Current Research Bulletin

Volume 01 Issue 01 July 2024

Page no: 01-08

Available Url: http://crbjour.org/index.php/crb/index

ISSN(e): Applied
ISSN (p): Applied

Open Access

Evaluation of the Qualitative Phytochemical Composition of Extracts of Picralima Nitida Seeds, Leaves and Bark

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Published Online:
11 July 2024

ABSTRACT: In West African traditional medicine, picralima nitida is frequently used to treat a variety of blood-related conditions, such as anemia and diabetes, as well as malaria, diarrhea, and inflammation. The ethno-medical use of several portions of this plant has been validated by strong scientific data; however, research on its contents has been restricted, and conflicting claims have been made regarding its safety profile. In this work, the qualitative phytochemical content of Picralima nitida seed, leaf, and bark extracts is determined.The results of the phytochemical study showed that the extractives had a high concentration of alkaloids, saponins, tannins, carbohydrates, and a moderate amount of flavonoids, ascorbic acid, and terpenoids. The bark showed 20% mortality at a dose of 5000 mg/kg, with an LD50 < 3,807.89 mg/kg, whereas the leaf and seed extracts had LD50 ≥ 5000 mg/kg (practically not dangerous).These abilities shown by the various extracts could be attributed to the phytoconstituents and some of the compounds identified in the GC-MS analysis.

KEY WORDS: qualitative, phytochemical, composition ,extracts , Picralima nitida seeds, leaves and bark.

INTRODUCTION

For millennia, people have used naturally occurring minerals, plants, and animals as remedies to treat various illnesses. It is a dynamic activity that has been documented in early practitioners' writings as well as folklore. Decoctions, poultices, ointments, and solutions of plants, animal parts, and minerals were common ingredients in recipes for treating illnesses [1]. The trend shifted towards the production of pure drugs from plant and animal precursors after the discovery of pure drugs like quinine, atropine, and reserpine from plants. While many of these remedies have vanished over time, some are still in use today and are used for the treatment of diseases by traditional medicine practitioners worldwide. The pharmacological potential of plants is still abundant despite the fact that this tendency was reversed with the introduction of exclusively synthetic medications. Of the 250 000 species of higher plants on the globe, only roughly 94 species have been or are now being used for drug production[2].Only a small portion of the 250 000 species of higher plants have been used as medicinal agents, even in the field of traditional medicine practice[3].Even with the widespread availability of synthetic pharmaceuticals, a sizable section of the populace in underdeveloped nations still receives their medical care from traditional practitioners. World Health Organization (WHO) estimates that 80% of people in developing nations rely almost exclusively on traditional medical methods, and that 60% of the world's population depends on traditional medicine [4]. Approximately 15-20 million people who practice traditional medicine in underdeveloped nations live in West Africa alone [5]. Over 30,000 higher plant species may be found in Africa, and of those, 60% of the population is thought to employ about 3000 of these species as medicines at the moment [6]. Furthermore, it's believed that 80% of people seek treatment from traditional healers initially for their health issues [7]. That 20,000 tonnes of over 700 different kinds of medicinal plants were traded in South Africa annually as of 1996, with a market worth of about $60 million (about R450 million), is therefore not surprising. [8]. Regretfully, only about 350 widely used and traded species have undergone chemical tests out of the enormous number of traditional plants utilized as traditional remedies, sometimes in conjunction with mainstream treatments[9].Worldwide, people employ herbal cures and alternative medicines. In the past, most pharmaceuticals' original sources were often herbs.A significant amount of commercial drugs used today to treat conditions including asthma, high blood pressure, heart disease, and

pain are still derived from herbs. Nowadays, a significant portion of prescription medications are still made with natural substances, with 25% of all medications containing one or m

ore active compounds sourced from plants. Herbs are commonly seen as "natural" and safe because they are plants. But as recent studies have demonstrated, there are hazards connected to the many forms of traditional medicine in addition to their many advantages. Even while traditional medicine treatments and therapies are widely available to consumers today, they frequently lack the knowledge of what to look for when utilizing them to prevent unneeded harm.The variety of traditional herbal products varies. Biologically active components of herbs, pollutants, and interactions between herbs and drugs can all result in side effects. Pyrolizidine alkaloids are complex compounds found in some plants that can be used or unintentionally added to herbal treatments (like comfrey, which is still accessible in the United States). This is a typical cause of toxicity to herbal medications.These alkaloids cause hepatotoxicity by causing a veno-occlusive illness, which can advance quickly and prove lethal [10]

