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Chemical Composition, Antioxidative and Antimicrobial Activities of Different Extracts of the Leaves of *Parquetinanigrescens* (Asclepiadaceae)

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Highlights

- Various extracts of *Parquetinanigrescens*leaves (PN) showed moderate to high activities against DPPH radical, nitric oxide and lipid peroxidation
- Ethyl-acetate extract of PN showed broad spectrum antibacterial activity, while *n*-hexane extract demonstrated narrow spectrum antifungal activity
- *P. nigrescens* leaves possess potentials as source of antioxidant and antimicrobial agents

ABSTRACT

Oxidative stress has been implicated in thepathogenesis of many diseases, while multiple drug resistance constitutes a major problem in management of infectious diseases. This stressrequires continuous investigations for development of novel antioxidant and antimicrobial agents. Here, phytochemical constituents, antioxidant, and antimicrobialactivities of *n*-hexane, ethyl-acetate, 70%-ethanol and methanol extracts of *Parquetinanigrescens* leaves were investigated. Antioxidant activities were evaluated by total phenolic content, total flavonoid content, total antioxidant capacity, reducing power, DPPH, nitric oxide, and lipid peroxidation assays. Antibacterial (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli*) and antifungal (*Aspergillus niger, Aspergillus flavus*, *Candida albican* and *Penicillium spp*.) activities of the extracts were examined using agar well diffusion method. Phytochemical investigations revealed presence of various phytochemicals, including alkaloids, tannins, flavonoids, cardiac glycosides, and terpenoids. Total phenolic contents were in the range 0.321±0.030–0.432±0.030 mg gallic acid/g extract, while total flavonoid contents were in the range 0.020±0.003–0.064±0.006 mg quercetin/g extract. The extracts displayed moderate total antioxidant capacity (TAC) and ferric reducing power; having TAC values in the range 0.820±0.060–0.876±0.030 mg ascorbic acid/g extract and reducing powers in the range 0.01–0.68. The extracts showed moderate to high activities against DPPH radical, nitric oxide and lipid peroxidation; having IC_{50} values in the range 59.3±10.8–87. 1±14.2 µg/mL, 52.7±10.6–56.9±10.1 µg/mL, and 58.9±18.6–101.3±10.2 µg/mL, respectively. Furthermore, ethyl-acetate extract showed broad spectrum antibacterial activities, while *n*-hexane extract demonstrated narrow spectrum antifungal activity.These findings suggest that *P. nigrescens* leaves have potentials as source of antioxidant and antimicrobial agents.

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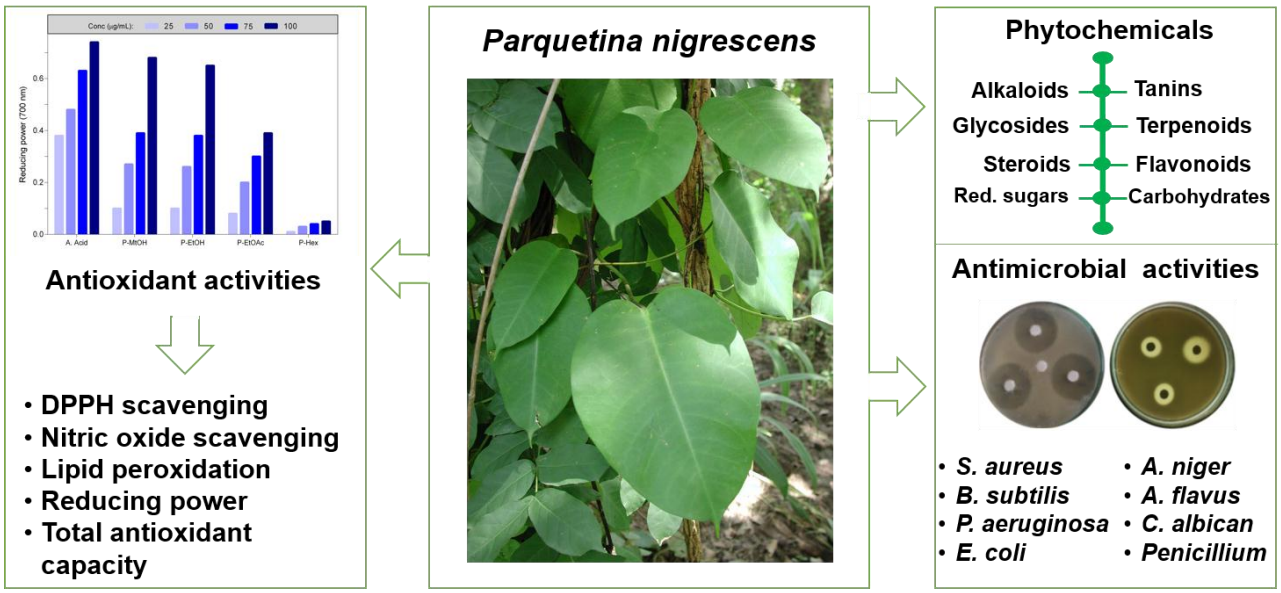
GRAPHICAL ABSTRACT



