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Extraction, Characterization, and Antioxidant Properties of *Salacia Pallescens* Essential Oil

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Abstract

Introduction: *Salacia pallescens*, a member of the Celastraceae family, has been traditionally used for its anti-inflammatory effects, but limited scientific evidence supports this claim. The high rate at which natural antioxidants have drawn attention to medicinal plants as safe, cost-effective and valuable sources of bioactive compounds.

Aim: The present study focused on the extraction of the essential oils of *S. pallescens* and its antioxidant potential. using the hydro-distillation method, characterisation of the essential oils

Methods: The plant material was collected, authenticated, and air-dried to remove the moisture content. The air-dried sample was weighed and subject to hydrodistillation using an all-glass Clevenger apparatus. The essential oil was characterized with a Gas Chromatography–Mass Spectrometer (GC-MS), and evaluation of the antioxidant properties were evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl), Nitric Oxide (NO), and Reducing Power assays.

Results: A greenish-yellowish oil with a yield of 0.25% was obtained having an aromatic odour. The essential oil components yielded eleven components corresponding to 98.2% of the total oil. The essential oil is dominated by 9-Nonadecene(44.66%), Oleic acid (21.42%), and Propanamide, 2,2-dimethyl-N-(3-methylphenyl) (13.72%). The constituents of the essential oils are majorly non-terpenoids. The antioxidant assays showed that the essential oils inhibited free radicals and the oxidant in a dose-dependent pattern.

Conclusion: The findings from this research validate the medicinal use of Salacia pallescens, promote its pharmaceutical relevance, and support the development of plant-based antioxidants.

Keywords: *Salacia pallescens*, antioxidant, 9-nonadecene, phytochemicals, essential oils, Nitric oxide

All co-authors agreed to have their names listed as authors.

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1.0 INTRODUCTION

Salacia pallescens, a member of the family Celastraceae, is a medicinal plant indigenous to parts of West Africa, especially in Nigeria, Ghana, Benin, Côte d'Ivoire, and parts of Cameroon, where it is widely used in traditional medicine for the treatment of fever, pain, inflammatory conditions, and other ailments [1]. In many Yoruba communities of southwestern Nigeria, the plant is popularly known as Elewekan. The genus Salacia consists of more than 200 species, many of which have been recognised for their medicinal importance due to the presence of biologically active phytochemicals with antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and anticancer properties [2-3].

Morphologically, *S. pallescens* is characterised by a woody stem with smooth to slightly rough bark and an extensive branching pattern. The leaves are simple, green, glabrous, and arranged alternately on the stem. They are generally elliptic to oblong in shape with entire margins, pinnate venation, and short petioles. The plant bears small, greenish to yellowish flowers which later develop into fleshy fruits containing seeds that facilitate natural propagation. *S. pallescens* produces a variety of secondary metabolites, including terpenoids, flavonoids, phenolic compounds, alkaloids, tannins, and other volatile constituents that contribute to its medicinal value [4]. These phytochemicals have attracted considerable scientific interest because of their potential applications in the development of natural antioxidant and therapeutic agents. Certain species of Salacia were reported to contain essential oil components. *S. senagalensis* is dominated by α-Terpinene (13.8 %), Germacrene-D (12.4 %), and α-phenandrene (11.6 %)[5]. Essential oil components in the methanolic extracts of *S. pallenscens* are thymol (29.79%), followed by 3-carene (15.97%), p-cymene (12.19%), and caffeine (8.28%)[6]. Therefore, terpenoids in the essential oils *S. pallenscens* are yet to be analysed and reported. This study investigates the essential oil components and the antioxidant potential using several models.

2.0 Methodology

2.1 Collection, Preservation, and Authentication of Plant Material

2.1.1 *Plant Collection and Taxonomy*

Fresh leaves of *S. pallescens* were collected from Idi Ayunre, Ibadan, Oyo State, Nigeria, during the appropriate harvesting season. The collected plant specimen was authenticated by a qualified taxonomist in the Department of Botany, Lagos State University, Ojo, Lagos State, Nigeria. A voucher specimen was prepared and deposited in the departmental herbarium for future reference. Authentication of the plant material ensured the correct botanical identity of the species used throughout the study and enhanced the scientific reliability and reproducibility of the research.

