

VALIDATION OF THE ANTIBACTERIAL EFFICACY OF THE ESSENTIAL OILS FROM THE LEAVES OF *PARQUETINA NIGRESCENS* (ASCLEPIADACEAE)

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ABSTRACT

Essential oils are complex mixtures of volatile compounds that are extracted from different parts of plants and possess diverse biological activity. In this work, the essential oils of *Parquetina nigrescens* leaves harvested in Ijebu-Ode, Ogun state, were extracted using hydrodistillation and the oils were analysed with Gas Chromatography Mass Spectrometry (GC-MS). Twenty six (26) different compounds were found in the oil with Caryophyllene (49.38 %) and α -Citral (14.49 %), being the most prevalent chemical compounds detected while Endo-Borneol (6.06 %), Citral (5.82 %), 3-Carene (4.18 %), 10-epi- γ -Eudesmol (3.75 %), Selin-6-en-4 α -ol (3.76 %), and α -Pinene (2.19 %) were present in smaller quantities and other compounds were only found available in negligible amounts. The oil was screened for its antibacterial activity against *K. pneumoniae*, *E. coli*, and *S. aureus*. In comparison to the standard drug, the oil displayed good activity against the bacteria screened. *E. Coli* was the most susceptible to the antibacterial effect of the oil, as the best activity was observed against it (22.00 ± 1.26 mm), followed by *K. pneumoniae* (19.00 ± 1.11 mm) and *S. aureus* (18.00 ± 1.23 mm). This result underscores the relevance of *Parquetina nigrescens* in traditional medicine practice.

Keywords: *Parquetina nigrescens*, essential oils, antibacterial, hydrodistillation.

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INTRODUCTION

Parquetina nigrescens is a medicinal plant native to the tropical region of Africa and commonly referred to as African wild calabash. It is known for its diverse biological activity as well as therapeutic potential (Oloyede *et al.*, 2018; Adeyemi *et al.*, 2019; Mahmud *et al.*, 2020). The plant is a deposit of numerous phytochemicals, which are responsible for its biological activities. Some of these include, but are not limited to alkaloids, anthraquinones, cardiac glycosides, cardenolides, flavonoids, saponins, and terpenes. The different parts of the plant have also been reported to be a rich deposit of essential oils which are composed of diverse chemical compounds. The first finding reported by Owolabi *et al.*, 2014, who identified five major compounds in the leaves of *Parquetina nigrescens* harvested in Badagry. A follow-up study on the same plant harvested in Ibadan revealed the presence of nine compounds in the leaves and fourteen compounds in the stem of the plant, all of which were different from those discovered in previous studies (Oghenejoboh and Nkop, 2018). This difference in composition may be attributed to varying factors such as time of harvest, geographical location or chemical transformation. *Parquetina nigrescens* is widely utilised in traditional medicine for the treatment of different ailments such as menstrual disorders, *diarrhoea*, pains, diabetes, with the most common use being the treatment of blood-related diseases such as anaemia and sickle cell disease (Imaga, *et al.*, 2010). Numerous reports of its potential as a remedy for medicinal application have also been published, indicating its cytotoxic (Oghenejoboh and Nkop, 2018), anti-*diarrhoea* (Mahmud *et al.*, 2020), antidiabetic activities (Ojuade, 2021), among others. While much attention has been focused on the phytochemical constituent and its pharmacological activities, there exists an increasing drive among scientists

to explore its essential oil constituents as well as its biological applications. The essential oils of *Parquetina nigrescens* have been shown to exhibit insecticidal activity (Osunsami *et al.*, 2016), cytotoxic activity (Oghenejoboh and Nkop, 2018), enzyme and oxidation inhibitory activities (Oloyede *et al.*, 2018). Despite the significant work that has been done on the plant, a major report on the antimicrobial activity of the essential oil of the plant is difficult to come across in the literature. In view of this, the present study is designed to reveal the chemical component of the essential oils of *Parquetina nigrescens* leaves harvested in Ijebu-Ode, Ogun State to ascertain the influence of geographical location on the composition and as well determine the antibacterial activity of the oil, which hitherto has not been previously reported.

MATERIALS AND METHODS

Plant material and essential oil extraction technique.