1-Introduction

The wide range of effects of reactive oxygen species (ROS) and related free radicals on biological systems has been broadly studied and documented. By and large, free radical-induced stress has been reported to be implicated in the pathogenesis of about 50 disease conditions including cancer, cardiovascular diseases, neurodegenerative diseases, diabetes mellitus, cataracts, and cirrhosis [1–4]. Naturally, the activities of ROS and related free radicals in the body are controlled by various protective mechanisms, referred to as antioxidative systems. It is when these protective systems have been compromised that free radical-induced stress become cumulative and pathological. Studies have shown that many plant extracts and isolated constituents possess antioxidant reinforcement properties and could exert protective effects against free radical-induced stress in biological systems [5, 6].

Multiple drug resistance in the management of infectious diseases remains a global medical problem, owing to a great extent to indiscriminate use of antibiotics. Currently, concerted efforts are being made to combat this problem; these include measures to control the use and/or misuse of antibiotics, research to elucidate the genetic mechanisms of resistance, and continuous studies for the development of novel antibiotics[7]. Studies have likewise shown that many plant extracts and isolated constituents have antimicrobial properties and could be employed for the treatment of infectious diseases and/or development of novel antimicrobial agents [8, 9].

Parquetinanigrescens(Afzel.) Bullockis a perennial herbaceous plant belonging to theAsclepiadaceae family. The plant is found in the tropical regions of Africa, across Senegal to Nigeria, and over the Congo basin to South tropical Africa [10]. *P. nigrescens*is planted around houses for wind breaking, for ornamental purposes and for its ethnomedicinal importance. The various parts of the plants are widely utilized for treating different conditions.The leaves are applied traditionally for treating helminthiasis and conjunctivitis, as insect repellant and to ease delivery in pregnancy, while the roots are used for treating rheumatism[11].*P. nigrescens*is also applied as a cardiac tonic, an aphrodisiacand remedies for stomach aches, snake bites, general fatigue,

menstrual troubles, mental disorders, diarrhea, skin infections, gonorrhea and erectile dysfunction[12–15].

Several scientific reports have been published attesting the medicinal properties of *P. nigrescens*. The plant has been reported to suppress haemorrhagic anemia and to have antidiabetic, haematinic, cardiotonic, and anti-ulcerative properties [10, 16–18]. The anti-inflammatory, analgesic, and antipyreticeffects of the leaf of the plant have also been documented [19].

Although several chemical, biochemical and pharmacological studies have been done on *P. nigrescens*, this study provides additional information on the phytochemical composition, antioxidative and antimicrobial activities of different extracts of the leaves of the plant.

2-Experimental

2-1-Collection of plant material

Fresh leaves of *P.nigrescens* were collected from the plant natural habitat at Federal University of Agriculture, Alabata, Abeokuta and identified at the University of Lagos Herbarium, where a voucher specimen was deposited with voucher number LUH: 7807.