Apocynaceae is a family of plants that includes Picralima nitida, which was initially identified as a genus in 1896. It is indigenous to tropical Africa, including Benin, Ghana, Ivory Coast, Nigeria, Gabon, Cameroon, Angola (Cabinda), Central African Republic, Republic of Congo, Zaire, and Uganda. Picralima nitida is the only species known to exist there. [11]

The Picralima nitida shrub or tree can grow up to 35 meters tall and has glabrous white latex throughout. Its bole can reach a diameter of 60 cm. The bark of the tree is thick and brittle, ranging from pale to dark greyish black or brown, smooth to slightly rough or finely striped. The leaves are opposite, simple, and whole. There are no stipules, and the petiole is 1-2 cm long. The blade is elliptical to oblong, measuring (5–)10–26 cm × 2–13 cm, with a cuneate base and an abruptly acuminate apex. It is pinnately veined, with 14–23 pairs of lateral veins.. The terminal or occasionally axillary inflorescence is a compound, umbel-like cyme that is 6–10 cm long and has 10–35 flowers; the peduncle is 2–35 mm long and has three main branches; the bracts are extremely small [12].

They are also often used to treat intestinal worms, pneumonia, chest and stomach issues, and pain [13]. For this purpose, the seeds or bark are typically chewed or crushed and consumed, or a decoction made from the roots, seeds, or bark is consumed.

A leaf decoction is eaten orally or applied topically to treat measles, while a bark or root decoction is used to treat jaundice in Côte d'Ivoire, Benin, and Nigeria. Hernias, vomiting fits, and diarrhea can all be treated by crushing and consuming the extremely bitter seeds with lemon juice. On abscesses, the crushed seeds are administered. Women with leucorrhea are treated by rubbing a paste made of ground seeds and Shea butter on their abdomens. A decoction of the seeds is used as an analgesic and enema in Ghana. Chewing the seeds has a tonic and stimulating effect. To treat guinea worm, dry leaves are boiled in water and consumed[14]. A fruit infusion is used to treat typhoid fever or cough in Cameroon; bark is used in the Democratic Republic of the Congo in a similar manner. The Pahouin tribe of Gabon uses a small amount of fruit and bark chewed during long marches through the jungle to ward off hunger. The decoction of the bitter bark is cooked with sugar and consumed to prevent food poisoning and sexually transmitted infections. In Congo, a decoction of bark is used to heal hernias, as a purgative, and to reduce gonorrhea when combined with other plants[15].A decoction made from bark is consumed by men in southern Cameroon and the Congo to treat infertility.To treat otitis, leaf sap is dripped into the ear. Additional materials for arrow poison include crushed seeds, roots, or fruit pulp. Mature fruits are mashed and thrown into the water as a fish poison in Ghana and the Democratic Republic of Congo.

The wood, known in commerce as "ebam," is used to produce a wide range of small implements, such as spoons, walking sticks, dolls, paddles, shuttles for weaving, combs, incense holders, bows and arrows, and spade handles. The firm fruit shell is also used to make dippers or spoons. The plant generates the alkaloids pericine and akuammine, among others. P. nitida seed powder was ground into standardized 250 mg capsules and sold by an innovative hospital in Ghana. This product quickly gained popularity as a palliative. This prompted scientists to look for the seeds' active ingredient. Animal investigations have shown that a combination of alkaloids found in P. nitida seeds has analgesic, antipyretic, and anti-inflammatory actions [14]. A number of these demonstrated poor affinity binding to opioid receptors in vitro, and two of these, akuammidine and ψ-akuammigine, were discovered to be μ-opioid agonists albeit not very selective [10].

