramatic rise in the use of

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traditional medicine in modern times, where the country's rich biodiversity may provide novel and cheaper therapies for a variety of ailments [2]. Wild plants have been used as sources of therapy, food security, and revenue generation for as long as man have existed [3, 4]. This is due to the immense potential for many wild plants, particularly for use in traditional medicine and pharmacopoeia medications. Due to the paucity and expensive expense of orthodox treatment, a sizable segment of the global population also relies on traditional medicine [5]. Therefore, these plants of natural origin provide not only essential components of human diet but also serve as medicine for treatment of diverse diseases and maladies [6, 7].

Thus, traditional societies and ethnic nationalities have applied medicinal plants in ethno-medicine over the years for the treatment of diverse diseases without any scientific knowledge of the physiologically active components known as phytochemicals, which were responsible for the plant's medicinal and pharmacological potentials [8]. Ethno-pharmacological uses of medicinal plants to treat and prevent numerous illnesses and disorder have some biochemical underpinnings, credit to the phytochemical investigations of these plants [9]. In addition to its laxative effects, Jung *et al*. [10] have demonstrated that anthraquinones found in plants reduce Tau aggregation and dissolve Alzheimer's paired helical filaments in-vitro and in cells. Their research suggested that the active phytochemical components of plants are what give them their therapeutic potential.

There are numerous vegetative natural resources in South Eastern Nigeria that are used as food and medicine. Since the majority of people still rely on botanical preparations as medication in this region, the treatment of diseases is not just confined to orthodox medications (3). There are always infusions, decoctions, macerations, and mixtures of these substances available. To properly comprehend the impacts and medicinal properties of these plants, it is vital to have a thorough understanding of the bioactive chemicals included in these medicinal plants and their bioactivity. Additionally, some of these herbal plants pharmacological properties have not yet been extensively characterized.

The only species of *Picralima*, a genus of plants belonging to the family "Apocynaceae", is *Picralima nitida*, and sometimes known as the "Akuamma plant" because akuammine is its most prevalent active alkaloid which is indigenous to tropical Africa, especially Nigeria [11]. It is also referred to as "Osu" or "Osi-Igwe" by the Igbo people of southern Nigeria. It is a species that lives in African forests and has expanded from Uganda to the Ivory Coast [12]. The plant's therapeutic benefits have been attributed to several of its components, and it is widely used as herbal medication by Nigerians [13]. In West Africa, particularly in countries like Ghana, Ivory Coast, and Nigeria, the dried form of different plant's part such as seeds, leaves, and stem bark are all utilized in traditional medication [14]. As well as anti-trypanosomiasis capabilities, it has been claimed to have anti-inflammatory, antipyretic, and other medicinal possessions (15). The fruits are used to cure dysmenorrhea and digestive problems, while the plant's bark is utilized as medication in treating malaria and erectile dysfunction in males [15]. The effectiveness of *Picralima nitida* plant extract as a treatment for fever, hypertension, jaundice, malaria, and other illnesses has been demonstrated in numerous studies [16]. Additionally, the bark and seed extracts potential for reducing pain and their hypoglycemic effects have been verified [13]. Akuammine was identified as the major alkaloid present in the seeds of *Picralima nitida* as well as its pain-relieving capabilities by Duwiejua *et al*. [17]. The bioactive profiles of *Picralima nitida's* stem bark aqueous extract have not yet been identified. In order to completely utilize *Picralima nitida's* medicinal potentials, this study was created to evaluate its phytochemical characteristics.

2. Materials and Methods

2.1. Chemicals and Reagents

All used chemicals and reagents in this study were obtained from certified suppliers such as Qualikems Laboratory reagents, BDH laboratory supplies, England etc and were of the highest analytical standard.

2.2. Collection and Authentication of Plant Material

The stem bark of *Picralima nitida* was obtained at Isuikwuato in Abia State. The plant was identified and authenticated by Mr. I. K. Ndukwe at the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.3. Extract Preparation of *Picralima nitida* Stem Bark

The plant material was sun-dried. The dried stem bark of *Picralima nitida* was pulverized into powder using Arthur Miller Machine and sealed in cellophane bags to avoid the effect of humidity and then stored at 27 ℃ until used. One-fifty grams (150g) of the powdered sample was extracted with 95% ethanol by continuous hot percolation using Soxhlet extractor for 48 hours. The extract was filtered through Whatman no.41 filter paper, and then concentrated in vacuum at 60 ℃ using a rotary evaporator to evaporate the ethanol. The extracts were kept on a water bath for 8 hours in other to evaporate the remaining solvent. The obtained dark brown residue was kept in airtight container and stored at 4 ℃ for further use [18].

