Phytochemistry and Antioxidant Evaluation of Methanol Seed Extract and Vacuum Liquid Chromatography Fractions of *Parquentina nigrescens* (Aflel) Bullock (Perplocaceae)

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ABSTRACT

In recent time, interest has been drawn to the possible pharmacologic properties of the fruits and seeds of *Parquetina nigrescens* (Periplocaceae). The aim of this study was to evaluate the phytochemical composition and antioxidant properties of the seeds. Phytochemical screening was done according to standard methods. Antioxidant evaluation was carried out by DPPH radical scavenging and FRAP assay on the crude extract and its Vacuum Liquid Chromatography (VLC) fractions. Phytochemical screening revealed the presence of alkaloids, saponins, phenols, flavonoids and proteins. Antioxidant scavenging activity in both DPPH and FRAP assays revealed a concentration-dependent activity with the best activity in the extract. Total phenol and flavonoid were determined as 112.34 mg gallic acid equivalent/g of extract (mgGAE/g) and 25.53 mg quercetin equivalent/g of extract (mgQE/g) respectively. Correlation between the antioxidant activity and the phenolics was significant ($P< 0.05$). This study has reported to the best of our knowledge, for the first time, the anti-oxidant properties of the methanol seed extract of *P. nigrescens* which was significantly associated with the phenolic constituents in the seeds.

***Keywords*:** *Parquetina nigrescens,* Seeds, Antioxidant, Methanol extract, VLC-fractions

INTRODUCTION

Parquetina nigrescens (Periplocaceae) is a shrub found in equatorial West Africa (Mabberly; 1987). It is a perennial plant with twining stems and a base tapering 10-15 cm long and 6-8 cm broad. It has smooth long stems on which are located the leaves. Its flower grows from its side branches having whitish outside and inner reddish colour (Gill; 1992). The fruit is composed of two parts; an outer woody and an inner softer part that house the feather-like seed (Awobajo and Olatunji; 2010). In some Nigerian languages, *P. nigrescens* is called kwankwanin (Hausa), mgbidimgbe (Igbo) inuwuelepe (Yoruba), Olilia or Ovieukpakoma (Etsako) (Ayoola *et al;* 2011; Konan *et al;* 2013). The leaves, roots and the latex of the plants are commonly used in traditional medicine (Kayode *et al;* 2009, Owoyele *et al;* 2011) Documented pharmacological activities of the plant have also been reported and available in literature (Owoyele *et al;* 2009, Aderibigbe *et al;* 2011). Reported pharmacological activities include their use as antiinfective, analgesic, anti-inflammatory, antidiabetic and in the treatment of sickle cell disease (Adebayo *et al;* 2010, Saba *et al;* 2010). The ethnomedicinal and established biological activity reported for the plant are majorly of the leaves, stem, and root. In recent time however, interest has been drawn to the possible pharmacologic properties of the fruits and seeds. The proximate and phytochemical analysis, anti-nociceptive activity (Uwaya *et al;* 2013) toxic effects (Uwaya *et al;* 2015) and haemopoietic effects (Olatunbosun *et al*; 2012) of the fruit bark has been reported. There is presently no information on activities of the seeds which informed this present study to evaluate the phytochemical content and antioxidant properties of the seeds of *P. nigrescens*.

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MATERIALS AND METHOD

Collection and preparation of plant materials

Fresh *Parquetina nigrescens* fruits were collected in November, 2016 from a forest near Benin City, Nigeria. The plant material was identified and authenticated by Dr F. Akinigbosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin city where a voucher specimen was prepared and herbarium specimen number UBHp 0233 was deposited. The fruits were air-dried under shade for 4 weeks, after which the seeds were separated from the wool and pericarp of the fruits and pulverised using a mechanical grinder. The crude powdered sample was stored in an air-tight container until ready for use.

Phytochemical screening of the seed of *P. nigrescens*

Simple chemical tests to determine the presence of alkaloids, tannins, saponins, carbohydrates, anthraquinones and other phenolic compounds were done in accordance with standard methods (Evans; 2002).

