Composition and antioxidant potential of leaf and stem essential oils from Nigerian *Indigofera spicata* Forssk

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The chemical compositions and antioxidant evaluation of the essential oils (EOs) obtained by hydrodistillation from the leaf and stem of *Indigofera spicata* Forssk (Fabaceae) grown in Nigeria have been studied. The EOs were analyzed using gas chromatography coupled with mass spectrometry (GC-MS) and the antioxidant potential was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability method. The essential oils from the leaf and stem obtained in 0.007% and 0.009% yield have been found to contain 18 and 17 compounds respectively. 13 compounds identified in the stem EOs make 90.2% of it. Sesquiterpenes (49.2%) and alcohols (30.7%) are dominant classes of compounds in the leaf EOs, with the most abundant compounds as caryophyllene (38.2%), humulene (6.2%) and m-eugenol (27.5%). Whereas esters (47.2%), and monoterpenoids (20.8%) dominate the stem essential oils with major constituents as linalyl acetate (23.9%), α-terpinyl acetate (12.8%), 3,5,5-trimethyhexyl acetate (9.4%), and linalool (20.8%). Common to both EOs were linalool, (leaf, 1.9%) caryophyllene (stem 5.9%), linanyl acetate (leaf, 2.5%). Comparison of the composition pattern of the leaf and stem EOs of *I. spicata* revealed significant qualitative and quantitative differences. Monoterpenes, sesquiterpenoid, diterpenoid, epoxide and ether were exclusive to the leaf oil while saturated, unsaturated hydrocarbons and anhydride were found only in the stem oil. The IC₅₀ values of antioxidant evaluations show the leaf EO (36.97 µg/mL) has more potential than the stem oil (39.89 µg/mL) and comparable to that of the controls Vitamin C and Butylhydroxyl Anisole with IC₅₀ values 24.20 and 24.21 µg/mL respectively. Most of these identified compounds have been known for various pharmacological activities such as antioxidant, antitumor, anti-inflammatory, even as fragrances and the antioxidant potential of the oils justify the ethno-medicinal uses of *I. spicata*.

**Key words:** *Indigofera spicata*, linalool, m-eugenol, caryophyllene, essential oil, sesquiterpene, antioxidant, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

**INTRODUCTION**

Essential oils research received prominent attention in natural product research due to its vast pharmacological importance. Even though, they represent a small fraction of plant’s composition, they confer the characteristics by which many aromatic plants are utilized in the food, cosmetic and pharmaceutical industries. Recent
researches into aromatic plants ascertain their age-long applications as antioxidant, antitumor, anti-inflammatory, and soothing effect. Even its fragrances are important in aromatherapy and their ability to prevent cardiovascular disease and cancer has recently been established (Miguel, 2010; Proestos et al., 2013). Oxidation is vital to many living organisms for metabolic processes. However, the uncontrolled production of oxygen-derived free radicals is involved in initiating many diseases such as arteriosclerosis, cancer, cirrhosis, rheumatoid arthritis as well as in degenerative processes associated with aging. Antioxidants are substances that prevent or delay the oxidation of the substrate even when present in low concentration in relation to the oxidant. Therefore, their presence is very important for healthy living. They are rich in phenolic substances, usually referred to as polyphenols, which are ubiquitous components of plants and herbs (Galego et al., 2008; Saleh et al., 2010).

Many ethnomedicinal plants of therapeutic importance have been widely unexplored for their essential oil constituents. Indigofera spicata, one of such plants has been identified and antioxidant activities of the essential oil composition of the leaf and stem have been carried out in this study.

I. spicata Forssk, specie of the Fabaceae family of plant is also known as “creeping or trailing indigo” belongs to the Leguminosae or legume family. It is the third largest family of flowering plants, comprising of over 650 genera and about 18,000 species (Bueno Pérez et al., 2013; Rahman et al., 2018). The Fabaceae are highly diverse, in general, they are characterized by the legume (pod) type of fruit that develops from a single carpel with marginal placentation (Rahman et al., 2018).

All species of this family possess characteristic fruits that are highly heterogeneous, typically dehiscent but occasionally indehiscent and are sometimes not easily recognized as part of the family. The flowers are also very dissimilar, commonly butterfly-like (papilionoid) with large or small petals but sometimes its petals are radially symmetrical and rose-like (non-papilionoid) (Schrire et al., 2009). Prominent only in tropical and sub-tropical climates, this species has been used for cover and erosion control in coffee, oil palm, rubber, sisal, and tea plantations. Indigofera spp. have been used widely as commercial dyes, for feeding livestock, and as ornamental and medicinal plants. The genus Indigofera is known for the medicinal importance due to a rich source of secondary metabolites such as flavonoids, triterpenoids, lignins and steroids (Rahman et al., 2018).