The healthy leaves of *Parquetina nigrescens* were collected in a home garden at Ijebu-Ode, Ogun state, Nigeria in September, 2023. The botanical identification and authentication were done in the Herbarium of the Department of Botany, University of Lagos, Nigeria (herbarium number LUH 6567). The fresh leaves of *Parquetina nigrescens* were air-dried for one week and pulverised using a mechanical grinder prior to extraction. The essential oils from the air-dried leaves were obtained by hydro-distillation of 300 g of each of the plant materials using the modified Clevenger-type apparatus (Njoku *et al.*, 2023). The oil was dried over anhydrous sodium sulphate and stored in a refrigerator prior to analysis.

GC–FID and GC/MS Analyses of Volatile Oils

The essential oil samples were analysed using a Varian CP-3800 gas chromatograph fitted with a flame ionisation detector (FID) and dimethylpolysiloxane (100%) column (CP Sil-5 CB: 50 m length × 0.25 mm i.d. × 0.4 µm film thickness) (Varian, Netherlands). Nitrogen was the carrier gas with a 16-psi inlet pressure. Samples (0.2 µL) were injected in split mode with a ratio of 1:100. The column was initially held at 60°C for 5 minutes, then heated to 220°C at a 5°C/minute ramp rate and was held for 3 minutes at that temperature. The temperature was further raised to 250°C at a 5°C/minute ramp rate and was held at this temperature for 4 minutes. The injector and detector temperatures were maintained at 250° and 300°C, respectively. The gas chromatography/mass spectrometry (GC/MS) analyses was performed on a Perkin Elmer Turbo mass Clarus 600 Instrument at 70 eV ionization energy with a mass range of 40–500 amu, employing an Elite-5 column (5 % phenyl and 95 % dimethylpolysiloxane) of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness (PerkinElmer, USA). Helium (1 mL/min) was used as a carrier gas. The initial temperature was 60 °C (1 min), which was increased to 240 °C at a rate of 6 °C/min, and remained at 240 °C for 6 min, and then continued to increase to 250 °C at a rate of 10 °C/min, with a final stage of 10 min at 250 °C. The oven temperature was programmed from 50 °C to 250 °C at a 5 °C/min dynamic rate, and remained for 15 min at 250 °C. A sample (0.1 µL) was injected with a splitless mode.

Identification of Volatile Oil Constituents

Component identification was accomplished by comparison of the retention indices (RI) of the GC peaks with those obtained using saturated n-alkanes (C8–C30) (Aldrich, USA),

those reported in the literature (Njoku *et al.*, 2020, Koenig *et al.*, 1998; El-Sayed *et al.*, 2012; NIST Library, 2020) and by comparison of the mass spectra of the peaks with those reported in the literature (Jennings and Shibamoto, 1989; Adams, 2007) and stored in the NIST library. Peak area percentages were calculated from GC–FID response without employing correction factors. RI values were calculated for all components using a homologous series of *n*-alkane. Mixtures (C7–C30) were injected under conditions similar to those of the samples and computer matched with the NIST libraries.

Antibacterial assay

The volatile oil of *Parquetina nigrescen* was tested on three different bacterial strains. The strains were maintained at 4 °C and were against *K. pneumoniae*, *E. coli*, and *S. aureus*. The bacterial strains were cultured in a Thermo Scientific (Waltham, MA, United States) Oxoid Nutrient agar (NA) at 37 °C for 24 hours. The disc diffusion method (Ha *et al.*, 2019) was used to determine the antimicrobial activities of the essential oils. Petri plates were prepared by pouring 20 mL Thermo Scientific Oxoid Nutrient agar (NA), and the solution was allowed to solidify. The plates were then dried, and 0.1 mL of the standardised inoculum containing 106-107 colony-forming units/mL of the bacterial suspension was poured, uniformly spread, and allowed to dry for 5 minutes. The volatile oil was prepared in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. 100 µL was taken from this stock solution and added to the respective wells. The control well received only 100 µL DMSO. Gentamycin (positive control) was used as the reference antibiotic. The plates were left at room temperature to allow diffusion and then incubated at 37 °C for 24 hours for bacterial growth. The antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition against the test

organisms. The experiments were repeated in triplicate and the results are expressed as average values. The MIC was determined using the broth microdilution method using 96-well microplates. The inoculum of the microbial strains was prepared from 24 to 48 broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Serial concentrations (500, 250, 125, 62.5, 31.3, 15.6, 7.81, 3.9, 1.95, 0.98, and 0.49 µg/mL) of essential oil were prepared. 100 µL from culture broth was mixed with 100 µL of different concentrations of the essential oils of *Parquetina nigrescens* in the corresponding well and plates were incubated at 37 °C for 24 hours for antibacterial activity. The lowest concentration of the tested oil showing no microbial growth was defined as the minimum inhibitory concentration (MIC).