2-2-Extraction of plant material

The collected leaves of *P. nigrescens* were air-dried and ground into fine powder with an impact mill. Approximately 500 g of the plant powder was extracted exhaustively and sequentially with 2.5 L each of *n*-hexane, ethyl acetate, 70% ethanol and methanol, using Soxhlet extractor. The resulting extracts were then concentrated*in vacuo* using a rotary evaporator and dried in an oven at 45°C to give the*n*-hexane (P-Hex), ethyl acetate (P-EtOAc), 70% ethanol (P-EtOH) and methanol (P-MtOH) extracts.

2-3-Phytochemical analysis

The extracts of *P. nigrescens* leaves were subjected to phytochemical analyses to detect the presence of flavonoids, saponins, alkaloids, anthraquinones, terpenoids, cardiac glycosides, steroids, reducing sugars, carbohydrates, and tannins using standard protocols [14,20].

2-4-Evaluation of antioxidant property

Estimation of total phenolic content

Total phenolic content was estimated using Folin-Ciocalteu reagent and according to McDonald et al [21]. Each extract (1 mL, 0.5 mg/mL) was transferred into a test tube, 0.4 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water) was added and the mixture was incubated for 5 min at room temperature. Following this, 4 mL of sodium carbonate solution (7.5% w/v) was added; the mixture was made up to 10 mL and further incubated for 90 min at room temperature. The absorbance of the mixture was measured at 765nm. The total phenoliccontent was determined with respect to the standard calibration curve ofgallic acid and expressed as gallic acid equivalent (GAE) (mg/g of extract).

2-5-Estimation of total flavonoid content

Total flavonoid content was estimated according to Chang et al[22]. Each extract (1 mL, 0.5 mg/mL) was transferred into a test tube, 2 mL of aluminum chloride (2% in ethanol) was added and the mixture was incubated at room temperature for 1 h. The absorbance of the mixture was measured at 510 nm. The total flavonoid content was determined with respect to the standard calibration curve of quercetin and expressed as quercetin equivalent (QE) (mg/g of extract).

2-6-Estimation of total antioxidant capacity

Total antioxidant capacity (TAC) was estimated according to Prieto et al[23]. Each extract (1 mL, 0.5 mg/mL) was mixed with 3 ml of TAC reagent (0.6 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a test tubeand incubated in a water bath at 95°C for 90 min. The mixture was left to cool to room temperature and the absorbance was measured

at 695 nm. The total antioxidant capacity was expressed as ascorbic acid equivalent (AAE) (mg/g of extract).

2-7-DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity

DPPH radical scavenging activity was determined according to Burits and Bucar[24]. Each extract (1 mL at four concentrations: 25, 50, 75 and 100 µg/mL) was taken into a test tube and 3 mL of DPPH solution (0.1 mM in ethanol) was added. The mixture was shaken, incubated at room temperature for 20 min and the absorbance was measured at 517 nm. Ascorbic acid was used as standard and a control mixture devoid of extract was maintained. The scavenging effect was calculated using the expression:

Percentage (%) DPPH radical scavenging activity = $[(A_0 - A_1)/A_0] \times 100$ (1)

Where A_0 is the absorbance of the control mixture and A_1 is the absorbance of the extract or standard.

2-8-Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined according to Alisi and Onyeze[25]. Each extract (4 mL at 25, 50, 75 and 100 µg/mL) was taken into a test tube, 1 mL of 5 mM sodium nitroprusside and0.5 mL of phosphate buffer saline were added and the mixture incubated for 120 min at 30°C. A 2 mL aliquot of the incubated mixture was added to 1.2 mL of Greiss reagent (1% sulphanilamide, 0.1% naphthylethylene-diaminedihydrochloride in 2% phosphoric acid), incubated for 30 min and the absorbance was read at 540 nm. Ascorbic acid was used as standard. The scavenging effect was calculated using the expression:

Percentage (%) nitric oxide scavenging activity = $[(A_0 - A_1)/A_0] \times 100$ (2)

Where A_0 and A_1 is the absorbance before and after the addition of Greiss reagent.

