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# Volatile constituent of sea purse, *dioclea reflexa* root

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**ABSTRACT**

Volatile oils of *Dioclea reflexa (Fabaceae*) roots were obtained by hydrostillation and analysed using GC and GC-MS. A total of 15 compounds were identified; hydrocarbons being the dominating group of the compounds representing 69.27 %. Oxygenated monoterpenoids identified included terpinen-4-ol (2.47 %), myrtenal (1.69 %), verbenone (1.25 %), trans-pinocarveol (2.86 %) and thymol (1.30 %); also stearyl iodide (10.33 %) was identified.



## Introduction

Essential oils are volatile, natural mixtures of complex compounds, mainly monoterpenoids and sesquiterpenoids, characterized by strong odour and possess some therapeutic properties [1]. Their utilization is influenced by the nature of their constituents which has widespread application in the pharmaceutical, agricultural, flavour and fragrant industries. In agriculture, they are used as food preservers and additives and as natural remedies, owing to their notable antimicrobial and antioxidant properties [2]. Essential oils extracted through steam distillation from aromatic and medicinal plants are well known for spasmolytic, carminative, antiviral, anticarcinogenic or hepatoprotective [3,4,5,6]. They also possess therapeutic properties such as anaesthetic (e.g *Peppermint*), anti-asthmatic (e.g. *Cupressus lusitanica*), anti-venomous (e.g. *Ocimum gratissium*) and anti-hypertension (e.g. *Hyssopus officinalis*) etc [1].

*Dioclea reflexa* (*Fabaceae*) is a woody climber with foliates phyllotaxy. Their bat-pollinated flowers and pods are produced on long, rope-like stems that hang from the forest canopy. The seed pods are covered with microscopic velvety hairs. In the Caribbean region and Central American, the hairs were stirred into honey or syrup as a remedy to dispel intestinal parasites. The seeds are also known as 'sea purse' because they are commonly carried by rivers into the ocean [8,9]. It is known as "Agbaarin" among the Yorubas of the western Nigeria, where it is used traditionally for treatment of various ailments such as backache, symptoms of stomach ulcers, asthma, head-lice, stimulant, dandruff and tuberculosis [19]. Extracts from the leave of this specie has shown pharmacological properties such as antimicrobial activity [7]. There is no previous report on the chemical analysis of essential oils from *Dioclea reflexa*. The study, therefore, was designed to analyse the essential oils from the root of the plant. Thus, we are reporting the volatile constituent of *D. reflexa* root for the first time.

## Experimental

### Plant Material

Root of mature and well established *D. reflexa* plant was collected from Eruwa township in Ibarapa East Local Government area of Oyo state, Nigeria (N 7°36', E 3°28'), in the month of October, 2010, and identified by a plant Taxonomist, at the Department of Botany and Microbiology, University of Ibadan, Nigeria. The root was cleaned, chopped and air dried at room temperature for 4 weeks.

### Extraction

The root (300 g) was hydrodistilled for 4 hours in an all-glass Clevenger-type apparatus in accordance with the British pharmacopoeia method [10]. The weighed oil collected in well capped bottle was dried over anhydrous Sodium sulphate and stored at 4 °C in the dark prior to analysis.

### Gas Chromatography (GC) Analysis

GC analysis of the oil extract was performed using a Shimadzu model QP 2010 chromatograph. An HP-Innowax FSC column (30 m x 0.25 mm, with 0.25 µm film thickness) was used with Helium as carrier gas at a flow rate of 1 ml/min. The GC oven temperature was kept at 60°C (hold for 0 min), and programmed to reach 140°C at a rate of 5°C /min, then kept constant at 280°C for 10 min being the final hold time. The split ratio was adjusted to 50:1. The injector temperature was set at 200°C.

### GC-MS Analysis

GC-MS analysis was carried out using a Shimadzu model QP 2010 chromatograph system with split/split less injector to a 5973 mass selective detector. HP-Innowax FSC column (30 m x 0.25 mm, with 0.25 µm film thickness) was used with Helium as carrier gas (1 ml/min). The GC operating parameters were identical with those of the GC-analysis above. Mass spectra were recorded at 70 eV. The acquisition mass range was 30-500 m/z. Data was analysed using MSD ChemStation software.

