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Phytochemical screening and GC-FID analysis of ethanolic extract of root bark of *Salacia nitida* L. Benth

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Abstract
This study was designed to determine the phytochemical compositions of the root bark of *S. nitida*, which is used for treatment of malaria and typhoid fever by the people of the Niger Delta of Nigeria, especially the Ogonis. Standard chemical methods were used for the screening and gas chromatography-flame ionization detector (GC-FID) instrument used for the analysis and quantification of phytochemicals present in the root bark. The phytochemical screening revealed the presence of alkaloid, tannin, saponin, phenol, anthocyanin, flavonoids, and absence of cardiac glycoside, while the results of GC-FID analysis revealed the presence of spartein (0.00172%), lunamarine (4.193%), %), ribalinidine (2.339%), tannins (6.104%), sapogenin (0.516%), epicatechin (7.295%), anthocyanin (0.3806%), catechin (37.553%), rutin (22.213%), kaempferol (18.561%), phenols (0.715%), and phytate (0.128%). The root bark of *S. nitida* contained pharmacologically active compounds which support its traditional use for the treatment of malaria and typhoid fever.

Keywords: GC-FID, ethanolic extract, phytochemicals, root bark, *Salacia nitida*

1. Introduction
Medicinal plants have made great contributions to human health which accorded plants as a source of novel drug compounds. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, etc [1, 2]. Medicinal plants are used as a common source for cures and preventing some diseases in traditional setting, especially in Africa [3, 4]. About 80% of the rural population in the developing world relies on traditional medicines for their health care. The presence of phytochemical compounds in medicinal plants has been reported [5-7]. In traditional medicines, *S. nitida* which is known as "Akorkon" in Khana (Ogoni) dialect is one of the plants used by the tribal people of Ogoni in the Niger Delta region of Nigeria for the treatment of typhoid fever and malaria.
Phytochemical screening is a qualitative test used to detect the present of secondary metabolites in plant materials and it is based on either formations of colour and/or precipitate. Analysis of phytochemicals by GC-FID is one of the modern techniques used to identify and isolate phytoconstituents. Phytochemical analysis of the extract of root bark will revealed its bioactive phytoconstituents, since no study on the phytochemical analysis of ethanolic extract of *S. nitida* is contained in any literature. Therefore, the present study is aimed to study the phytochemical screening and GC-FID analysis of ethanolic extract of root bark of *S. nitida*.

2. Materials and Methods
2.1 Collection and Preparation of Plant Material
This study was carried out in the month of April, 2016. *Salacia nitida* ("Akorkon") plant was collected from Wiiyor farmland in Nyogor-Beeri, Khana Local Government Area of Rivers State, South-South Nigeria, and was identified by Dr. N. L. Edwin-Wosu of the department of plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. The herbarium voucher number is UPHV-1033. Fresh plant roots were uprooted with a spade into bacco bag and taken to the laboratory, where they were properly washed in clean water and air dried. The barks were removed from the roots with knife onto a clean leather material. Part of the root barks were used for GC-FID phytochemical analysis, while the remaining portion of the root barks were then cut into smaller pieces with knife and air dried under shade for one week. The dried root barks were ground into powder material with a grinding machine (corona-16D).

2.2 Preparation of Ethanolic Extract

Extraction of the ethanolic extract of root bark of *S. nitida* was done using soxhlet extractor. 96.2g of the powdered material of root bark of *S. nitida* was carefully loaded in the main chamber of the soxhlet extractor and was added 50 ml of ethanol. Extraction was done at a temperature of 80 ºC using water bath for about 20 hours and then concentrated to dryness using same water bath at 80 ºC for about one week.

2.3 Phytochemical Analysis

2.3.1 Phytochemical Screening

Standard qualitative screening methods [6, 8, 9], were used to screen the phytochemical constituents present in the root bark of *S. nitida*.

2.3.1.1 Test for alkaloids: The test was carried out using Wagner's reagent, by adding 1.27g of iodine and 2g of potassium iodide to 100ml of distilled water and stirred. 2ml of the Wagner's reagent was added to a solution of the extract in a test tube. Formation of reddish-brown colour precipitate was observed.

2.3.1.2 Test for Tannins: 3ml of 10% alcoholic ferric chloride ($FeCl_3$) solution was added to a solution of the extract in a test tube. Formation of dark-blue colour compound was seen.

2.3.1.3 Test for Saponins: Few portion of the extract was vigorously shaken with 5ml of distilled water. Foam-like substance (froth) was formed. 3 drops of olive oil was then mixed with the froth and formation of emulsion was observed.