2-9-Lipid peroxidation inhibition

Lipid peroxidation inhibition was evaluated as described by Okhawa[26]. Each extract (0.1 mL at 25, 50, 75 and 100 µg/mL) was transferred to a test tube, 0.1 mL of liver homogenate and 1.6 mL of Tris-HCl buffer were added. To the resulting mixture, 0.5 mL of 10% trichloroacetic acid and 0.5 ml of 0.75% thiobarbituricacid were added. The mixture was boiled for 1 h at 90˚C, then cooled in ice and centrifuged for 15 min at 3000 rpm. Absorbance of the supernatant was measured against the corresponding blank at 582 nm. Ascorbic acid was used as standard and a control mixture without extract was maintained. The inhibitory effect was calculated using the expression:

Percentage (%) lipid peroxidation inhibition = $[(A_0 - A_1)/A_0] \times 100$ (3)

Where A_0 is the absorbance of the control mixture and A_1 is the absorbance of the extract or standard.

2-10-Reducing power assay

Reducing power was determined according to Burits and Bucar[24]. Each extract (1 mL at 25, 50, 75 and 100 µg/mL) was transferred to a test tube, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferric cyanide were added and the mixture was incubated in a water bath at 50˚C for 30 min. Following this, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. To a 2.5 mL aliquot of the upper layer, 2.5 mL distilled water and 0.5 mL 0.1% ferric chloride solutions were added and the absorbance of the mixture was measured at 700 nm. Ascorbic acid was used as standard.

2-11-Evaluation of antimicrobial property

Test microorganisms

Four bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli*) and four fungal strains (*Aspergillus niger, Aspergillus flavus*, *Candida albican* and *Penicillium spp*.) were used andobtained from their stock cultures at the Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutics and

Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos.

2-12-Antibacterial and antifungal assays

The antibacterial and antifungal activities of the extracts were examined using agar well diffusion protocols in accordance with NCCLS methodology [27]. Mueller-Hinton agar was used as the growth medium for bacterial organisms, while Sabouraud Dextrose agar was used for fungal organisms. Media were prepared according to manufacturers' instructions and 25 mL each of sterile medium was poured in sterile petri plates. After solidification, five uniform wells were bored in each plate with a 10 mm diameter sterile cork-borer and 150 µl portion of various concentrations of the extracts (75, 150, and 300 mg/mL) and standards (6.25, 12.5, 25, and 50 µg/mL) were dispensed. The plates were incubated micro-aerobically for 24 h at 37°C for the bacterial strains and 25°C for fungal strains. Each treatment was replicated three times and the average zone of inhibition was determined. Antibiotics, levofloxacin and bifonazole were used as standards for the antibacterial and antifungal assays, respectively. Control wells were maintained for 5% ethanol, asthe solvent.

2-13-Statistical analysis

Values are presented as mean ± standard error of mean (SEM)and visualized using tidyverse package [28] in R code [29] on RStudio Version 1.3.1093 [30]. Statistical analysis was done on GraphPad Prism Version 6.0 (GraphPad Software, San Diego, USA) using one-way analysis of variance (ANOVA) followed byTukey's post-hoc test;confidence level was 95% and $p \leq 0.05$ was considered significant.

3-Results and discussion

3-1-Phytochemical analysis

Phytochemical analyses indicated high levels of alkaloids, tannins and flavonoids; moderate levels of cardiac glycosides, terpenoids, carbohydrates, and reducing sugars; and low level of steroids in P-MtOH and P-EtOH. Similar phytochemicals were detected in P-EtOAc and P-Hex at moderate to low levels. Saponins and anthraquinones were not detected in the four extracts of *P. nigrescens* leaves (Table 1).