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**Identification of constituents**

Identification of constituents was done by comparing the retention times and mass spectra of the chromatographic peaks with those of standards analysed under the same conditions. The peak assignment of the other volatile components was based on computer matching of the mass spectra obtained with the WILEY 275, NIST 08 and ADAMS libraries, taking into account the coherence of the retention indices of the analysed compounds with those reported by Adams and NIST08 libraries [11, 12].

**Result and discussion**

The essential oil yield based on the dry weight of the sample was 0.56 %. From the volatile oils obtained by hydrodistillation, a total of 15 compounds were identified representing 89.17 % with analysis of GC and GC-MS data (Table 2). The volatile oil constituents were dominated by hydrocarbons (69.27 %); such as heptacosane (10.40 %), octacosane (13.42 %), triacontane (8.99 %), hexacosane (8.41 %), pentacosane (6.44 %), nonacosane (4.84 %), tetracosane (3.61 %) and heptacosane (2.09 %). These oils also contained a number of oxygenated monoterpenoids including trans-pinocarveol (2.86 %), terpinen-4-ol (2.47 %), myrtenal (1.69 %), thymol (1.30 %) and verbenome (1.25 %). Other constituents found in high quantities are stearyl iodide (10.33 %) and 3 unidentified compounds (5.75 %).

Monoterpenoids are known to exhibit a diverse array of pharmaceutical and therapeutic properties. Quantity of monoterpenoids in this specie may be more relevant in cases where plant parts and preparation are applied topically or mixed with animal fat to form ointments; particularly as monoterpenoids, in addition to any direct pharmacological actions, are known to permit or enhance trans-dermal penetration of other non-lipophilic compounds and drugs which do not normally penetrate the layers of the skin [13].

Some of the constituents identified (e.g. terpinen-4-ol, thymol) have been shown to be an effective antimicrobial, especially antifungal and antiviral [13,14,16]. Research also shown that myrtenal and verbenome are effective in treatment of Alzheimer's disease [15]. Terpinen-4-ol has been demonstrated to be a potential anticancer drug [18] also used in flavour and fragrance for citrus and spice types including reconstitutions of essential oils such as lavender and geranium [17].

In conclusion, *D. reflexa* essential oils could hold promise for future application in therapy as an anti- anticancer agent, antimicrobial, especially antifungal and antiviral, in addition to the food and flavour and fragrance industry.Given the potential for economic interest in this plant and its continuing widespread traditional medicinal use, scientific evaluation of its active principles should be given consideration. This would involve characterization and elucidation of bioactive constituents responsible for therapeutics properties. This data provides information on the essential oil constituents of *D. reflexa* root collected in Nigeria, which hitherto unavailable.

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**TABLE 1. Percentage composition of volatile constituents of *D.reflexa* root.**

| No | Components | Retention Time (min) | % composition |
|---|---|---|---|
| Hydrocarbons | | | |
| 1. | Heptadecane | 23.27 | 2.09 |
| 2. | Tetracosane | 24.27 | 3.61 |
| 3. | Pentacosane | 26.38 | 6.44 |
| 4. | Hexacosane | 27.85 | 8.41 |
| 5. | Heptacosane | 29.27 | 10.40 |
| 6. | Octadecane | 30.63 | 11.07 |
| 7. | Triacontane | 33.35 | 8.99 |
| 8. | Octacosane | 36.09 | 13.42 |
| 9. | Nonacosane | 39.73 | 4.84 |
| Oxygenated Monoterpenes | | | |
| 10. | Terpinen-4-ol | 2.88 | 2.47 |
| 11. | Myrtenal | 2.95 | 1.69 |
| 12. | Verbenome | 3.05 | 1.25 |
| 13. | Trans-pinocarveol | 2.56 | 2.86 |
| 14. | Thymol | 32.68 | 1.30 |
| Others | | | |
| 15. | Stearyl Iodide | 31.95 | 10.33 |
| 16. | Unidentified compound | 18.69 | 3.32 |
| 17. | Unidentified compound | 28.66 | 1.26 |
| 18. | Unidentified compound | 32.40 | 1.17 |
| Total | | | 94.92 |