2.3.1.4 Test for Cardiac glycosides: 2ml of glacial acetic acid (CH_3COOH) containing a drop of ferric chloride solution was added to a few drops of the extract solution in a test tube. The mixture was carefully added to 1ml of concentrated sulphuric acid (H_2SO_4) in another test tube such that the conc. H_2SO_4 is directly beneath the mixture. No colour change was observed.

2.3.1.5 Test for Flavonoids: Few drops of 1% dilute ammonia solution (NH_4OH) were added to a small of the solution of the extract in a test tube, followed by the addition of few drops of conc. H_2SO_4 solution. Formation of a yellow colour was observed.

2.3.1.6 Test for Phenols: Few drops of 5% ferric chloride solution were added to few portion of the solution of the extract in a test tube and the formation of a greenish colouration was observed.

2.3.1.7 Test for Anthocyanin: 2ml of 2M sodium hydroxide (NaOH) solution was added to few extract in a test tube. The formation of blue-green colour compound was observed.

2.3.2 GC-FID Identification and quantification of Phytochemical Constituents

For the GC-FID analysis, fresh root barks of *S. nitida* were crushed in a container and 1g of the crushed sample was weighed and transferred into a test tube. 15 ml of ethanol and 10 ml of 50% w/v potassium hydroxide were added to the crushed root bark in the test tube. The test tube was allowed to stand in a water bath at 60 ºC for 60 minutes. Then the content of the test tube was carefully transferred into a separatory funnel and the tube rinsed into the same funnel with 10ml of cold water, 10ml of hot water, 20ml of ethanol and 3ml of hexane. The extract in the test tube was washed three times with 10ml of 10% v/v ethanol solution. The extract solution was then dried with anhydrous sodium sulphate and the solvent was evaporated. A sample of the extract was then made soluble in 100µl of pyridine of which 20µl was transferred into a vial on the Gas Chromatography machine for phytochemical analysis.

The GC-FID phytochemical analysis was performed on a BUCK M910 Gas Chromatograph (GC) (BUCK Scientific, USA), equipped with a flame ionization detector (FID). A RESTEK 15 meter MXT-1 column (15m x 250µm x 0.15µm) was used. The injector temperature was 280 ºC with splitless injection of 2 µl of sample and a linear velocity of $30cms^{-1}$, Helium 5.0 Pas was the carrier gas with a flow rate of $40mlmin^{-1}$. The oven operated initially at 200 ºC, it was heated to 330 ºC at a rate of 3 ºC min^{-1} and was kept at the temperature of 320 ºC.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals.

3. Results

Informations on the phytochemical constituents of plant materials are generally required for the discovery of novel drugs. The results of the phytochemical screening of ethanolic extract of root bark of *S. nitida* showed the presence of alkaloids, tannins, saponins, flavonoids, phenols, and anthocyanin, and absence of cardiac glycosides, which are compounds with different therapeutic effects.

Table 1: Results of the phytochemical screening test of ethanolic extract of root bark of *S. nitida*.

Phytochemicals	Result
Alkaloids	+
Tannins	+
Saponins	+
Flavonoids	+
Phenols	+
Anthocyanin	+
Cardiac glycosides	-

- = not present; + = present

The GC-FID analysis revealed the following sparteine, lunamarine, ribalinidine, tannins, sapogenin, epicatechin, anthocyanin, catechin, rutin, kaempferol, phenols, and phytate, as some of the compounds present in the extract of root bark of *S. nitida* (Figure 1.0 and table 2.0 below).

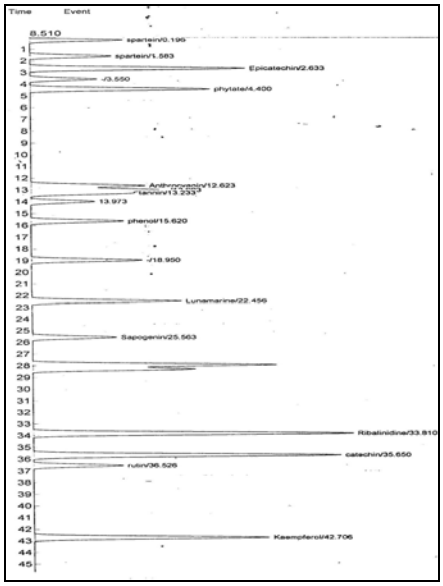

Fig 1: Chromatogram showing the phytochemical constituents of extract of *S. nitida*

Table 2: Phytochemical components identified in the root bark extract of *S. nitida* by GC-FID.