Table 1. Results of phytochemical analyses of the extracts of *P. nigrescens* leaves

Phytochemical group	P-MtOH	P-EtOH	P-EtOAc	P-Hex
Alkaloids	+++	+++	++	-
Tanins	+++	+++	-	+
Cardiac glycosides	++	++	++	++
Terpenoids	++	++	++	++
Steroids	+	+	++	++
Carbohydrates	++	++	+	++
Reducing Sugars	++	++	++	++
Flavonoids	+++	+++	++	+
Saponins	-	-	-	-
Anthraquinones	-	-	-	-

Key: +++ = High level, ++ = moderate level, + = low level, − = not detectable
P-MtOH: methanol, P-EtOH: 70% ethanol, P-EtOAc: ethyl acetate,
P-Hex: *n*-hexane extracts

3-2-Total phenolic and total flavonoid contents

The total phenolic contents of the different extracts of *P. nigrescens* leaves were in the range of 0.321 ± 0.030 to 0.432 ± 0.030 mg gallic acid/g extract, while the total flavonoid contents were in the range of 0.020 ± 0.003 to 0.064 ± 0.006 mg quercetin/g extract as presented in Table 2.

3-3-Total antioxidant capacity

The four extracts of *P. nigrescens* leaves displayed comparable total antioxidant capacity (TAC), having TAC values in the range of 0.820 ± 0.060 to 0.876 ± 0.030 mg ascorbic acid/g extract (Table 2).

3-4-DPPH radical scavenging activity

The extracts of *P. nigrescens* leaves showed moderate to high scavenging effect against DPPH radical, having scavenging activity in the range of54.27–79.13% at the maximum concentration: 100 µg/mL (Fig. 1A).The ethyl acetate extract (P-EtOAc; IC_{50} value of 59.3 ± 10.8 µg/mL) had the highest activity,which was not significantly different ($p > 0.05$) from that of ascorbic acid (IC_{50} of 46.0 ± 8.0 µg/mL).

3-5-Nitric oxide scavenging activity

The nitric oxide scavenging activitiesof the extracts of *P. nigrescens* leaves wereconcentration dependent as presented in Figure1B. The four extracts of the leaves had similar scavenging activities (IC_{50} in the range of 52.7 ± 10.6 to 56.9 ± 10.1 µg/mL), which were comparable ($p > 0.05$)to that of ascorbic acid (IC_{50} of 38.3 ± 7.0 µg/mL).

Table 2. Thetotal phenolic content, total flavonoid contentand total antioxidant capacity of the extracts of *P. nigrescens* leaves

Extracts	Total phenolic content[b]	Total flavonoid content[c]	Total antioxidant capacity[d]
P-MtOH	0.401 ± 0.070	0.042 ± 0.003	0.876 ± 0.030
P-EtOH	0.321 ± 0.030	0.028 ± 0.006	0.873 ± 0.060
P-EtOAc	0.432 ± 0.030	0.064 ± 0.006	0.820 ± 0.060
P-Hex	0.428 ± 0.030	0.020 ± 0.003	0.862 ± 0.050

Values are presented as mean ± SEM (n=3). [b] presented as mg gallic acid/g extract,
[c] presented as mg quercetin/g extract,and[d] presented as mg ascorbic acid/g extract

Table 3. Values of IC_{50} (µg/mL) of the extracts of *P. nigrescens* leaves and ascorbic acid for DPPH, nitric oxide and lipid peroxidation assays

Samples	IC_{50} (µg/mL)		
	DPPH	**Nitric oxide**	**Lipid peroxidation**
Ascorbic acid	46.0 ± 8.0	38.3 ± 7.0	40.4 ± 7.2
P-MtOH	76.2 ± 8.6*	54.3 ± 6.7	101.3 ± 10.2**
P-EtOH	83.3 ± 3.6*	52.7 ± 10.6	74.0 ± 11.3
P-EtOAc	59.3 ± 10.8	55.1 ± 11.1	69.4 ± 13.6
P-Hex	87.1 ± 14.2**	56.9 ± 10.1	58.9 ± 18.6

Values are represented as mean ± SEM.
* $p < 0.05$, ** $p < 0.01$, significantly different compared to ascorbic acid