Extraction of crude powdered sample

The powdered plant material (390 g) was extracted with 1.5 L of methanol by maceration at room temperature for two days. The extract was concentrated to dryness using a rotary evaporator at reduced pressure. The concentrated extract was weighed and the percentage yield calculated. The extract was stored in an air-tight container kept in a refrigerator at 4 ^{o}C until further experiment.

Solvent-Solvent extraction (Pre-fractionation/partitioning technique)

Vacuum liquid chromatography (VLC) was performed in a column (25 cm x 15 cm) packed with silica gel of particle size (0.04 to 0.06 mm) 200 to 400 mesh. The crude extract (30 g) was subjected to vacuum liquid chromatography using gradient solvent elution (Harborne *et al;* 1984). The column was successively eluted with 100 % petroleum ether, mixture of ethyl acetate/petroleum ether (1:1), ethyl acetate 100 %, mixture of ethyl acetate/methanol (1:1), 100% methanol and a mixture of methanol/water (9:1). Five fractions each of 1.5 L were collected on the basis of VLC profile. The fractions were concentrated using rotary evaporator at 40^{o}C and tested for antioxidant properties.

Determination of antioxidant activity

All assays were carried out in triplicates and results expressed as means ± SEM.

DPPH radical scavenging assay

The scavenging effect of crude methanol extract and fractions of *P. nigrescens* seed on DPPH radical was estimated with method described by Jain *et al*., (2008). The absorbance of the mixture was measured using a spectrophotometer at 517 nm. Ascorbic acid was used as reference standard. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = $[(A_0 - A_1)/ (A_0)] \times 100$

Where; A_0 was the absorbance of DPPH radical + methanol,

A_1 was the absorbance of DPPH radical + sample extract /standard (Kaushik *et al;* 2012).

The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radical (Benzie and Strain; 1996).

Total antioxidant activity (FRAP Assay).

A modified method of Benzie and Strain (1996) was adopted for the ferric reducing antioxidant power (FRAP) assay. This assay depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Briefly, 1.5 ml of freshly prepared FRAP solution (i.e. 25 ml of 300 mM acetate buffer at pH 3.6 + 2.5 ml of 10 mM 2,4,6-tripyridyls-triazine (TPTZ) in 40 mMHCl + 2.5 ml of 20 mM ferric chloride ($FeCl_3.6H_2O$) solution) was mixed with 1 ml of the extracts separately, and the absorbances read at 593 nm. The standard curve was linear between 100 and 500 μM $FeSO_4.7H_2O$. Results are expressed in μM Fe (II)/g dry plant material and compared with that of ascorbic acid.

Determination of polyphenolic content

Total phenol

Total phenol contents in the extracts were determined by the method described by Kim *et al*., (2003). The absorbance was measured by spectrophotometer at 750 nm. The total phenolic content was expressed as

milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). The standard curve was prepared by gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/l).

Total flavonoid

Total flavonoid contents were estimated using the method described by Ebrahimzadeh *et al*., (2008). The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared using quercetin at six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/l).

RESULTS AND DISCUSSION

Phytochemical screening

The result of the phytochemical screening of the powered seeds of *Parquetina nigrescens* is shown in table 1. The result indicates the presence of alkaloids, saponins and flavonoids and the absence of tannins and phytosterols.

Table 1: Phytochemical composition of *Parquetina nigrescens* seeds.

Phytochemical	Inference
Alkaloids	+
Carbohydrate	+
Reducing sugars	+
Saponins	+
Tannins	-
Phytosterols	-
Proteins	+
Phenolic compounds	+
Flavonoids	+

+ indicates presence of component, – indicates absence of component

Table 2: IC_{50} values of *Parquetina nigrescens* seed extract and fractions.

Sample	IC_{50} Value (µg/ml)
Ascorbic acid	6.50
Petroleum ether/Ethyl acetate	-
Ethyl acetate	-
Ethyl acetate/ Methanol	10.00
Methanol	4.50
Methanol: water	-
Crude	11.00

Values are as extrapolated from the plots in figure 1.

Percentage yield of methanol extract and fractions

The percentage yield of the methanol extract was 12.89 %, while the percentage yield of the petroleum ether/ethyl acetate, ethyl acetate, ethyl acetate /methanol, methanol and methanol/water fraction with respect to the crude methanol extract were 17.97 %, 1.29 %, 46.21 %, 41.16 % and 1.05 % respectively.