2.1.2 *Plant preparation*

The freshly collected leaves of *S. pallescens* were thoroughly washed with clean water to remove dust, soil particles, and other foreign materials. The leaves were subsequently air-dried in the shade at room temperature for an appropriate period until a constant weight was obtained. After drying, the leaves were pulverised to increase the surface area for extraction. The prepared plant material was stored in clean, dry, airtight containers until required for essential oil extraction.

2.1.3 *Essential Oil Extraction*

The essential oil was extracted from the prepared leaves of *Salacia pallescens* using the hydrodistillation method with a Clevenger-type apparatus. 300 g of the air-dried plant material was placed into a round-bottom flask, and an adequate volume of distilled water was added to immerse the plant material completely according to the modified method of Avoseh et al[7].

2.2 Characterisation of the essential oils

2.2.1 ***Gas chromatography***

The essential oil was comprehensively analysed for its constituents with the aid of gas chromatography-flame ionisation detector (GC-FID) and gas chromatography- mass spectrometry (GC/MS). Gas chromatography (GC-FID) analysis was accomplished with

an HP-5890 Series II instrument equipped with an HP-Wax and HP-5 capillary column (both 30 m x 0.25 mm, 0.25 µm film thickness), working with the following temperature program: 60 °C for 10 min, rising at 5 °C / min to 220 °C. The injector and detector temperatures were maintained at 250 °C; carrier gas was nitrogen (2 mL/min); detector: FID; split ratio 1:30. The volume injected was 0.5 µL. The relative proportions of the oil constituents were percentages obtained by FID peak area normalisation.

2.2.2 Mass Spectrometry

Gas chromatography-mass spectrometry (GC-EIMS) analysis was performed with a Varian CP-3800 gas chromatograph equipped with an HP-5 capillary column (30 m x 0.25 mm; film thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures of 220 °C and 240 °C, respectively; oven temperature programmed from 60-240 °C at 3 °C /min; carrier gas helium at a flow rate of 1 mL/min. Injection volume 0.2 µL (10% n-hexane solution); split ratio 1:30. Mass spectra were recorded at 70 eV. The acquisition mass range was m/z 30-300 at a scan rate of 1 scan/sec. The identification of the constituents of the *S. pallescens* was based on a comparison of the retention times with those of authentic samples, comparing their linear indices relative to a series of n-alkanes (C6-C36).

3.5 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

2.3 Antioxidant analysis

2.3.1 DPPH scavenging Assay

0.1g of the extract was mixed with 25ml 0f 99% methanol and covered; the mixture was placed in a shaking water bath of 100 rpm at room temperature and left for 2 ½ h. 4 g of 1 M DPPH was mixed with 100ml of 99% methanol in a separate beaker and covered with foil. The sample solution was poured into different sample bottles and placed in a centrifuge of 6000 to 7000 rpm for 20 minutes. The top solution was filtered. A solution series from 1ml to 5ml was prepared using the residue and methanol. 3 mL of DPPH solution was added to each of the extract series

They were made up to 10ml using 99% methanol. The fraction series were kept in the dark for 10 minutes, and the spectroscopic analysis was done using ascorbic acid as the

standard at different concentrations (20, 40, 60, 80 and 100) ug/mL at 517nm of wavelength[8].

2.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

0.1 mg of the plant extract was dissolved in methanol. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl3 in a 10:1:1 (V/V). A reaction mixture containing 1 mL of extract solution and 3 mL of FRAP reagent was incubated at 37°C for 10 minutes, after which absorbance was measured at 593 nm. Ascorbic acid (20, 40, 60, 80, and 100) µg/mL served as the standard, and antioxidant power was expressed relative to the standard curve [9].