2.4. Phytochemical Analysis of the Plant Extract

Using standard methods outlined by Harbone [19] and Sofowora [20], the extract was used for quantitative identifi-

cation of various phytochemicals such as saponins, alkaloids, flavonoids, tannins, phytate, anthracene glycosides, cyanogenic glycosides, and cardiac glycosides.

2.5. GC-MS (Gas Chromatography - Mass Spectrometry) Analysis

The major function of Gas Chromatography - Mass Spectrometry (GC-MS) is in the analysis of unrevealed plant components. The chemical composition of ethanolic extracts of stem bark of *P. nitida* was subjected to GC-MS analysis.

GC. MS analysis of this extract was conducted using the equipment GCMS-QP 2010 plus Shimazu Japan with column oven temperature of 60 ℃, injection mode was split, flow control mode was linear velocity, carrier gas pressure was 100.2 kpa, total flow was 6.2ml/min, column flow was 1.61ml/min, linear velocity was 46.3 cm/sec, purge flow was 3.0ml/min and split ratio was 1.0. Also, in source temperature was 200 ℃, interface temperature was 250 ℃, solvent cut time was 2.5 min., detector gain was 0.00KV, detector gain mode was relative and the threshold was 1000. The relative percentage amount of each component of the plant extract was calculated by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature [23].

2.6. Statistical Analysis

All the qualitative test/analysis was performed in triplicate.

3. Results

3.1. Phytochemical Composition

The phytochemical constituents in aqueous extracts of stem bark of *P. nitida* was evaluated quantitatively and revealed in Table 1. The following active biochemical compounds like alkaloids (2.43%), flavonoids (6.05%), tannins (6.25%), saponins (3.22%), phytate (2.87%), oxalate (12.70%), anthracene glycoside (2.14%) and cyanogenic glycosides (1.37%) were present in the aqueous extract of stem bark of *P. nitida.*

Table 1. *Phytochemical Constituents of Ethanolic Extract of Picralimanitidastem bark.*

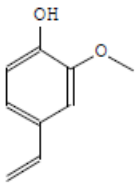
S/no.	Phytochemicals	Concentration (%)
1	Saponins	3.22 ±0.01
2	Alkaloid	2.43 ±0.03
3	Flavonoids	6.05 ±0.01
4	Tannins	6.25 ±0.01
5	Oxalate	12.70 ±0.01
6	Phytate	2.87 ±0.02
7	Anthracene glycoside	2.14 ±0.01
8	Cyanogenic glycosides	1.37 ±0.02

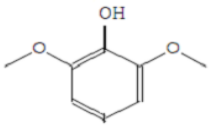
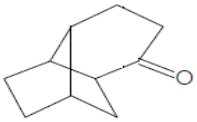
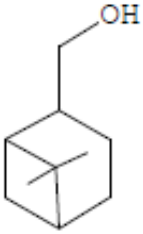
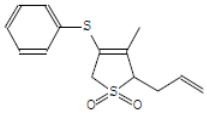
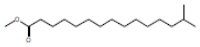
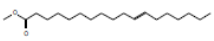
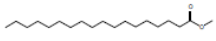
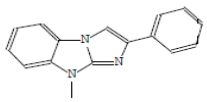
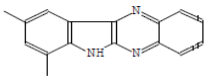
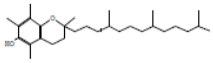
Data are means ±standard deviation of triplicate determinations.

3.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Analysis of different bioactive compounds in ethanolic extracts of *P. nitida* stem bark was carried out using GC-MS. The chromatograms of the extract was shown in Figure 1 and summarized in Table 2. GC-MS chromatogram of *P. nitida* stem bark revealed the presence of 11 peaks which signified 11 different bioactive compounds Figure 1. The results revealed that the percentage of major bioactive compounds viz., vitamin E (69.31%), Cis-Myrtanol (5.57%), Octadecanoic acid methyl ester (4.52%), 11-Octadecenoic acid methyl ester (4.42%), 9-Methyl-2-phenyl-9H-imidazo (1,2-a) benzimidazole (3.70%), Pentadecanoic acid 14-methyl- methylester (3.21%) and 7,9-Dimethyl-6H-Indolo (2,3-b) quinoxaline (3.11%) were found as the major compounds in the ethanolic extract of stem bark of *P. nitida* Table 2.