Antioxidant activity

DPPH radical scavenging activity

The crude extract, methanol (MeOH) and ethyl acetate/methanol (EA/MeOH) fractions demonstrated high concentration-dependent DPPH radical scavenging activity which was comparable to ascorbic acid at concentrations between 25-100 µg/ml. The petroleum ether/ethyl acetate (PE/EA), ethyl acetate (EA) and methanol/water ($MeOH/H_2O$) fractions had low antioxidant activity. Figure 1 shows the plot of the percentage radical scavenging activities versus the concentrations of the extract and fractions. IC_{50} values obtained from figure 1 are shown in table 2.

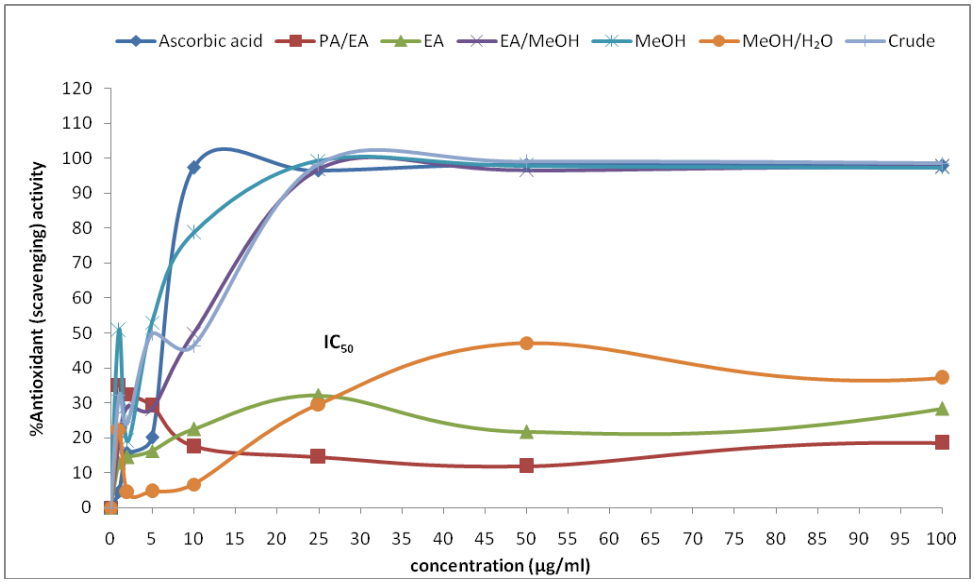


Figure 1: DPPH radical scavenging activity of crude extract and fractions of *Parquetina nigrescens* seed compared with standard (Ascorbic acid)

Table 3: FRAP assay result of extract and fractions

Sample	Result (µM/g dry weight of extract)
Ascorbic acid	1790.00 ± 0.00[a,c]
Petroleum ether: ethyl acetate	436.67 ± 3.33[b]
Ethyl acetate	433.33 ± 3.33[b]
Ethyl acetate: Methanol	1780.00 ± 0.00[a,c]
Methanol	1780.00 ± 0.00[a,c]
Methanol: water	406.00 ± 24.04[b]
Crude	1790.00 ± 0.00[a,c]

Values are expressed in Mean ± S.E.M (n=3). c= values significantly different from b values P< 0.05, *P*< 0.01, same alphabets indicate statistically comparable results.

Total phenolic and flavonoid assay

Figure 2 shows the result of the total phenolic and flavonoid contents of crude extract and fractions of *Parquetina nigrescens*. Total phenolic content was reported as mg gallic acid equivalent/g of extract by reference to a standard curve (y = 0.0037x; R^2 = 0.9768) (Figure 3). The total flavonoid content was reported as mg quercetin equivalent/g of extract by reference to a standard curve (y = 0.0132x; R^2 = 0.9761) (Figure 3).

