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Chemical Composition, Cytotoxicity and Antioxidant Activities of Essential Oils of Parquetina nigrescens (afz.) Bullock from Ibadan, Nigeria

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Abstract *Parquetina nigrescens* belonging to the *Periplocaceae* family is a plant reported to have diverse medicinal uses, this study was aimed at determining the chemical composition, antioxidant activity and cytotoxicity of the essential oils of the leaves and stems of the plant. The chemical composition of the essential oils determined using the Gas Chromatography-Mass Spectrometry method revealed a total of 9 compounds for the leaves and 14 compounds for the stems, with neophytadiene being the major constituent in the leaf oil while lanosterol acetate and α-amyrin acetate were the major constituents in the stem oil. The antioxidant and cytotoxic activities of the oils were tested using the DPPH radical scavenging assay and brine shrimp lethalithy assay respectively. The essential oils displayed good antioxidant activity, their ability to scavenge free radicals was comparable to that of the standard antioxidant used. The essential oils all had lethal concentration at 50% (LC_{50}) values less than 100 µg/mL indicating a moderate cytotoxic activity. The essential oils of *Parquetina nigrescens* could be exploited as potential therapeutic agents especially in cancer therapy because of their promising antioxidant and cytotoxic activities.

Keyword: *Parqueina nigrescens*, essential oils, antioxidant, cytotoxicity

Introduction

Essential oils are a class of secondary metabolites, they contain complex mixtures of chemical compounds, the most common of which is terpenes either in the hydrocarbon or oxygenated form [1]. Essential oils act as part of plant defence system, this forms a basis for their biological use in humans [2]. One very important biological use of essential oils are their ability to act as free radicals scavengers, this is due to the fact that they most often contain phenolic compounds [3].

Essential oils with antioxidant properties can be used as dietary supplements or applied topically to help prevent or slow down both internal and external free radical damages, such as the development and progression of cancer and ageing [4–7]. When an essential oil has cytotoxic properties in addition to antioxidant properties, it can help to destroy cells already damaged by oxidative radical species, this prevents their ploriferation [8].

Parquetina nigrescens is a plant in the Periplocaceae family; it is a climbing shrub with soft woody base. It is mostly found in West and Central Africa [9]. Almost all the plant parts have been used in traditional medicine as remedy for a varieties of ailments [10–12]. The most common use of the plant is in the treatment of blood related disease such as anemia and sickle cell disease [13]. The solvent extracts of the plants have been found to have good antithyhoid and gastroprotective activities [14,15], and the plant itself have been found to be rich in protein [16].

The chemical composition of the essential oil of the leaves of *Parquetina nigrescens* from Badagry, Lagos State was found to contain five compounds, with citral being the major compound identified in the essential oil [17]. The leaves essential oils displayed good insecticidal activity against adult maize weevil *Sitophilus zeamais* [18]. The

current study is aimed at determining the chemical constituents of the essential oils of the leaves and stems of *Parquetina nigrescens* extracted separately and to test the cytotoxic and antioxidant properties of the essential oils.

Materials and Methods

Plant Collection and Extraction

Parquetina nigrescens plant was collected from a local residence in Ibadan North Local Government Area of Oyo State, Nigeria in late July and early August, 2017. The leaves were separated from the stem, air dried and pulverized. The essential oils were extracted using an all glass Clevenger designed following the specifications of the British Pharmacopeia [19].

GC-MS

The chemical constituents of the essential oils of the leaves and stem of *Parquetina nigrescens* was determined using GC-MS Agilent Technologies, model-7890A gas chromatogram, coupled with a 5975C mass spectrometer Agilent Technology. The gas chromatogram capillary column type was HP-5MS, with column length 30 m, internal diameter 0.320 mm and film thickness 0.25 µm. Temperature programming started with an intial column temperature set at 80 ^{0}C/min for 2 mins and was increased to 240 ^{0}C at rate of 10 ^{0}C/min. The constituents of the essential oils were identified by comparing the GC-MS data with published mass spectra database (NIST 11.L).

Cytotoxic Activity

The cytotoxic activity of the leaves and stem essential oil of *P. nigrescens* was done using the brine shrimp lethalithy assay method [20–22]. The essential oils were diluted into a concentration of 1000, 100 and 10 ppm. The 1000 ppm stock solution was prepared by adding 0.02ml of essential oil to 1.8 ml sea water and 0.2 ml of DMSO. 0.5 ml was taken from the 1000 ppm in triplicates. 0.2 ml of the remaining 0.5 ml (1000 ppm) was taken and made up to 2 ml using sea water to give 100 ppm concentration. 0.5 ml from the 100 ppm solution was drawn in triplicates and kept aside; another 0.5 ml was taken from it and made up to 2 ml using sea water to give 10 ppm solution. Also 0.5 ml from the 10 ppm solution was transferred into three test tubes. All concentrations were labeled; a blank solution was also prepared using just two drops of DMSO and 5 ml of sea water. 10 brine shrimps were added to all solution using a Pasteur pipette, and made up to 5 ml using sea water. After 24 hours the number of dead shrimps in each concentration was counted, the lethal concentration at 50% (LC_{50}) was extrapolated with the Probit Finery computer program.