2.3.3 Nitric Oxide Radical Scavenging Assay

0.1 mg of the plant extract was dissolved in methanol. The reaction mixture contained 1 mL of extract solution and 2 mL of 10 mM sodium nitroprusside prepared in phosphate buffer (pH 7.4) and was incubated at 25°C for 150 minutes. After incubation, 1 mL of the reaction mixture was combined with 1 mL of Griess reagent, allowed to stand for 10 minutes, and absorbance was measured at 546 nm. Ascorbic acid (20, 40, 60, 80, and 100) µg/mL was used as the reference standard, and percentage nitric oxide scavenging activity was calculated [10].

3.0 RESULTS AND DISCUSSION

3.1 Chemical constituents of the essential Oils

In this study, a pale-yellow oil with an aromatic odour was obtained at a yield of 0.25% v/w, indicating a relatively low plant oil yield. Characterisation of the essential oil yielded a total of 12 components, resulting in 99.75% of the total oil components, as shown in **Table 1** below. The oil is dominated by the presence of several volatile compounds with varying relative abundances. The identified constituents consisted mainly of long-chain hydrocarbons, fatty acids, phenolic compounds, alcohols, and other oxygenated organic compounds, indicating that the essential oil contains chemically diverse bioactive constituents.

The predominant compound identified in the essential oil was 9-Nonadecene, representing 44.66% of the total identified constituents. This high percentage suggests

that 9-Nonadecene is the principal volatile constituent of the essential oil and may contribute significantly to its physicochemical and biological properties. Long-chain alkenes have been reported to exhibit antimicrobial and antioxidant activities, although their biological effects are often enhanced through interactions with other constituents present in essential oils. The second most abundant compound was Oleic acid (21.42%), a monounsaturated fatty acid widely recognized for its antioxidant, anti-inflammatory, and cardioprotective properties. Oleic acid has been reported to reduce oxidative stress by modulating lipid oxidation and protecting biological membranes against free radical-induced damage. Its relatively high abundance in the essential oil may therefore contribute substantially to the antioxidant activity observed in this study.

Another notable constituent identified was Propanamide, 2,2-dimethyl-N-(3-methylphenyl), which constituted 13.72% of the total composition. Other compounds identified included 1-Nonadecene (7.05%), 11-Octadecenoic acid (3.23%), 2-Hexyl-1-decanol (2.56%), 1,2-Benzenedicarboxylic acid (2.50%), Dibutyl phthalate (1.36%), Tricontane (1.11%), Phenol (1.09%), and 2-Dodecen-1-yl(-)succinic anhydride (1.03%). Although these compounds occurred at lower concentrations, they may collectively contribute to the essential oil's biological activities through synergistic interactions.

In comparison with previous reports, *S. pallenscens* methanolic extract reported thymol (29.79%), 3-carene (15.97%), and p-cymene (12.19%) as the most abundant components[6]. In another study, polyphenols, di- and triterpenes, and salacinol were isolated from the roots of *S. reticulata* and lipolysis. (–)-Epigallocatechin and (–)-epicatechin as well[11]. The leaves of *S. senegalensis* Lam. (DC) afforded 38 compounds containing α-Terpinene (13.8 %), Germacrene D (12.4 %), α-phenandrene (11.6 %), α-Pinene (11.5 %), and α-Caryophyllene (11.2 %)[12].

The components of the essential oils in the present research are different from other reported components, which could result from the plant species and the geographical locations of the plant. Abiotic factors such as weather, temperature, rainfall, etc., play a major role in the composition, quality, and quantity of plant phytochemicals[13].

Overall, the GC–MS profile demonstrates that *S. pallescens* essential oil contains a complex mixture of volatile bioactive compounds. The combined action of these constituents is likely responsible for the antioxidant potential of the essential oil,

supporting its possible application in pharmaceutical, nutraceutical, cosmetic, and food preservation industries.