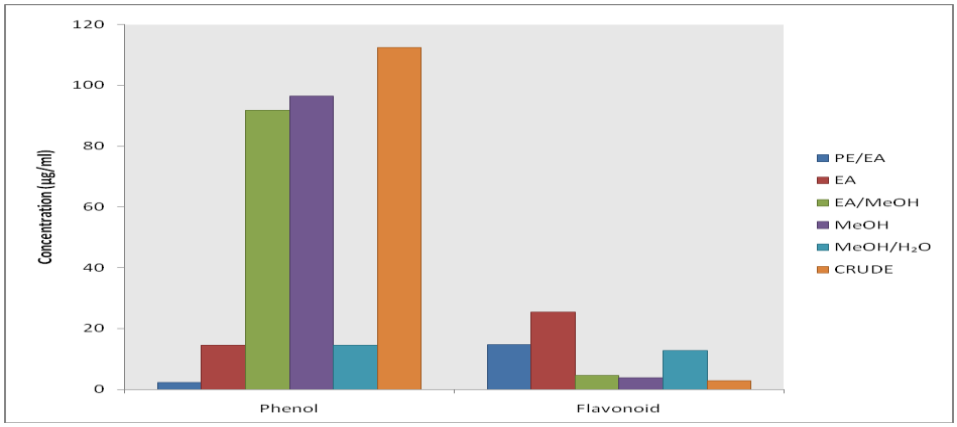
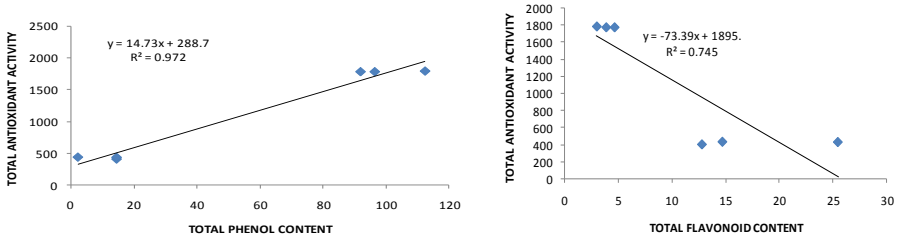


Figure 2: Total phenolic and flavonoid contents of extract and fractions of *P. nigrescens* seed



(A) **(B)**

Figure 3: Correlation plots: **(A)** Total antioxidant activity vs Total phenol content; **(B)** Total antioxidant activity vs Total flavonoid content. Significant correlation *(P < 0.05)* exists in **(A)**, in plot **(B)** there was no correlation.

DISCUSSION

Phytochemical screening

Phytochemical screening of *P. nigrescens* seed revealed the presence of alkaloids, flavonoids, phenols, proteins, saponins and carbohydrate with the absence of tannins and phytosterols. Imaga *et al*., (2010) had earlier reported the presence of alkaloids, flavonoids, glycosides, tannins, saponins and anthraquinones in the leaves of *P. nigrescens*. Phytochemical screening of the fruit bark by Uwaya *et al*., (2013) also showed the presence of sugars, reducing sugars, alkaloids, cardiac glycosides and saponins and the absence of flavonoids, tannin, and anthracene glycosides. Okunrobo *et al*., (2014) also reported alkaloids, saponins, flavonoids and phenolics as phytochemicals present in the methanol extract of *Parquetina nigrescens* seeds. These results show that the phytochemical constituents are evenly distributed in the plant with exception to tannins which are absent in the seeds. Alkaloids are present in most parts of the plant.

Antioxidant assay

In this study, a dose-dependent scavenging activity was observed for the crude extract and fractions as seen in figure 1. The plateau observed might be associated with the fact that the crude extract and fractions have exhibited their maximum scavenging effects at concentrations used. A significantly low

value was observed for the petroleum ether/ethyl acetate fractions with the activity decreasing with increasing concentration. Maximum scavenging activity was observed for the crude extract, ethyl acetate/methanol and methanol fractions.

Natural antioxidants present in plants are responsible for preventing or inhibiting the deleterious consequences of oxidative stress (Bhuiyan and Hoque; 2009, Gill; 2011). Plants contain free radicals like polyphenols, flavonoids and phenolic compounds. Polyphenols are the major plant compounds with antioxidant activity. The activity is due mainly to their redox properties (Wichi; 1988, Khalaf *et al*; 2008), which plays an essential role in neutralizing and absorbing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Kaushik *et al*; 2012).