Table 2. *GC - MS analysis result of Picralimanitidastem bark extract.*

Chromatogram peak	Compound name	Molecular Formula	Structural Formula	Molecular Weight	Retention Time (Mins)	Percentage Composition (peak Area) (%)	Nature of Compound
1.	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$		150	8.511	0.60	Phenol

Chromato-gram peak	Compound name	Molecular Formula	Structural Formula	Molecular Weight	Retention Time (Mins)	Percentage Composition (peak Area) (%)	Nature of Compound
2.	2,6-Dimethoxyphenol	$C_8H_{10}O_3$		154	8.963	1.45	Phenol
3.	Tricyclo (4.4.0. (2,8) decan-5-one	$C_{10}H_{14}O$		150	10.505	1.21	Ketone
4.	Cis-Myrtanol	$C_{10}H_{18}O$		154	11.110	5.57	Alcohol
5.	3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5-di hydrothio-phene1,1-dioxide	$C_{14}H_{16}O_2S_2$		280	12.531	2.90	Thio Com-pound
6.	Pentadecanoic acid 14-methyl- methylester	$C_{17}H_{34}O_2$		270	16.678	3.21	Fatty Acid Esther
7.	11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$		296	19.818	4.42	Fatty Acid Esther
8.	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$		298	20.183	4.52	Fatty Acid Esther
9.	9-Methyl-2-phenyl-9H -imidazo (1,2-a) ben-zimidazole	$C_{16}H_{13}N_3$		247	23.973	3.70	Alkaloid
10.	7,9-Dimethyl-6H-Indol o (2,3-b) quinoxaline	$C_{16}H_{13}N_3$		247	24.455	3.11	Alkaloid
11.	Vitamin E	$C_{29}H_{50}O_2$		430	24.768	69.31	Vitamin E

Note: The components were identified by matching the chromatogram peaks with computer Willey MS libraries and confirmed by comparing mass spectra of the peaks and those from literature [23].

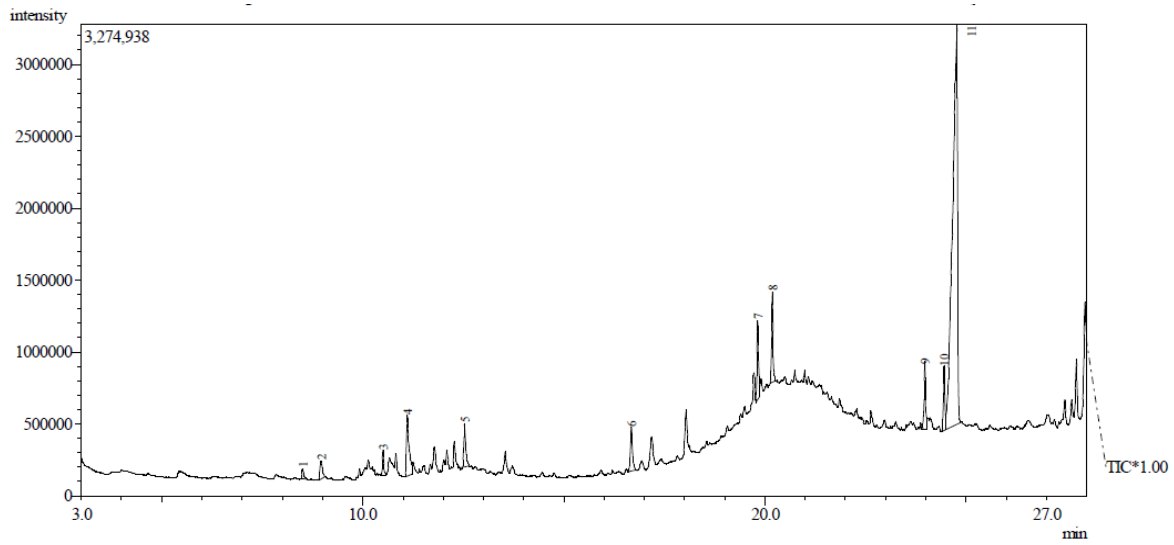


Figure 1. *GC-MS Chromatogram of ethanolic extract Picralimanitidastem bark.*

4. Discussion

The negative side effects of synthetic drugs are driving up interest in phytochemical research and its use. For the purpose of choosing the plant under research, local knowledge and literature regarding the plant's therapeutic capabilities were helpful. A plant native to West Africa, *Picralimanitida* (fam. Apocynaceae), has a variety of uses in traditional African medicine. As a traditional medicine, the plant's various parts have been used to treat fever, hypertension, jaundice, dysmenorrhea, gastrointestinal ailments, and malaria [16].

