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

Barine I Nwiloh, Augustine A Uwakwe and Joyce O Akaninwor

**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 Wiyor 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 was 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 °C using water bath for about 20 hours and then concentrated to dryness using same water bath at 80 °C 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 (FeCl₃) 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 (CH₃COOH) 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 (H₂SO₄) in another test tube such that the conc. H₂SO₄ is directly beneath the mixture. No colour change was observed.

2.3.1.5 Test for Flavonoids: Few drops of 1% dilute ammonia solution (NH₄OH) were added to a small of the solution of the extract in a test tube, followed by the addition of few drops of conc. H₂SO₄ 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 °C 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 °C 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 °C, it was heated to 330 °C at a rate of 3 °C min⁻¹ and was kept at the temperature of 320 °C.

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*](image)

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
</tbody>
</table>

* = 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*](image)
The extract is rich in flavonoids, which are the most common polyphenols found in plants and are reported to show antioxidant properties [32, 33]. 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 antimarial activities [36-38]. Spartein, lunamarine and ribalinidine are quinoline alkaloids. Quinoline alkaloids are pharmacologically active compounds with biological activities such as antimarial, 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 and ribalinidine have been shown to exhibit anti-inflammatory and cholesterol lowering effects [39, 40]. 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 their therapeutic activity and the elucidation of their mechanism(s) of action is needed.

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

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>PK</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>Conc (µg/ml)</th>
<th>% composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spartein</td>
<td>1</td>
<td>0.196</td>
<td>3389.4154</td>
<td>303.261</td>
<td>0.0028</td>
<td></td>
</tr>
<tr>
<td>Spartein</td>
<td>2</td>
<td>1.583</td>
<td>4707.9376</td>
<td>267.691</td>
<td>0.0030</td>
<td>0.00172</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>3</td>
<td>2.633</td>
<td>12159.6737</td>
<td>687.959</td>
<td>24.6059</td>
<td>7.295</td>
</tr>
<tr>
<td>Phytate</td>
<td>5</td>
<td>4.400</td>
<td>10204.4489</td>
<td>578.745</td>
<td>0.4325</td>
<td>0.128</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>6</td>
<td>12.623</td>
<td>6422.3298</td>
<td>370.657</td>
<td>1.2840</td>
<td>0.3806</td>
</tr>
<tr>
<td>Tannin</td>
<td>7</td>
<td>13.233</td>
<td>4546.7058</td>
<td>336.241</td>
<td>20.5901</td>
<td>6.104</td>
</tr>
<tr>
<td>Phenol</td>
<td>9</td>
<td>15.620</td>
<td>5349.4632</td>
<td>303.667</td>
<td>2.1427</td>
<td>0.715</td>
</tr>
<tr>
<td>Lunamarine</td>
<td>11</td>
<td>22.456</td>
<td>8544.8521</td>
<td>484.073</td>
<td>14.1437</td>
<td>4.193</td>
</tr>
<tr>
<td>Sapogenin</td>
<td>12</td>
<td>25.563</td>
<td>4879.4752</td>
<td>277.078</td>
<td>1.7417</td>
<td>0.516</td>
</tr>
<tr>
<td>Ribalinidine</td>
<td>15</td>
<td>33.810</td>
<td>18146.3787</td>
<td>1015.923</td>
<td>7.8886</td>
<td>2.339</td>
</tr>
<tr>
<td>Catechin</td>
<td>16</td>
<td>35.650</td>
<td>17401.4000</td>
<td>975.639</td>
<td>126.6694</td>
<td>37.553</td>
</tr>
<tr>
<td>Rutin</td>
<td>17</td>
<td>36.526</td>
<td>5140.1202</td>
<td>292.941</td>
<td>74.9289</td>
<td>22.213</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>18</td>
<td>42.706</td>
<td>18247.1778</td>
<td>748.069</td>
<td>62.6072</td>
<td>18.561</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>337.3104</td>
<td></td>
</tr>
</tbody>
</table>

PK = Peak; RT = Retention time

### References

7. Sofowora A. Medicinal plants and traditional medicine in...