A variety of Picralima nitida plant parts, including the fruit, leaves, stem bark, and seeds, are prepared for the purpose of treating various illnesses [16]. The seeds have antipyretic and aphrodisiac properties, and they are used to treat pneumonia, malaria, and other respiratory tract ailments. Fever, dysmenorrhea, and gastrointestinal issues are all treated with the fruit. The leaf sap is administered to the ears to treat otitis, and the leaves are used as a vermifuge. Bark preparations are used as febrifuges, anthelmintics, laxatives/purgatives, and for the treatment of hernias and venereal disorders. Its root extract is used to treat gastrointestinal issues, malaria, pneumonia, and vermifuge, aphrodisiac, and febrifuge conditions.

To our knowledge, there is no published information on the toxicity profile of P. negida, despite the fact that characteristics like diuretic, carminative, and anthelmintic capabilities have been described. Therefore, the purpose of this study is to assess the toxicity effects, both acute and sub-acute, of the ethanol extract of P. Nitida's seeds, bark, and leaves. Many phytochemical components found in plants contribute to their poisonous and therapeutic qualities. Primary components, which include amino acids, proteins, common sugar, and chlorophyll, among others, and secondary metabolites, which include alkaloids, flavonoids, tannins, terpenoids, saponins, phenolic compounds, essential oils, and so forth, are the two basic categories into which they are divided[17].

One technique that aids in clarifying the phytochemical components of the plant is phytochemical analysis. Understanding a plant's bioactive chemicals aids in determining its beneficial or harmful properties, and this study will also focus on the phytochemistry of this magnificent plant.

The public and rural populations have paid close attention to the long-term use of herbs due to their broad application in the treatment of many ailments. Health issues related to toxicity can arise from the careless use of therapeutic plants and their poisonous metabolites. When consumed, plants that contain secondary metabolites such cyanide, alkaloids, tannins, saponins, phenolic chemicals, and terpenoids can result in acute or chronic illnesses that impair normal physiology and productivity and cause significant losses in livestock [18]. When a chemical is taken through food, inhalation, or skin contact and causes dose-related reactions, its hazardous effects are evaluated.

The majority of what is currently known about the uses of plants is the outcome of many years of human research and selection of the most successful, lustrous, and desirable plants that are available in the surrounding environment at any given moment. The advantages of phytopharmacy are well acknowledged, and medicinal plants already play a significant role in both plant research and medicine.

The present worldwide paradigm for obtaining pharmaceuticals from plant sources makes the use of plants in medicine even more significant. As a result, attention has been drawn to the medical benefit of herbal remedies for their safety, efficacy, and affordability. In my community of Umunoha, Mbaitoli, a plant known for its purported hematinic and hypoglycemic qualities is called picralima nitida. Despite its widespread use, there is a lack of scientific evidence validating these traditional claims. Hence, the need to check its phytochemicals

MATERIALS

Plant material

Picrilima nitida leaves, seed and stem-bark were used for the study

Equipment/Instruments

The under listed equipment/ instruments were used in the course of the study:

Metler weighing balance, LA 164 (B. Bran Scientific & Instrument Company England), Vacuum Rotary Evaporator (BUCHI Labortechnik, Switzerland), Spectrophotometer (Bibby Scientific Ltd, U.K.), water bath (Techmel and Techmel, Texas, USA), micropippete (Labsystems, Finland), ceramic mortar and pestle, Gas chromatography-mass spectra (GC-MS) (Agilent 7890A).

Consumables

Consumables used for the research include: Whatman No. 1 filter paper (England), grower's mash (Vital®, Nigeria), disposable hand gloves and protective masks, distilled water, needles and syringes, sample bottles, hematocrit tubes.

Glass wares

Beakers, test –tubes, glass rods, funnels, measuring cylinders, pipettes, bijou bottles.

METHODS

Plant collection, identification and extraction

The leaves, seed and stem-bark of Picralima nitida were collected from Umunoha village in Mbaitoli local government area of Imo state and identified by a taxonomist (Prof. F.M. Mbagwu) in the Department of Botany, Imo State University, Owerri.