The phytochemicals that make up the plant stem bark may be held accountable for its curative properties. According to Varadarajan *et al*. [24], the secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants explains their medicinal importance. For instance, it have been reported that saponins; glycosides of both triterpene and steroids exhibited hypotensive, hypocholesterolemic, cardio-depressant and anti-diabetic properties [25], while cardiac glycosides are naturally cardioactive drugs used traditionally for the treatment of congestive heart failure and cardiac arrhythmia [26]. Saponins are also thought to possess biological effects that include cytotoxic, antibacterial, and anti-inflammatory properties [27]. Alkaloids, tannins, polyphenols, and steroids have all been found in *P. nitida*, and studies have linked these substances to the species' anti-cancer properties [28]. Phenolic molecules, which are employed as antioxidants, are abundant in plant tissues. Due to the existence of hydroxyl groups, which are crucial to their capacity to scavenge free radicals, this antioxidant activity is beneficial and efficacious for various disorders [29]. Hence, they are capable to react with active oxygen radicals for instance hydroxyl radicals [30]. Phenolics are made up of one or more polar hydroxyl functional groups [31], therefore it makes sense that their high quantities in the extract is responsible for the plant's antioxidant properties. The biological and physiological features of phenolics, including their anti-microbial, antioxidant, anti-inflammatory, cardio-protective, and vasodilatory activities, have been discovered through studies [32, 33]. These poly-phenolic phyto-constituents are present in the plant extract, which suggests a variety of potential health benefits. Flavonoids are poly-phenolic compounds, holding several phenolic groups, and are responsible for some of the health benefits possess by vegetables and fruits [34]. The majority of oxidizing molecules, including singlet oxygen and other free radicals [35] linked to a number of illnesses, have been demonstrated to be effectively neutralized by flavonoids. The capacity of flavonoids to inhibit enzymes and their protective effects on mucous membranes make them significant as well [36, 37]. Vegetables high in flavonoids are utilized as functional foods frequently because they can be used to treat cardiovascular disorders [38]. As a result of their high bioavailability, flavonoids have been linked to pharmacologically relevant plasma concentrations in humans when consumed consistently through diet [39]. Additionally, quite a number of studies have revealed that flavonoids may have cardio-protective benefits against ischemia reperfusion [40, 41]. Tannins, on the other hand, are high molecular weight poly-phenolic substances that possess the best antioxidant properties [42]. Researchers have since learned that tannins' beneficial properties rely on their dose and chemical makeup, contrary to long-held beliefs that they are anti-nutritional. The existence of tannins in plants may also suggest that they are effective astringents, or that they have anti-parasitic and wound-healing properties [43]. The antioxidant, anti-allergic, anti-inflammatory, antibacterial, and anticancer activities of flavonoids and tannins have been established [44]. While tannins lessen the permeability of the mucosa to chemical irritation, saponins may activate protective mechanisms for the mucous membrane. As a result, they lessen inflammation,

protect the stomach mucosa by acting as an astringent, and lower acidity. Alkaloids play a significant role in the antibacterial, analgesic, and other antispasmodic activities of several drugs [45-47]. Alkaloid and terpenoid compounds have also been demonstrated to possess strong anti-ulcer properties [48, 49]. Terpenoids found in vitamin E have been discovered to relax cardiovascular smooth muscle by preventing Ca^{2+} influx in vascular smooth muscle or by squelching ROS and stimulating synthesis of nitric oxide (NO) [50]. The presence of the various phyto-constituents in the plant extract showed a higher likelihood that these structurally distinct substances would interact chemically and alter biological activity by either boosting or lowering it. Therefore, these secondary metabolites aid in the effective use of plants in the pharmaceutical sector.