Antioxidant Assay

The antioxidant activity of the essential oil was tested using a modified form of the 2,2- diphenylpicryl hyddraizne (DPPH) method [23]. Three different concentrations of the volatile oils were prepared (1.0, 0.5, 0.25 mg/ml).

A stock solution of 1.0 mg/ml concentration was prepared by dissolving 0.5 ml of the essential oil in 2 ml of methanol. 1 ml was drawn from the stock solution and kept aside; 2 ml pure methanol was added to the remaining stock solution to give 0.5 mg/ml concentration. From the 0.5 mg/ml concentration 1 ml was drawn and 2 ml methanol was added to the remaining solution to give 0.25 mg/ml concentration.

Tocopherol and Butylated hydroxyanisole which are standard antioxidants were used as the positive control. 2 mg of the standards was dissolved in 2 ml of methanol to give stock solution of 1.0 mg/ml. the 0.5 mg/ml and 0.25 mg/ml concentrations were then prepared from the stock in the same manner in which those of the essential oils were prepared.

The DPPH solution was prepared by dissolving 1.97 mg of DPPH in 100 ml of methanol to give 0.05 mM concentration. 2 ml of the DPPH solution was added to all the samples. A blank sample was made with just DPPH and methanol this served as the negative control.

All samples were kept in dark room immediately after preparation to incubate for 30 mins; absorbance reading was taken at 517 nm using spectrumlab S22PC visible spectrometer with model No 721S.. The percentage inhibition of the samples was calculated using the measured Absorbance values.

Result and Discussion

Gas Chromatography-Mass Spectrometry

The results of the chemical constituents of the essential oils of *Parquetina nigrescens* leaves and stems are found in table 1. Nine compounds were detected in the volatile oils of *Parquetina nigrescens* leaves representing a total composition of 90.91%, four terpenes and five non-terpenes. The major component of the oil is the diterpene neophytadiene (41.91%) a branched chain hydrocarbon belonging to the class of compounds called phytanes [24]. The result of the chemical composition of the essential oils of the leaves differed greatly from those of owolabi from Badagry Lagos, Nigeria who detected five compounds in the leaf essential oil of *Parquetina nigrescens* and found citral to be the major compound [17]. The difference in chemical composition could be because both plants were from different environmental source [25]. Another possibility could be that both plants though belonging to the same species may be of different chemotype as a result of either one of the plant adapting to its environment over time which leads to it having distinct chemical components in its essential oils [26, 27]. The presence of the diterpene neophytadiene as the major component of the essential oil of the leaves of *Parquetina nigrescens* could make it very useful in insect control, because diterpenes have been found to have good insect growth regulation, antifeedant and insecticidal properties [28].

The volatile oils of *Parquetina nigrescens* stem contained 6 non-terpenes and 8 terpenes. Out of the eight terpenes present, four were hydrocarbon terpenes while the other four were oxygenated triterpenoids. Two of the oxygenated triterpenes α- amyrin acetate (19.15%) and lanosterol acetate (18.43%) were the major compounds present in the oil. α-amyrin has been found to exhibit various pharmacological activites both in in-vitro and in-vivo studies against different diverse health related conditions such as inflammation, microbial infections and cancer [29].

Table 1: Chemical Constituents of Essential oils of *Parquetina nigrescens* leaves and stems

S/N	RT*	Compounds	% Composition	
			Leaf oil	Stem oil
1	16.322	Hexahydrobenzofuran		1.47
2	16.419	Pentadecanal		4.29
3	16.436	Acorenone	7.66	-
4	16.619	β-vertivenene	-	2.98
5	17.804	Widrol	-	1.28
6	18.158	Cis-pinane	-	1.98
7	18.164	Trans-pinane	4.36	-
8	18.342	Hexahydrofarnesyl acetate	20.16	-
9	18.347	Phytone	-	8.61
10	22.295	Henicosane	-	1.21
11	22.775	Neophytadiene	41.91	16.08
12	27.917	Docosane	2.52	-
13	27.920	Octacosane	-	1.06
14	29.877	Heptacosane	2.42	0.84
15	30.547	Lanosterol acetate	-	18.43
16	31.079	Squalene	4.93	4.62
17	31.537	Nonadecane	5.20	-
18	31.542	Hexacosane	-	12.80
19	31.657	α-amyrin acetate	-	19.15
20	33.007	Eicosane	1.75	-
Total			90.91%	96.2%

* Retention time in order of elution from an HP-5MS capillary column

Antioxidant

The essential oils ability to scavenge free radicals of DPPH was investigated and compared with standard known antioxidant: Butylated hydroxyl anisole (BHA). The percentage inhibition of DPPH radical by the essential oils was concentration dependent, it increased with decreasing concentrations of the essential oils. The antioxidants activities were either better than or comparable to that of Butylated hydroxyl anisole. The antioxidant activity exhibited by

Parquetina nigrescens essential oils could make the oils useful in the formulation of drugs and creams used for the treatment of inflamatory conditions since most inflamatory disease are caused by reactive oxygen species.