The ethno-medical uses of Indigofera spp. include the application of the crushed leaf to the skin to soothe itching, while the fruits are utilized for ophthalmic purposes, and the roots are employed for the treatment of poisoning (Nwachukwu and Mbagwu, 2006). Indirubin isolated from I. suffruticosa proved to be an excellent inhibitor in mice against lewis lung carcinoma and walker 256 carcinosarcoma (Bakasso et al., 2008). Indispicine isolated from both I. spicata and I. endecaphylla possess good hepatotoxic and teratogenic activity (Rahman et al., 2014; Rahman et al., 2018). While bovinicadin obtained from I. endecaphylla has showed moderate activity against mycobacterium tuberculosis (Rahman et al., 2018), louisfieserone isolated from I. suffruticosa has antibacterial stroke against vague gram-positive and gram-negative microorganisms (Rahman et al., 2018).

In countries of Africa, some Indigofera species are valued as insecticides and fish poisons (Lima et al., 2012). In addition, several Indigofera species (e.g., I. tinctoria L., I arrecta Hochst. ex A. Rich., and I. suffruticosa Mill.) have been used extensively to obtain indican, the source of the blue dye, indigo (Wahyuningsih et al., 2017). However, I. spicata Forssk, contains only low concentrations of indican, and is not grown commonly for this purpose (Bueno Pérez et al., 2013). Planting of I. spicata has been encouraged for erosion control and it is also a valuable fodder plant. If eaten in large quantities, however, it is reported to cause abortion in cattle and sheep (Lima et al., 2012). The roots of I. spicata have been used in the treatment of diarrhea, stomachache, cough, toothache, malaria, tinea nigra, meningitis, evil eye, headache, intestinal parasite, retained placenta, boils and external wounds in Ethiopian folklore medicine (Birhane, 2013; Giday et al., 2009).

Because of the vast potentiality of aromatic plants as sources of antioxidant activity, the present investigation was undertaken to determine the main constituents and the antioxidant activities of the leaf and stem of I. spicata essential oils.

**MATERIALS AND METHODS**

**Plant materials**

Fresh growing plant of I. spicata was collected, identified and authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria (3°55'2.3268"E, 7°24'7.063-2"N) in June, 2019, and a specimen with voucher number 112657 was equally deposited at the herbarium section of the institute. The plant was sorted into leaf and stem parts.

**Apparatus**

Temperature regulated heating mantle, Clevenger apparatus, water
Essential oils of the leaf and stem part were subjected to GC-MS analysis on an Agilent 7809A Gas Chromatography Mass Spectrometer having a split/splitless injector interfaced to mass selective detector operating at 70 eV. The ion source temperature was set to 200°C over a range of m/z 50-700 mass spectral at a scan rate of 1428 amu/s. The column used was HP-5MS with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The oven temperature was programmed as follows: initial temperature 80°C for 2 min, increased at 10°C/min to a temperature of 240°C for 6 min. The carrier gas (Helium) was at a 1 mL/min flow rate. Injection volume for 1 mL was 1.0 µL, 56.2 KPa and 362 cm/s respectively. The oven temperature was set at 60°C, hold for 1 min to 180°C for 3 min at 10°C/min, the final temperature was 280°C for 2 min at 10°C/min both the injector and detector temperatures were fixed at 250°C.

Table 1 shows the retention time (Figures 1 and 2), structure, mass spectra data and identities of the EOs constituents. Not less than 18 and 17 compounds made up of 1000, 500, 250, 125, 62.5 and 31.25 µg/mL of the essential oils and crude extract were mixed with 100 µM methanolic DPPH solution prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol to give a purple colour solution. The mixture was vigorously shaken and left to incubate in the dark for 20 min. The absorbance at 517 nm was recorded as Abs (sample) using a GS UV -12, UV-Vis spectrophotometer. In its radical form, DPPH absorbs but upon reduction by antioxidant species, its absorption reduces. A blank experiment was carried out applying the same procedure but without the sample (DPPH and Methanol) and the absorbance was recorded as Abs (control). Each experiment was done in triplicates and the antioxidant potentials of the essential oils were calculated as percentage inhibition according to the formula;

\[
\% \text{ Inhibition} = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}} \times 100
\]

Where: Abs (Control) = Absorbance of control (that is, without sample); Abs (Sample) = Absorbance of sample.