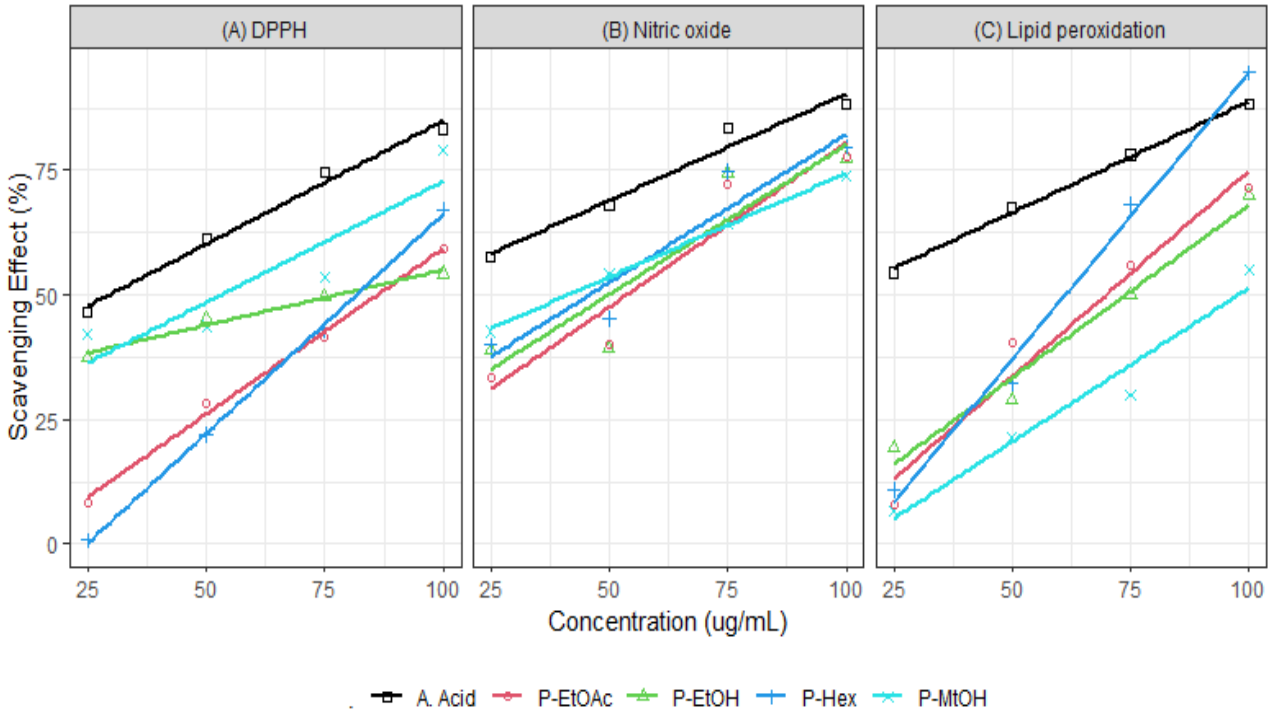


Fig. 1.Scavenging activities of the extracts of *P. nigrescens* leaves and ascorbic acid against (A) DPPH, (B) nitric oxide, and (C) lipid peroxidation

Lipid peroxidation inhibitory effect

The inhibitory effects exerted by the extracts of *P. nigrescens* leaves on lipid peroxidation are presented in Figure1C. P-EtOH, P-EtOAc, and P-Hex (IC_{50} in the range of 58.9 ± 18.6 to 74.0 ± 11.3 µg/mL)displayed high inhibitory effects, which were comparable ($p > 0.05$) to that of ascorbic acid (IC_{50} of 40.4 ± 7.2 µg/mL), while P-MtOH(IC_{50} of 101.3 ± 10.2 µg/mL) showed inhibitory effect significantly lower ($p < 0.05$) than those of the other extracts and ascorbic acid.

Reducing power

All the extracts of *P. nigrescens* leaves showed ferric reducing powers, whichprogressively increased with increase in concentrations as presented in Figure 2. P-MtOH, P-EtOH, and P-EtOAc demonstrated high reducing powers (0.08–0.68), which were comparable ($p > 0.05$) to that of ascorbic acid (0.38–0.74), while P-Hex showed significantly lower ($p < 0.05$) reducing power (0.01–0.05) than the other extracts and ascorbic acid.