Minimum bactericidal concentration (MBC) values were determined by taking a part of the liquid from each well that showed no growth and incubating on agar plates at 37 °C for another 24 hours. The lowest concentration that disclosed no visible growth of bacteria or fungi was confirmed as MBC.

RESULTS AND DISCUSSION

Chemical composition of the essential oils of *Parquetina nigrescens*

The essential oils of *Parquetina nigrescens* were obtained by hydrodistillation and analysed using the GC-MS. The chemical compositions of the essential oils are reported in Table 1.

Table 1: Composition of the essential oils of *Parquetina nigrescens*

Compound	RI	Rical	%
α-Pinene	933	936	2.19
β-cis-Ocimene	1028	1032	0.23
3-Carene	1006	1003	4.18
1,5,5-Trimethyl-6-methylene-cyclohexene	1338	1338	1.96
p-Cymen-8-ol	1163	1165	0.39
Linalool	1086	1094	0.21
L-α-Terpineol	1172	1170	1.63
Citral	1241	1244	5.82
Geraniol	1237	1235	0.30
endo-Borneol	1151	1152	6.06
α-Citral	1241	1240	14.42
Ethanol,2-(3,3-dimethylcyclohexylidene)-, (Z)-	1225	1220	0.31
2,6-Octadien-1-ol,3,7-dimethyl-, acetate	1352	1350	0.26
Nerolidol	1551	1554	0.30
cis-β-Farnesene	1447	1443	0.26
Isocaryophyllene	1409	1404	0.56
Caryophyllene	1419	1416	49.38
Humulene	1451	1446	0.99

Caryophyllene oxide	1574	1571	0.82
Humulene epoxide II	1599	1556	0.15
Selin-6-en-4 α -ol	1593	1590	3.77
Neointermedeol	1601	1605	1.36
Ledol	1586	1582	0.21
Viridiflorol	1582	1580	0.10
10-epi- γ -Eudesmol	1615	1612	3.75
Globulol	1579	1576	0.39
Total identified	Total%		100

The percentage compositions of each compound from GC-MS analysis indicate the relative abundance of each compound within *Parquetina nigrescens* leaves oil. The result indicates that the oil of the *Parquetina nigrescens* leaves is a rich deposit of terpenes. Twenty-six (26) different compounds were detected in the essential oils of *Parquetina nigrescens* leaves representing a total composition of 100 %. Eighteen (18) of the compounds were identified as terpenoids and nine (8) were terpenes. This result differs greatly from the first two previous reports by Owolabi and Oghenejoboh, respectively. The former identified five compounds in the volatile oil of the plant's leaves (Owolabi et al., 2014) while the latter obtained nine compounds in the leaves (Oghenejoboh and Nkop, 2018). This difference in chemical composition in the different studies could be attributed to differences in geographical location where the plant is domiciled (Amorati et al., 2013), period of harvest (Chelghoum, et al., 2021, Osinubi et al., 2023), chemotypic difference (Oghenejoboh and Nkop, 2018), or even chemical transformation that may have occurred over time, leading to variations in the chemical compounds recovered in the different studies.

The GC-MS analysis of essential oil indicated Caryophyllene (49.38 %) and α -Citral (14.49 %), as the most prevalent chemical compounds detected in the oil while Endo-

Borneol (6.06 %), Citral (5.82 %), 3-Carene (4.18 %), 10-epi- γ -Eudesmol (3.75 %), Selin-6-en-4 α -ol (3.76 %), and α -Pinene (2.19 %) were present in smaller quantities. Other compounds were only found in negligible amounts. Caryophyllene, a sesquiterpene known to exhibit various biological activities (Francomano et al., 2019) was found to be the most abundant component of the oil with a percentage composition of 49.38 %. α -Citral, also known as geranial (Pengelly, 2004; Tajidin et al., 2012) is the next abundant compound in the essential oil evaluated and is well known for its pharmacological activity. Additionally, Endo-Borneol, a monoterpene with reported antinociceptive and anti-inflammatory activity (Shareef et al., 2016), was discovered present in the oil. The presence of citral, a mixture of two isomeric aldehydes (geranial and neral) with a strong lemony scent, possessing antimicrobial and antioxidant properties, corroborates previous studies, which reported the presence of geranial and neral (Owolabi et al., 2014) in the plant leaves. α -Pinene is a monoterpene that contributes to the characteristic pine-like aroma of the essential oil and possess reported biological activity ranging from antioxidant to anti-inflammatory, analgesic and antitumor among others (Salari et al., 2019). The presence of these compounds may be attributed to the unique aroma and potential therapeutic properties of the essential oil of *Parquetina nigrescens*.