Table 1: Components of the GC- MS characterization of *S. pallenscens* essential oils

S/No.	KI value	Components	Concentration (%)
1.	429.45	Heptacosane	0.02
2.	467.01	Tricontane	1.11
3.	1513	Phenol	1.09
4.	1524	Propanamide, 2,2-dimethyl-N-(3-methylphenyl)	13.72
5.	1540	2-Hexyl-1-decanol	2.56
6.	1893	9-Nonadecene	44.66
7.	1894	1-Nonadecene	7.05
8.	1966	2-Dodecen-1-yl(-)succinic anhydride	1.03
9.	1968	Dibutyl phthalate	1.36
10.	2116	11-Octadecenoic acid, (Z)-	3.23
11.	2117	1,2-Benzenedicarboxylic acid	2.5
12.	2140	Oleic Acid	21.42

3.2 *Antioxidant Activity of S. pallescens Essential Oil*

The antioxidant activity of the essential oil extracted from *Salacia pallescens* leaves was evaluated using the α, α-diphenyl-β-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), Nitric oxide (NO), and hydrogen peroxide (H_2O_2) assays. These assays describe the mechanisms of free radical scavenging capacity, which could be attributed to oxygen capture, reduction, oxidation, and other mechanistic approaches [14].

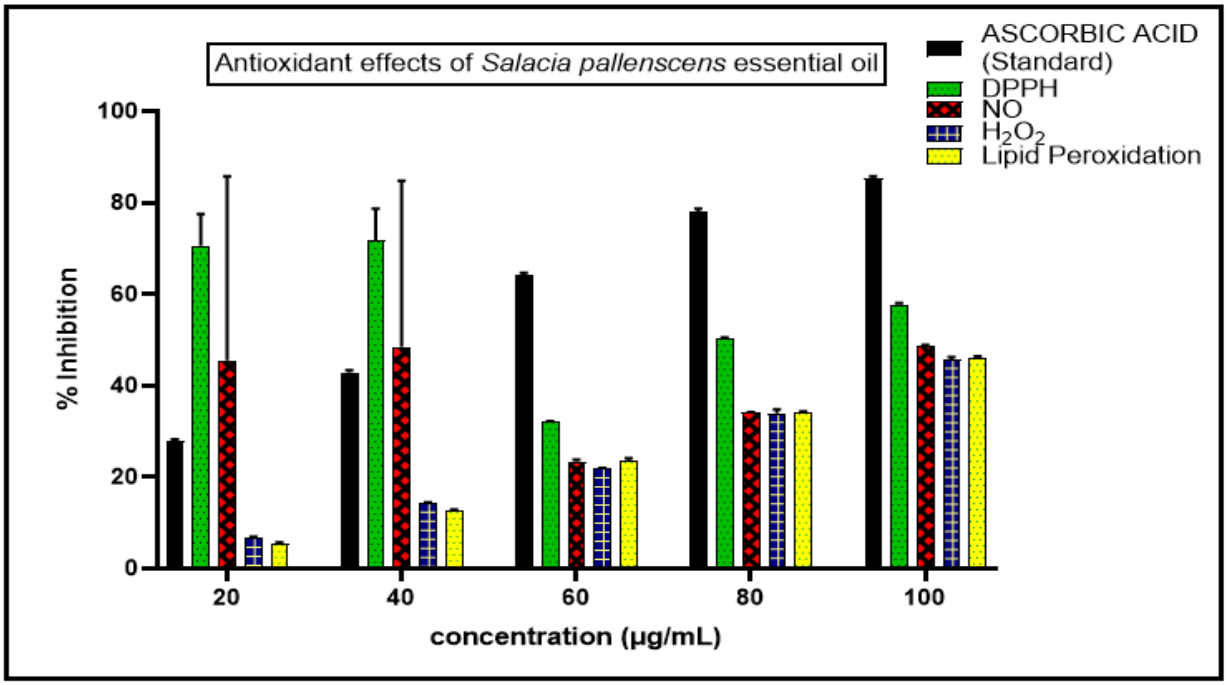


Figure 2: Antioxidant activities of *S. pallenscens* essential oils against DPPH, NO, H_2O_2, and Lipid Peroxidation.