The plant samples were dried on laboratory bench and pulverized into coarse powder using hammer mill (Muliner®). Five hundred gram (500 g) each of the plant material were macerated in 2 litres of 80% methanol for 48 hours with intermittent shaking at 3 hours intervals and filtered with Whatman No. 1 filter paper. The filtrates were concentrated in vacuo using rotary evaporator and finally dried in hot air oven (40°C). The extracts were stored in the refrigerator (4°C) throughout the period of the experiment. Percentage yield was calculated using the formula:

% yield = a – b/ a × 100

Where, a = weight of original material (i.e. coarse powder) used for the extraction and b = weight of the dried extract.

Gas chromatography-mass spectra (GC-MS) Analysis of the extracts

The GCMS analysis of the extracts were conducted using GC-MS (Agilent 7890A) equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 um film thickness, J & W Scientific, Folsom, CA). The initial oven temperature was 60 °C. Helium was used as the carrier gas at the rate of 1.0 mL/min. The eluent of the GC column was introduced directly into the source of the MS via a transfer line (250 °C). Ionization voltage was 70 eV and ion source temperature was 230 °C. Scan range was 41- 450 amu. The components were identified by comparing their retention times to the reference in the National Institute of Standards and Technology (NIST, ver. 2.0, 2008) mass spectral database.

In vitro antioxidant assay of the

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) photometric assay

The free radical scavenging activity of the extracts were investigated by the DPPH assay using spectrophotometer. The crude extracts at concentrations (25, 50, 100, 200 and 400) µg/mL each were mixed with 1 mL of 0.5 mM DPPH (in methanol) in a cuvette. The

absorbance at 517 nm were taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activities were calculated as follows.

% antioxidant activity (AA) = 100-[{(ABS sample—ABS blank) ×100}/ABS control]

One mililitre of methanol plus 2.0 mL of the test extract was used as the blank while 1.0 mL of the 0.5 mM DPPH solution plus 2.0 mL of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard

Ferric reducing antioxidant power

The ferric reducing antioxidant power. The protocol involved is as follows:

Reagents:

a. Acetate buffer (300 mM), pH 3.6 (3.1 g sodium actate.$3H_2O$ and 16 mL glacial acetic acid in 1000 mL buffer solution).

b. 2, 4, 6-triphridyl-s-triazine (TPTZ) (10 mM) in 40 mM HCL.

c. $FeCl_3$ $6H_2O$ (20 mM) in distilled water.

FRAP working solution was prepared by mixing solution 1, 2 and 3 in the ratio of 10:1:1, respectively. The working solution was freshly prepared.

The FRAP reagent (3 mL) and 100 µl sample solution at concentrations of 25, 50, 100, 200 and 400 µg/mL was mixed and allowed to stand for 4 minutes. The absorbance was recorded at 593 nm, at 37°C. The ascorbic acid was tested in a parallel process. The absorbance of each test tube was taken at 0 and 4 minutes after addition of sample.

FRAP value = abs 4 minutes − abs 0 minute

Statistical Analysis

Data obtained were presented as mean (± S.E.M.) in tables and chart. They were analyzed using one-way analysis of variance (ANOVA) (SPSS software). The variant means were separated by Least Significant Difference (LSD) of the different groups. Significance was accepted at the level of $p < 0.05$.

RESULTS

Table 4.1: GC-MS result of acetone extract of P. nitida seed showing isolated compounds

S/No.	RT	%	Identified compound	Molecular formular	Molecular weight (g)
1	3.673	3.94	N-(2-Methylbutylidene) isobutylami	-	-
2	4.387	2.11	2-Methoxy-N-methylethylamine	-	-
3	5.796	50.77	Acetic acid, pentyl ester	$C_7H_{14}O_2$	130.1849
4	11.601	1.29	3-Hydroxy-7,8-dihydro-. beta. -ionol	$C_{13}H_{20}O_2$	208.2967
5	11.982	3.76	7-Oxabicyclo [4.1.0] heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	$C_{13}H_{22}O_3$	226.3120
6	13.068	4.65	Trimethylsilylpyrazole	-	-
7	14.539	0.59	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507
8	14.663	1.72	Palmitoleic acid	$C_{16}H_{30}O_2$	254.4082
9	14.854	12.61	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241
10	15.559	1.20	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.4614
11	16.311	6.04	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614
12	16.487	3.51	Cyclopropaneoctanal, 2-octyl-	-	-

13	18.140	6.19	Oxayohimban-16-carboxylic acid, 16,17-didehydro-19-methyl-, methyl ester, (19α, 20α)-	-	-
14	20.297	1.62	1H-Indole, 5-methyl-2-phenyl-	-	-

Table 4.2: GC-MS result of acetone extract of P. nitida stem bark showing isolated compounds.