Antibacterial Activities of the Essential Oils of *Parquentina nigrescens*

The antibacterial potential of the essential

oils of *Parquentina nigrescens* was tested against *K. pneumoniae*, *E. coli*, and *S. aureus*. The result of the biological screening is presented in table 2.

Table 2: Antibacterial activity of the Essential Oils of *Parquentina nigrescens* against selected Pathogen

Microorganisms	Diameters of zone of inhibition(mm)		MICs	MBCs
	Volatile oil	Antibiotics	($\mu\text{g}/\text{mL}$)	($\mu\text{g}/\text{mL}$)
<i>K. pneumoniae</i>	19.00 \pm 1.11	22.00 \pm 0.3	80.40	140.22
<i>E. coli</i>	22.00 \pm 1.26	22.00 \pm 0.2	72.30	165.20
<i>S. aureus</i>	18.00 \pm 1.23	20.00 \pm 0.2	68.30	133.60

Diameters of zone of inhibition (ZI), Minimal Bactericidal Concentration (MBC); Minimum Inhibitory Concentration (MIC). Results were mean \pm SD of triplicate values. The antibiotics used was gentamicin.

The ability of the essential oils of the plant of study to inhibit bacterial growth /activity of *K. pneumoniae*, *E. coli* and *S. aureus* was investigated and compared with a known standard antibacterial drug, gentamicin. The biological activity of the plant is a factor of the ZI which represented the potential of the plants to be an antibacterial agent. The larger the ZI, the greater the potential of the plant's oil extract to inhibit bacterial growth. *P. nigrescens* was observed to display good activity against all three bacterial strains screened with the highest ZI recorded against *E. coli* (22 mm) while a ZI of 19 and 18 mm were recorded against *K. pneumoniae* and *S. aureus*, respectively. The relatively high ZI against *E. coli* corroborates the work of Banwo *et al.*, 2020, who reported a dose-dependent reduction in the faecal *E. coli* load of experimental rats upon administration of the plant's leaf extract after 48 hours. Mahmud *et al.*, 2020, reported a maximal inhibitory activity of the extract against castor oil-induced diarrheal. The essential oil of *P. nigrescens* showed the highest MIC on *S. aureus*, while the lowest MIC was recorded for the oil on *K. pneumoniae*. The minimal bactericidal concentration was

calculated to ascertain the least possible concentration at which the essential oil will be active. The result from this assay showed that the essential oil of *P. nigrescens* gave the best bactericidal concentration against *S. aureus* (Table 2). According to Olaleye *et al.*, 2021, the ethyl-acetate extract showed *P. nigrescens* displayed broad-spectrum antibacterial activities, while the n-hexane extract demonstrated narrow spectrum antifungal activity. In another research, the tested leaves extract of *P. nigrescens* produced activity in only one of the *C. diphtheriae* isolated from the ear swab of one of the otitis media cases with 16 ± 0.0 mm zone of inhibition at 100 mg/mL when compared with 30 ± 0.0 mm at 10 $\mu\text{g}/\text{mL}$ of gentamicin (positive control). They also reported that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the tested extract for the isolate were 25 and 400 mg/mL, respectively as compared to the respective MIC and MBC of 40 and 320 $\mu\text{g}/\text{mL}$ for gentamicin (Oyagbemi *et al.*, 2013). The essential oil of the plant in this research showed promising antibacterial activity when compared to that of the standard drug gentamicin.

CONCLUSION

The essential oils of *Parquetina nigrescens* leaves were extracted using hydro distillation and analysed for their chemical composition using GC-MS. A total of twenty-six (26) compounds were identified in the oil, with Caryophyllene being the most abundant of the compounds identified. The plant's oil showed promising antibacterial activity. The result of the antibacterial screening showed that the essential oil of *P. nigrescens* leaves has stronger inhibitory effects on all the bacterial strains tested. The antibacterial activity exhibited by the *P. nigrescens* oil by comparison with that of the standard drug can be said to support its use in traditional medicine practice as well as point to the plant as a promising lead for drug development against diseases like diarrheal, which is a major cause of morbidity/mortality among infants.

Declaration of conflict of interest

The authors declare no competing interests, financial or otherwise.

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