The antioxidant activity of Ascorbic acid and Butylatedhydroxylanisole (BHA) were used as standards for comparison.
up the essential oils of the leaf and stem respectively. All the compounds in the leaf oil were identified. However, in the oil of the stem, 13 compounds were identified making 90.2% of it.

The most abundant compounds in the leaf essential oil were caryophyllene (38.2%), m-eugenol (27.5%), humulene (6.2%), eugenol acetate (4.7) \(\sigma\)-Cadinene (3.4%) 3-allylguaiacol (3.2%) (Table 2).

In the stem essential oil, the most abundant compounds were linalyl acetate (23.9%), linalool (20.8%), \(\alpha\)-terpinyl acetate (12.8%), 3,5,5-trimethylhexyl acetate (9.4%), caryophyllene (5.9%), iso-caryophyllene (3.8%), i-docosene (3.8%) and (5\(\alpha\),13\(\alpha\))-homoandrostan-3(7)-one (3.7%) (Table 2).

### Class of compounds in leaf and stem essential oils of *Indigofera spicata*

Leaf EO is rich in sesquiterpenes (49.2%) and alcohols (30.7%) whereas esters (47.2%), and monoterpenoids (20.8%) dominate the stem essential oils; this class of compounds are also found in an appreciable amount in the two EOs (Table 3). Prominent sesquiterpenes found in the EOs of the leaf were caryophyllene (38.2%), humulene (6.2%), and \(\sigma\)-Cadinene (3.4%) while cadina-1(6),4-diene (0.4%), \(\alpha\)-cadinaene (0.6%), \(\alpha\)-farnesene (0.6%) were found in minute proportion.

This result is consistent with report on the essential oil procured from *Indigofera microcarpa*, which has caryophyllene and-humulene-sesquiterpenes which has major constituents (Arriaga et al., 2008). Presence of esters of fatty acids were also reported in the oil of *Indigofera suffruticosa* (Arriaga et al., 2013). However, unlike the oils from I. suffruticosa which has the linear diterpenes (78.5%) as the most abundant class of compounds (Arriaga et al., 2013), diterpenoids found in the oil of I. spicata leaf was only 3% of the essential oil composition. This shows the chemical variations in the oils of the different species of the genus Indigofera. Caryophyllene, the major sesquiterpene have been reported to have strong antitumor activity (Ferraz et al., 2013). The principal composition of the alcohols found in the leaf EOs was m-eugenol (27.5%) and 3-allylguaiacol (3.2%). Oxygenated sesquiterpene found in leaf oil were caryophyllene oxide (1.1%) and 6,10,14-trimethyl 2-pentadecanone (0.8%). While monoterpenoid has only linalool (1.9%) as the component found in the leaf oil, \(\alpha\)-terpinolene (2.5%) was the only monoterpene identified in the leaf oil (Table 2).

![GC-MS chromatogram of *Indigofera spicata* leaf essential oil.](image)
The stem oil is rich in esters and monoterpenoid component with major ester constituents as Linalyl acetate (23.9%) α-Terpinyl acetate (12.8%) and 3,5,5-Trimethylhexyl Acetate (9.4%) while the only monoterpenoids identified in the stem EO was Linalool (20.8%). Caryophylene 5.9% and isocaryophylene (3.8%) were the only sesquiterpenes found the stem EO.

Comparison of the composition pattern of the leaf and stem EOs of *I. spicata* revealed significant qualitative and quantitative differences. Monoterpenes, sesquiterpenoid, diterpenoid, epoxide and ether were exclusive to the leaf oil while saturated unsaturated hydrocarbons and anhydride were found only in the stem oil (Table 3).

Monoterpenes are known to suppress the assemblage of toxins in biological system (SBI, 2017). This terpene is present in an appreciable amount in stem essential oils. Esters dominate stem oil as they are essential constituents of perfumes, cosmetics, food flavours, and surfactants e.g. in soap and detergents.

Antioxidants activities of essential oils of *Indigofera spicata*

The DPPH test provides information on the reactivity of the test oils with a stable free radical. The change in color of DPPH from purple to yellow suggests the ability of these oils to act as donors of hydrogen atoms or electrons in the transformation of DPPH into its reduced form DPPH-H. Percentage inhibition and IC50 values were obtained for each part (Figure 3). The assay yielded IC50 of 36.97 and 39.89 µg/mL for leaf and stem EOs respectively. The leaf oil has the highest antioxidant activity while the stem gave a relatively high activity. Both possessed relatively better activity compared to the antioxidant potential reported for the extract from the aerial part of this plant (47.13 µg/ml) (Bitew et al., 2018).