These secondary metabolites present in the stem barks of *Picralima nitida* matches with the reports of Kouitcheu-Mabeku *et al.* [51], Igboasoiyi and Essien [52], and Teugwa *et al.* [53] who also reported the presence of flavonoids, saponins, tannins, alkaloids and glycosides. Obasi *et al*. [12] reported similar compounds with the dearth of phenol in the peel of *picralima nitida* whereas Aghedo *et al.* [54] observed the same with tannins absent. However, Ngassona *et al*. [55] found terpenoids in polar extracts of the barks of the trunk and the root of *Picralima nitida* in addition to the previously described phyto-compounds. The seeds of the species discovered in Ivory Coast were also found to contain terpenoids and alkaloids [53, 56, 57]. Akuammine, akuammidine, akuammicine, akuammine, and pseudoakuammine are among the noteworthy indole alkaloids with opioid analgesic activity that have been discovered from the seeds of *P. nitida* [16].

High separation efficiency and sensitive detection are factors that qualified GC–MS technique as a powerful and suitable tool for volatile compounds determination [58]. Analysis of the ethanolic extract of *Picralima nitida* stem bark by GC-MS technique indicated the existence of phyto-compounds that are responsible for the pharmacological effects. Studies have shown that some of these constituents are biologically active substances. These constituents were shown to have pharmacologic properties that might add to the plant's capacity for healing. In spite of the advantage of the recent drug discovery and screening techniques, knowledge of traditional medicine have also given clues to the discovery of important drugs [59]. There is rising responsiveness in linking the phytochemical compounds and their biological activities [60]. Aja *et al*. [61] on GC-MS analysis of *Moringa oleifera* leaf and seed revealed that 9 - octadecenoic acid (20.89 %) constitutes the major constituent of the leaf extract. Three coumestan glycosides namely: 3-hydroxy-9-methoxy-2-[2'(E)-3'-methyl-4'-O-β-D-3'-methyl-4'-O-β-D glucopyranosyl-butenyl]-8-[2"(E)-3"-methyl-4"-oxobutenyl]coumestan and 3-hydroxy-9-methoxy-4-[2'(E)-3'-methyl-4'-O-β-Dglucopyranosylbutenyl]-8-[2"(E)-3"-methyl 4"oxobutenyl] coumestan were isolated from the roots of *P. nitida* by Kouam *et al.* [62]. Dodecanoic acid (lauric acid), octadecanoic acid (stearic acid), n-hexadecanoic acid (palmitic acid), phytol, and 5-cholesten-3-Beta, 2,6-dioic-16-one were all found in the n-Hexane extract of *P. nitida* by Aghedo*et al*. [54]. Octadecanoicacid (Stearic acid) is also known as a potent anti-inflammatory lipid and antiviral agent. This fatty acid has profound and diverse effects on liver metabolism [63, 64]. Gas Chromatography – Mass Spectrometry analysis of ethanol extract have led to discovery of twenty-eight compounds from *Macrotyloma uniflorum* Linn by comparison of their retention indices and mass spectra fragmentation [65].

The primary volatile components in the *Picralima nitida* stem bark ethanolic extract that possess antiulcer, anti-inflammatory, anti-arthritic, antidiabetic, hypolipidemic, and cytotoxic effects are phenols, esters, and ketones [66]. Phytol, which may be produced when vitamin E is broken down, was found to possess antioxidant, neuroprotective, antibacterial, anticancer, anti-inflammatory, and anti-diuretic properties [66, 67]. The methyl ester of octadecenoic acid exhibits anti-inflammatory, anti-cancer, anti-arthritic, hepatoprotective, anti-androgenic, antioxidant, hypocholesterolemic, nematicide, 5-alpha-reductase inhibitor, antihistaminic, anti-coronary, insectifuge, anti-eczemic, and anti-acne effects [68, 69].

5. Conclusion

In this current study, *Picralima nitida* stem bark has revealed various secondary metabolites possessing many pharmacological properties of which antioxidant activity is one of it. The GC-MS analysis showed the presence of 11 bioactive compounds which contribute to activities like antibacterial, antioxidant, anti-cancer, anti-arthritic, anti-inflammatory, hypocholesterolemic and other activities. Hence, the presence of the identified phytochemicals makes the plant pharmacologically active. These findings open the door to separating out certain bioactive substances from the stem bark of *P. nitida* that have medicinal utility for commercial purposes. Further research is therefore necessary for development of novel drugs using some of the bioactive compounds with medicinal values found in *P. nitida*.

Conflicts of Interest

The authors declare no conflicts of interest.

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