S/No	RT	%	Identified compound	Molecular formular	Molecular weight (g)
1	3.663	2.80	N-(2-Methylbutylidene) isobutylami	$C_9H_{19}N$	141.25
2	4.901	5.91	1,2-Propanediol, 3-chloro-	$C_3H_7ClO_2$	110.539
3	5.625	33.74	Hexanal, 5-methyl-	$C_7H_{14}O$	114.1855
4	6.663	3.03	1,3-Disilacyclobutane, 1,1,3,3-tetramethyl-	$C_6H_{16}Si_2$	144.3622
5	11.735	6.56	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-	$C_{13}H_{20}O_2$	208.2967
6	13.054	3.00	3-(4-Fluoroanilino)-1-(3-nitrophenyl)-1-propanone	-	-
7	14.535	0.62	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507
8	14.844	11.65	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241

Table 4.3: GC-MS result of acetone extract of P. nitida leaf showing isolated compounds.

S/N	RT	%	Identified compound	Molecular formular	Molecular weight (g)

1	11.587	9.89	1,2,3,5-Cyclohexanetetrol, (1 α, 2 β, 3 α, 5 β)	$C_6H_{12}O_4$	148
2	12.773	2.15	Acetamide, 2,2,2-trifluoro-N-(1-methyl-1H-imidazol-2-yl)-	C_2H_5NO	59.06
3	13.897	40.67	2-O-Methyl-D-mannopyranosa	-	-
4	14.539	5.25	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507
5	14.854	6.11	n-Hexadecanoic acid	$C_{17}H_3O_4$	270
6	15.978	4.50	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.4879
7	16.135	23.92	Phytol	$C_{20}H_{40}O$	296.5310
8	16.211	7.51	Methyl stearate	$C_{19}H_{38}O_2$	298.5038

Table 4.4: Phytochemical screening of different parts of P. nitida plant

Phytochemicals	**Leaf**	**Seed**	**Stem bark**
Alkaloids	++	+++	++
Cyanogenic glycoside	-	-	-
Cardiac glycosides	-	-	-
Ascorbic acid	+	-	+
Saponin	+	+++	+
Steroids	+	+	+
Carbohydrate	+	+	++
Tannins	+	++	+

Terpenoids	+	-	+
Protein	+	-	+
Flavonoids	+	+	+
Fats and oil	-	+	-

Note: + mild; ++ moderate; +++ strongly present

DISCUSSION

The methanol leaf, seed, and stem-bark extracts of P. nitida underwent phytochemical analysis, which showed the presence of modest levels of flavonoids, ascorbic acid, terpenoids, and other compounds, along with a high concentration of alkaloids, saponins, tannins, and carbohydrates. The results of the phytochemical investigation appear to support the conclusions made in [5]. These phytochemicals function as potent antioxidants that shield cells from harm caused by highly reactive oxygen species or free radicals to red blood cells [19]. It was mentioned that flavonoids have biological properties that defend against cancers, hepatotoxins, allergies, free radicals, platelet aggregation, and microbes. The degree of hematinic activity in the various extracts may have been influenced by the presence of these phytochemicals.

CONCLUSION

The results show great therapeutic potential and support the traditional usage of Picralima nitida for its hematinic and hypoglycemic qualities. The plant extract's traditional use as a blood-boosting agent was supported by its effective counteraction of the hematological abnormalities caused by phenylhydralazine.

These results support the use of Picralima nitida in complementary and alternative medicine and add to the growing body of knowledge in the field of ethnopharmacology by highlighting the plant's potential as a natural treatment for diabetes and anemia.

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