3.2.1 Mechanism of the α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity

The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine [14]. In the scheme below, the DPPH radical is represented by Z• and the donor molecule by AH (natural product/ antioxidants); the primary reaction is where ZH is the reduced form, and A• is the free radical produced in the first step. In this study, the DPPH assay demonstrated that the essential oil exhibited concentration-dependent free radical scavenging activity. As the concentration of the essential oil increased, its ability to neutralize DPPH free radicals also increased, indicating that the oil contains bioactive compounds capable of donating hydrogen atoms or electrons to stabilize free radicals. This concentration-dependent response is characteristic of natural antioxidants and suggests that the essential oil possesses appreciable antioxidant properties.

$$Z^{\cdot} + HA = ZH + A^{\cdot} \qquad \textbf{Scheme 1}$$

Similarly, the FRAP assay showed that the essential oil possessed ferric reducing antioxidant power. The observed reducing activity indicates that constituents present in the essential oil were capable of converting ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), thereby demonstrating their electron-donating ability. This finding further confirms the antioxidant potential of the essential oil and complements the results obtained from the DPPH assay.

The antioxidant activity observed in both assays may be attributed to the phytochemical constituents identified by GC–MS analysis. Compounds such as oleic acid, phenolic compounds, and other oxygenated constituents have previously been reported to possess antioxidant properties through mechanisms involving free radical scavenging, inhibition of lipid peroxidation, and reduction of oxidative stress. Although some constituents were present in relatively low concentrations, synergistic interactions among the various compounds may have enhanced the overall antioxidant activity of the essential oil.

The hydrogen peroxide assay: Is the simplest peroxide, characterised by its oxygen–oxygen single bond. H_2O_2 and $O_2^{\cdot-}$. H_2O_2 production is closely linked to the electron transport chain in the various organelles, including mitochondria, chloroplasts, endoplasmic reticulum, and plasma membrane, as well as to photorespiration metabolism and the β-oxidation of fatty acids[15]. The inhibition rate increases with concentration. This is directly related to the phytochemicals' and essential oil components' ability to capture reactive oxygen species. Previous studies revealed that *S. pallenscens* exhibited high antioxidant properties, revealing an IC_{50} of 21.47±1.96 and 49.49±1.24 for the DPPH and the nitric oxide assays, respectively[6].

The findings obtained in this study are consistent with previous reports that essential oils rich in biologically active phytochemicals exhibit significant antioxidant activity. The ability of the essential oil of *Salacia pallescens* to scavenge free radicals and reduce ferric ions suggests that it may serve as a promising natural source of antioxidant compounds for pharmaceutical, nutraceutical, food preservation, and cosmetic applications [16-17].

Overall, the combined results of the DPPH and FRAP assays demonstrate that the essential oil of *Salacia pallescens* possesses measurable antioxidant activity. These findings support the traditional medicinal use of the plant and provide scientific evidence that its essential oil contains bioactive constituents capable of protecting biological systems against oxidative damage.

4.0 Conclusion

This study successfully demonstrated that the leaves of *S. pallenscens* contain essential oils, though in very low quantities, characterised by a high number of hydrocarbons. Plants' terpenoids are always reported in small amounts, which is dependent mainly on the family, age of the plant, geographical location, species, weather, and the soil type[18]. The observed antioxidant effects of the essential oils of *S. pallenscens* may be attributed to the combined action of the volatile phytochemicals, which either donate electrons or hydrogen atoms to neutralise reactive species and reduce oxidative stress. Overall, the findings of this study provide scientific evidence that the essential oil of *Salacia pallescens* contains bioactive constituents with antioxidant potential. These results support the traditional medicinal use of the plant and suggest that its essential oil may have promising applications in the pharmaceutical, nutraceutical, food, and cosmetic industries. Furthermore, the study contributes to the limited scientific information available on the essential oil of *Salacia pallescens* and provides a foundation for future research on its pharmacological properties.

Competing interests

Authors have declared that no competing interests exist.

Authors' Contributions

ONA designed the study, wrote parts of the protocol and condensed the first draft; OOA, AAS. FTA collected, processed and analysed samples; FTA conducted the statistical analysis of the data; AAS wrote the first draft of the manuscript, as well as assisted with sample collection and protocol; OAA, FTA assisted with study design and sample analyses, and the final copy was proofread by all authors.

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