The result of the DPPH radical scavenging assay showed that *P. nigrescens* extracts have an appreciable DPPH scavenging effect with the crude extract being the most active. The free radical scavenging power of the crude extract and fractions increased with increasing amount of the extract (Figure 1). The methanol, ethyl acetate/ methanol and crude extract showed antioxidant activity, with IC_{50} values of 4.5 µg/ml, 10 µg/ml and 11 µg/ml. The 50% inhibition concentration (IC_{50}) in the methanol fraction was lower than the values obtained for the polar fractions and crude, this may be due to the fact that the polar solvent systems used prior to the methanol/water system had exhaustively taken up most active constituents of the extract or hydrolysis of active constituents. The IC_{50} values show that the methanol fraction (4 µg/ml) was more potent than the standard drug (ascorbic acid, 6.5 µg/ml). However, the crude extract, ethyl acetate/methanol and methanol fractions had percentage scavenging activities that were comparable to that of the standard ascorbic acid. A concentration dependent relationship was observed in the radical scavenging activity of the ethyl acetate/methanol fraction with the scavenging activity increasing as the concentration of this fraction was being increased. The high antioxidant activity observed in the crude, ethyl acetate/methanol and methanol is related to the phenolic compounds but not the flavonoids compounds present in the plant.

In the FRAP assay as seen in table 3, the crude extract, petroleum ether/ethyl acetate, ethyl acetate, ethyl acetate/ methanol, methanol and methanol/water fraction had 1790.00, 436.67, 433.33, 1780.00, 1780.00, 406.00 µM/g dry weight of extract respectively. Ferric reducing antioxidant activity (FRAP) which depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)-TPTZ) at low pH was determined (Ou *et al*; 2005). The results showed that the crude extract (1790 µM/g dry weight of extract) has antioxidant activity comparable to that of the standard ascorbic acid (1790 µM/g dry weight of extract). The ethyl acetate/ methanol and methanol fractions also had comparable antioxidant activity (1780, 1780 µM/g dry weight of extract) with the petroleum ether/ethyl acetate, ethyl acetate and methanol/water fraction having low antioxidant activity (436, 433, 406µM/g dry weight of extract respectively). The result corresponds to that obtained using DPPH scavenging activity. This confirms that the seeds of *Parquetina nigrescens* have antioxidant activity as seen in the leaves (Prior *et al;* 2005). The results obtained from both method of assay of antioxidant activity were comparable with the crude methanol extract, ethyl acetate/methanol and methanol fractions having the highest antioxidant activity.

Total phenol and flavonoid contents

As shown in figure 2, the results of the total phenolic and flavonoid contents indicated that the crude extract contained more phenol (112.34 mgGAE/g extract) contents while the ethyl acetate fraction had more flavonoid (25.53 mgQE/g extract) contents than the other fractions. The Folin-Ciocalteu assay has for many years been used as a measure of total phenolics in natural products. The mechanism is an oxidation/reduction reaction and, as such, can be considered a measure of antioxidant capacity provided the specified conditions are followed to minimize variability and eliminate erratic results. The

presence of hydroxyl groups in the phenolic compounds may directly contribute to the antioxidant activity and is a key determinant of their radical scavenging and metal chelating activity (Sathishsekar and Subramanian; 2005, Elmastas *et al*; 2007).

In this study, high content of total phenolic compounds in *P. nigrescens* seeds may be responsible for its free radical scavenging ability (Figure 4). In addition, the differences in activities of the different solvents may have been due to higher solubility of phenolic components in the crude and methanol solvent (polar) than in ethyl acetate/methanol solvent, ethyl acetate (semi-polar) and petroleum ether/ ethyl acetate solvents (non polar). In figure 3, an inverse relationship was observed for the flavonoids and phenolics. Non-polar fractions had higher flavonoid content than phenolics and *vise versa* for the polar fractions.

CONCLUSION

This study has demonstrated that the seeds of *Parquetina nigrescens* possess antioxidant activity and could potentially serve as a source of natural plant antioxidants. However, further studies are pertinent.

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