Table 2: 2,2-Diphenyl picryl hydrazine antioxidant assay

Concentration (mg/ml)	Leaf oil	Stem oil	BHA
1.0	76	73	71
0.5	73	68	71
0.25	69	66	69

Cytotoxicity assay

The essential oils of both the leaves and stem of *Parquetina nigrescens* demonstrated moderate cytotoxic activity this was deduced from the fact that the LC_{50} values (lethal concentration at 50%) were all less than 100 µg/mL [30, 31]. The essential oils can be used in cancer therapy, because they can be toxic to cancer cell lines. Studies into the use of essential oils as chemotherapeutic agent have been of great interest lately, since essential oils contain a mixture of complex chemical compounds which could work in synergy to enhance their chemotherapeutic activity ac [32].

Table 3: Brine shrimp cytotoxicity of *Parquetina nigrescens* essential oils

Plant parts	No	Of	Dead	Shrimps
	1000 ppm	100 ppm	10 ppm	LC_{50} (µg/mL)
Leaf oil	24	24	12	14.90
Stem oil	30	24	4	38.04

Conclusion

The essential oils of *Parquetina nigrescens* leaves and stems were analyzed using GC-MS and a total of nine and 14 compounds was identified in the oils respectively with neophytadiene being the most abundant compound in the leaf oil while Lanosterol acetate and α-amyrin acetate were the most abundant compounds in the stem oil. The oils showed promising antioxidant and cytotoxic activities, these could make the essential oils of *Parquetina nigrescens* very useful in drug formulations for the treatment of diseases caused by reactive oxygen species and associated with abnormal cell proliferation.

References

1. Sharifi-rad, J., Sureda, A., Tenore, G. C., Daglia, M., Shaeifi-rad, M., Valussi, M., Loizzo, M. R., Ademiluyi, A. O., Sharifi-Rad, R. & Ayatollahi, S. A. (2017) Biological Activities of Essential Oils : From Plant Chemoecology to Traditional Healing Systems. Molecules, 22(7): 1–55.
2. Ibrahim, M., Kainulainen, P., Aflatuni, A., Tiilikkala, K & Holopainen, J. (2001) Insecticidal repellent, antimicrobial activity and phytotoxicity of essential oils with special reference to Limonene and its suitability for control of insect pests. Agric food Sci. Finl., 10: 243–259.
3. Papas, A. (1999). Diet and antioxidant status. Food Chem. Toxicol, 37:999–1007.
4. Elaissi, A., Rouis, Z., Salem, N. A., Mabrouk, S., Ben-Salem, Y., Salah, K. B., Aouni, M. B., Farhat, F., Chemii, R., Harzallah-Skhiri, F. & Khouja M. L. (2012). Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC Complement. Altern. Med, 12(1):1080.
5. Tate, S. (1997). Peppermint oil: a treatment for postoperative nausea. J. Adv. Nurs, 26(3): 543–549.
6. Carson, C. F., Hammer, K. A. & Riley, T. V. (2006). *Melaleuca alternifolia* (Tea Tree) oil: a review of antimicrobial and other medicinal properties. Clin. Microbiol. Rev, 19(1): 50–62.
7. Srivastava, J. K., Shankar E. & Gupta, S. (2010). Chamomile: A herbal medicine of the past with bright future. Mol. Med. Rep, 3(6): 895–901.
8. Miguel, M. G. (2010). Antioxidant and Anti-Inflammatory Activities of Essential Oils: A Short Review. molecules, 15: 9252–9287.