The antioxidant potential was concentration-dependent. Percentage inhibitions of each oil sample were calculated from the absorbance (Figure 3).

The antioxidant assay of *I. spicata* EOs shown in Figures 3 and 4 indicates the activities of the tested oils and standard compounds which increases as follows: Vitamin C>BHA>Leave oil> Stem oil with IC₅₀ values in order 24.20, 24.21, 36.97 and 39.89 µg/mL (Figure 4). Ascorbic acid showed significantly higher activity (93.05 - 90.40) compared to others at the tested concentration of 1000 - 31.5 µg/mL, followed by BHA which showed better activity at 92.60%.

Both essential oils of *I. spicata* show good percentage
## Table 2. Essential oil composition of leaf and stem part of *Indigofera spicata*.

| S/N | RT (min) | Structure of identified compounds | Identified compounds | KIa | %TIC Leaf | %TIC Stem | Class of compounds | MS |
|-----|----------|----------------------------------|----------------------|-----|-----------|-----------|-------------------|----|
| 1   | 3.5      | ![Linalool](image)               | Linalool             | 1100| 1.9       | 20.8      | Monoterpenoid     | 71,93,55,43,69 |
| 2   | 4.5      | ![3,5,5-Trimethyhexyl Acetate](image) | 3,5,5-Trimethyhexyl Acetate | 1162| -         | 9.4       | Esters            | 57,70,43,115,61 |
| 3   | 5.6      | ![Linalnyl acetate](image)       | Linalnyl acetate    | 1257| 2.5       | 23.9      | Esters            | 93,43,80,121,69 |
| 4   | 6.9      | ![α-Terpinolene](image)         | α-Terpinolene       | 1297| 2.5       | -         | Monoterpenone     | 161,105,119    |
| 5   | 6.9      | ![α-Terpinyl acetate](image)    | α-Terpinyl acetate  | 1332| -         | 12.8      | Esters            | 43,121,93      |
| 6   | 7.2      | ![m-Eugenol](image)             | m-Eugenol           | 1339| 27.5      | -         | Alcohol           | 164,149,103,137|
| 7   | 7.3      | ![3-Allylguaiacol](image)       | 3-Allylguaiacol     | 1362| 3.2       | -         | Alcohol           | 164,149,77,103 |
| 8   | 7.6      | ![Isocaryophyllene](image)      | Isocaryophyllene    | 1407| -         | 3.8       | Sesquiterpene     | 41,93,79,133,55 |
| 9   | 7.9      | ![Caryophyllene](image)         | Caryophyllene       | 1419| 38.2      | 5.9       | Sesquiterpene     | 93,133,79,69,41 |
| No. | Time (min) | Retention Time (s) | Component Name                  | Mass (amu) | Relative Intensity (%) | Type          | Molecular Formula  |
|-----|------------|--------------------|---------------------------------|------------|------------------------|---------------|-------------------|
| 10  | 8.3        |                    | Humulene                       | 1449       | 6.2                    | Sesquiterpene | 93,121,80,41      |
| 11  | 8.5        |                    | Cadina-1(6),4-diene            | 1523       | 0.4                    | Sesquiterpene | 161,105,204,119   |
| 12  | 8.6        |                    | α-Cadinene                     | 1526       | 0.6                    | Sesquiterpene | 105,161,204,94,41 |
| 13  | 9.0        |                    | α-Farnesene                    | 1528       | 0.6                    | Sesquiterpene | 4193,69,55,102    |
| 14  | 9.2        |                    | σ-Cadinene                     | 1524       | 3.4                    | Sesquiterpene | 161,105,81,204,91 |
| 15  | 9.3        |                    | Eugenol acetate                | 1541       | 4.7                    | Esters        | 164,149,43        |
| 16  | 9.9        |                    | Caryophyllene oxide            | 1570       | 1.1                    | Sesquiterpenoids | 43,79,93,109   |
| 17  | 11.4       |                    | Pentadecanal                   | 1695       | 3.1                    | Aldehydes     | 82,57,43,85,97    |
| 18  | 11.7       |                    | UI                             | ND         | 2.3                    | Aldehydes     | 57,71,43,85,97    |
| 19  | 12.4       |                    | 2,6,11-Trimethyl dodecane      | ND         | 0.8                    | Saturated Hydrocarbon | 71,57,43,85 |
| 20  | 12.8       |                    | UI                             | ND         | 2.8                    | Sesquiterpenoids | 85,57,43        |
| 21  | 12.8       |                    | 6,10,14-Trimethyl 2-pentadecanone | 1832   | 0.8                    | Sesquiterpenoids | 85,57,43        |
Table 2. Cont’d.