Phytoconstituents	PK	RT	Area	Height	Conc (µg/ml)	% composition
Spartein	1	0.196	3389.4154	303.261	0.0028	.
Spartein	2	1.583	4707.9376	267.691	0.0030	0.00172
Epicatechin	3	2.633	12159.6737	687.959	24.6059	7.295
Phytate	5	4.400	10204.4489	578.745	0.4325	0.128
Anthocyanin	6	12.623	6422.3298	370.657	1.2840	0.3806
Tannin	7	13.233	4546.7058	336.241	20.5901	6.104
Phenol	9	15.620	5349.4632	303.667	2.4127	0.715
Lunamarine	11	22.456	8544.8521	484.073	14.1437	4.193
Sapogenin	12	25.563	4879.4752	277.078	1.7417	0.516
Ribalinidine	15	33.810	18146.3787	1015.923	7.8886	2.339
Catechin	16	35.650	17401.4000	975.639	126.6694	37.553
Rutin	17	36.526	5140.1202	292.941	74.9289	22.213
Kaempferol	18	42.706	18247.1778	748.069	62.6072	18.561
Total					337.3104	

PK = Peak; RT = Retention time

4. Discussion

It has been reported that plants used for medicinal purposes are very rich in variety of bioactive compounds [2]. Alkaloids play some important metabolic role in living organisms, causing some physiological changes and are involved in protective function in animals, thus are used in making medicines. They have been shown to have important pharmacological functions such as anticancer, psychedelics and antimalarial [10], analgesic, antispasmodic and bactericidal [11, 12], antioxidant and stimulating activities [13]. Tannins, which are polyphenols, are importance because of their physiological potentials. They have been reported to exhibit antibacterial, antioxidants, antimicrobial, anti-inflammatory, antitumor, antivirus, antidiarrheal, antihaemorrhoid, and antimalarial activities [14-20]. Saponins are reported to exhibit broad range of pharmacological actions, such as ability to heal wounds and inflamed mucous membranes [12]. It also has anti-hyper cholesterol and haemolytic effects [21, 22]. The extract is rich in flavonoids, which are the most common polyphenols found in human diet and which have been implicated in many human diseases including lipid lowering, hepatoprotective, anti-inflammatory, antioxidant, antimalarial and antimicrobial activities by acting as antioxidant [23-31]. Anthocyanins are flavonoid found in virtually all vegetables, fruits and other plant parts and are reported to show antioxidant properties [32, 33]. They also exhibit anti-inflammatory activity [34]. Anthocyanins have been reported to play a beneficial role in visual acuity, treatment of cancer, heart disease, age-related neurodegenerative disorders and in angiogenesis [35]. Phenols are commonly found in plants and have diverse physiological functions, including anti-inflammatory, antioxidant and antimalarial activities [36-38].

Spartein, lunamarine and ribalinidine are quinoline alkaloids. Quinoline alkaloids are pharmacologically active compounds with biological activities such as antimalarial, anti-inflammatory, and antimicrobial [39, 40]. The quinoline alkaloids also have anti-protozoal, antioxidant and metal chelating activities [41, 42]. Lunamarine and ribalinidine have been reported to have radical scavenging function [43]. Lunamarine is also said to possessed anti-amoebic activity [44]. The flavonoid epicatechin, is a strong antioxidant [45], while catechin which is the major constituent of the extract is hemostatic in nature [46]. Rutin is digested in the body to quercetin, an antioxidant with antimicrobial activity [47]. Kaempferol has also been implicated with anti-microbial activity [48, 49]. Phytate has been shown to exhibit anti-inflammatory and cholesterol lowering effects [50]. It also act as an antioxidant and metal chelator [51-53].

The present of these phytoconstituents in the ethanolic extract of root bark of *S. nitida* showed that the plant part under investigation has therapeutic activity. The GC-FID elucidated bioactive compounds present in the root bark have been shown to possess antioxidant activities. So the root bark is very rich in secondary metabolites with therapeutic activity, and could be a good source of novel drugs. This justifies the use of the root bark of *S. nitida* in folk medicine for treatment of malaria, typhoid fever and other ailments.

5. Conclusion

The results of the present study on the phytochemical screening and GC-FID analysis of ethanolic extract of root bark of *S. nitida* showed that the plant extract contained some phytoconstituents which are pharmacologically important. This plant part could represent potential source of lead molecules with pharmacological activities for the development of new novel pharmaceutical products for treatment of malaria and other diseases. Also, the presence of compounds with biological activities justifies the traditional use of root bark of *S. nitida* for the treatment of malaria and other diseases. However, further studies into the isolation and identification of the individual bioactive compounds responsible for its therapeutic activity and the elucidation of their mechanism(s) of action is needed.

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