9. Burkill, H. M. (1997). The useful plants of West Tropical Africa Volume 4, Families M-R, Royal Botanic Gardens Kew Richmond, United Kingdom. 2nd Edition.
10. Adeyemi, S. (1993). Study of the atiarhematic plants in parts of Oyo Africa, Spectrum Books, Ibadan. 2nd ed.
11. Olatunbosun, L. O., Caxton-Martin, A., Jimoh, O. R., Biliaminu, S. A. & Ghazal, O. K. (2012). Proliferative effect of aqueous extracts of *Parquetina nigrescens* on haemopoietic multipotent stem cells in irradiated guinea pigs. J. Appl. Pharm. Sci., 2(6): 108–111.
12. Gills, L. (1992). Ethnomedical uses of plants in Nigeria., Uniben Press, Benin. 1st Ed, pg 276.
13. Imaga, N., Gbenle, G., Okochi, V., Adenekan, S., Edeoghon, S., Kehinde, M. & Obinna, A. (2010) . Antisickling and toxicological profiles of leaf and stem of *Parquetina nigrescens* L . J. Med. Plants Res., 4(8): 639–643.
14. Akinyemi, O. I. & Dada, E. O. (2013) phytochemical screening and antityphoid properties of ethanolic leaf extracts of *Parquetina nigrescens*. ARPN J. Agric. Biol. Sci., 8(1)1: 732–739.
15. Kayode, A., Kayode, O. & Odetola, A. (2009). Anti-Ulcerogenic Activity of Two Extracts of *Parquetina nigrescens* and their Effects on Mucosal Antioxidants Defence System on Ethanol-Induced Ulcer in Rats. Res. J. Med. plant, 3(3): 102–108.
16. Awa Imaga, N., Gbenle, G. O., Okochi, V. I., Adenekan, S., Duro-Emmanuel, T., Oyeniyi, B. & Ekeh, F. C. (2014). Phytochemical and antioxidant nutrient constituents of Carica papaya and Parquetina nigrescens extracts. *Sci. Res. Essays*, 5(16): 2201–2205.
17. Owolabi, M. S., Lawal, O.A., Hauser, R. M. & Setzer,W. N. (2014.) The volatile constituents of *Parquetina nigrescens* from southwestern Nigeria. Nat. Prod. Commun., 9: 6857–856.
18. Osunsami, A. A., Sotubo, S. E., Lawal,O. A., Ogunwande, and O. I. Eresanya, I. A. (2016). Insecticidal Activity of Essential oil of *Parquetina nigrescens* (Afzel) Bullock," *I*nt. J. Pharm. Chem., 2(2): 20–23.
19. British Pharmacopoeia. (1980) H. M. Stationary Office," London, "Vol II.
20. Micheal, A. S., Thompson, C. G. & Abramovitz, M. (1956). *Artemia salina* as a test organism for a bioassay. Science, *80*(123): 464, 1956.
21. Vanhaecke, P., Persoone, G., & Sorgeloos, R. (1981). Proporsal for a short-term toxicity test with *Artemia nauplii*. Ecotoxic Env. Saf., 5: 382–387.
22. Sleet, R. B. & Bendel, K. (1988). Improved methods for harvesting and counting synchronous populations of *Artemia nauplii* for use in developmental toxicology. Ecotoxic Env. Saf., 7: 435–446.
23. Mensor, L. I., Menezes, F. S., Leitao, G. G., Reis, A. S., Santos, T. C., Coube, C. S. & Leita, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 15: 127–130.
24. Hernandez-Matamoros, A., González, E., Garcia-Casco, J. M. & Tejeda, J. F. (2013). Determination of neophytadiene in the subcutaneous fat of Iberian pigs from different feeding systems. Grasas Y Aceites, 64(2): 173–180.
25. Amorati, R., Foti, M. C. & Valgimigli, L. (2013). Antioxidant Activity of Essential Oils. J. Agric. Food Chem., 61: 10835-10847 .
26. Clarke, S. (2008). Composition of essential oils and other materials. Churchill Livingstone. pp. 123–229.
27. Ingrid, M. (2007). Aromatherapy for Massage Practitioners. Lippincolt William Wilkins Baltimore, USA.
28. Lanzotti, V. (2013). Diterpenes for Therapeutic use. Spinger-Verlag, Berlin. pp. 3173–3191.
29. Vázquez, L. H., Palazon, J. & Navarro-ocaña, A. (2011). The Pentacyclic Triterpenes Alpha, Beta-amyrins: A Review of Sources and Biological Activities, in Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, InTech, Shanghai. pp. 487–502.
30. Meyer, B., Ferrigini, N., Putnam, J., Jacobson, L. & Nichols, D. (1982). Brine shrimp: a convenient general bioassay for active plants constituents. Planta. Med., 45: 31–34.
31. Alanis-Garza, B.,Gonzalez-Gozalez, G., Salazar-Aranda, R., waksman de Torres, N. &. Rivas-Galindo, V. (2007) Screening of antifungal activity of plants from the northeast of Mexico. J. Ethnopharmacol., 114:

468–471.

32. Russo, R., Corasaniti, M. T., Bagetta, G. & Morrone, L. A. (2015) Exploitation of Cytotoxicity of Some Essential Oils for Translation in Cancer Therapy. Evidence-Based Complement. Altern. Med., 2015:1–9.