|   | RT (min) | % TIC   | Compound Description | Retention Time | % Identified | % Unidentified (UI) | Total (%) |
|---|---------|---------|-----------------------|----------------|--------------|---------------------|-----------|
| 22 | 13.0    | 1966    | 2-Dodecen-1-yl(-) succinic anhydride | -              | 1.9          | Acid anhydride       | 41,55,69,83,97 |
| 23 | 13.4    | ND      | 1-(Ethenyloxy)-cis-1,2-cyclododecane | 0.3            | -            | Vinyl ether          | 57,43,69,83,97 |
| 24 | 13.4    | 1909    | Cis,cis,cis-7,10,13-hexadecatrienal | 0.3            | -            | Aldehydes            | 79,67,41  |
| 25 | 13.6    | ND      | (5α,13α)-D-homoandrostan | -              | 3.7          | Saturated Hydrocarbon | 95,55,69,259 |
| 26 | 13.8    | UI      | 1-Decanol,2-hexyl       | ND             | -            | 2.8                 | 95,259,109,55,69 |
| 27 | 14.4    | UI      | Phytol                 | ND             | -            | 1.9                 | 57,83,97  |
| 28 | 15.4    | ND      | 1-Decanol,2-hexyl       | ND             | -            | 0.8                 | 57,43,69,83,97 |
| 29 | 15.5    | 2099    | Phytol                 | -              | 3.0          | -                   | Diterpenoid |
| 30 | 16.3    | -       | 1-Decosene             | -              | 3.8          | Hydrocarbons         | 57,43,97,83,69 |
| 31 | 16.6    | ND      | Tetratriacontylpentfluoropropionate. | -              | 1.2          | Esters               | 57,71,97,43,83 |
| 32 | 17.5    | 2324    | Tricosane              | -              | 1.5          | Hydrocarbons         | 57,43,71,85,99 |

% Identified 100.0  90.2
% Unidentified (UI) - 9.8
Total (%) 100.0  100.0

RT: Retention time in minutes. %TIC: Percentage Total ion concentration in; UI = unidentified compound; MS: mass to charge values of fragment ions with base peak 1st stated, and other most prominent ions. #: Kovat Index; ND: Not determined.
### Table 3. Class of organic compounds in leaf and stem EOs of Indigofera spicata.

| Class of organic compounds         | Amount in the essential oil (%) | Leaf | Stem |
|-----------------------------------|---------------------------------|------|------|
| Monoterpene                       | 2.5                             |      |      |
| Monoterpenoid                     | 1.9                             |      | 20.8 |
| Sesquiterpene                     | 49.2                            |      | 10.0 |
| Sesquiterpenoids                  | 1.9                             |      |      |
| Diterpenoid                       | 3.0                             |      |      |
| Esters                            | 7.2                             | 47.2 |      |
| Alcohol                           | 30.7                            |      | 0.8  |
| Aldehydes                         | 3.4                             |      |      |
| Saturated Hydrocarbon             |                                 | 6.0  |      |
| Unsaturated Hydrocarbons          |                                 | 3.8  |      |
| Acid anhydride                    |                                 | 1.9  |      |
| Vinyl ether                       | 0.3                             |      |      |
| UI                                |                                 | 9.88 |      |

UI = unidentified compound

### Figure 3. DPPH scavenging potential of essential oils from leaf and stem of Indigofera spicata.

The graph shows the % inhibition of DPPH scavenging potential at various concentrations (µg/mL) for leaf and stem oils, as well as VITC and BHA as controls. The graph demonstrates a high antioxidant inhibition which is comparable to the controls used. Eugenol, which has been reported to have very good free radical scavenging activity by Gülçin (2011) dominates the leaf essential oil of *I. spicata* and may be...
Figure 4. IC\textsubscript{50} values of essential oils from leaf and Stem of \textit{Indigofera spicata}. 

Conclusion

The chemical compositions of the essential oils reported in this study are unique and show the leaf oil is sesquiterpene rich while the stem oil is rich in esters and monoterpenoids. The observed antioxidant activity of the oils is an indication that they can be important in combating several free-radical mediated diseases and this may be attributed to the vast ethno-medicinal applications of \textit{I. spicata}.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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