Antibacterial and antifungal activities

The antibacterial and antifungal activities of the extracts of *P. nigrescens* leaves against the employed bacteria and fungi were assessed by determinations of zone of inhibition as depicted in Tables 4 and 5. P-MtOH, P-EtOH and P-EtOAc showed some antibacterial activities at the maximal concentration, 300 mg/mL but, showed no antifungal activity. Conversely, P-Hex displayed antifungal property but exhibited no antibacterial activity.

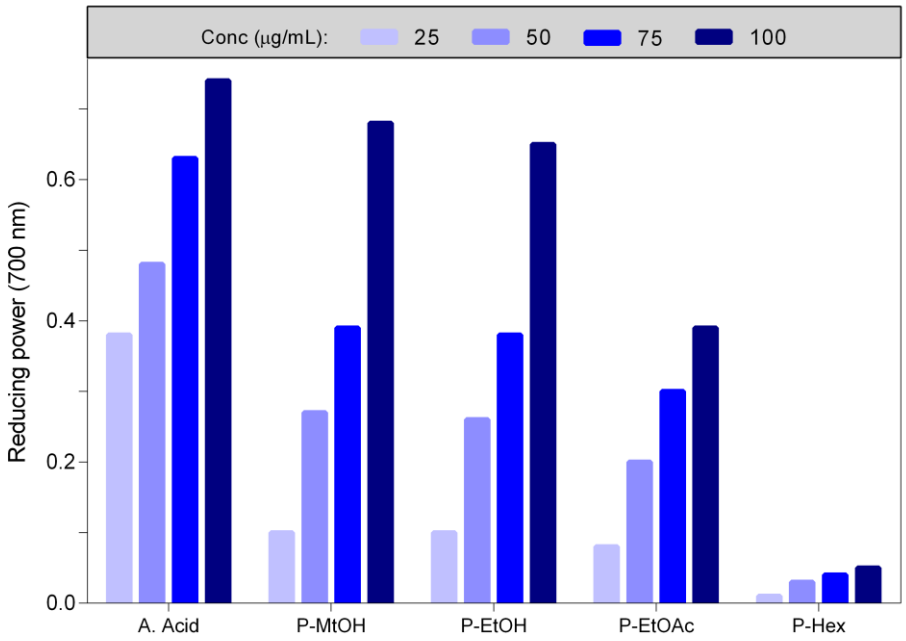


Fig. 2. Reducing power of the extracts of *P. nigrescens* leaves and ascorbic acid

Table 4. Zone of inhibition (mm) produced by the extracts of *P. nigrescens* leaves and levofloxacin in plate cultures of various bacteria

Samples		**Bacterial organisms**			
	Conc .	***S. aureus***	***B. subtilis***	***P. aeruginosa***	***E. coli***
P-MtOH	300 (mg/mL)	-	15.0 ± 0.0	-	-
	150	-	-	-	-
	75	-	-	-	-
P-EtOH	300 (mg/mL)	24.3 ± 0.03	24.3 ± 0.1	-	-
	150	-	16.0 ± 0.0	-	-
	75	-	-	-	-
P-EtOAc	300 (mg/mL)	18.0 ± 0.0	19.5 ± 0.03	21.5 ± 0.09	-
	150	-	-	-	-
	75	-	-	-	-
Levofloxacin	50 (µg/mL)	34.0 ± 1.2	37.5 ± 0.3	35.5 ± 0.9	-
	25	29.0 ± 0.7	35.3 ± 2.1	31.3 ± 2.1	-
	12.5	28.7 ± 0.5	34.0 ± 2.5	30.0 ± 1.4	-
	6.25	22.0 ± 1.0	29.7 ± 1.4	20.0 ± 2.3	-
Control	-	-	-	-	-

Values are represented as mean ± SEM, - = no inhibition

N.B.: P-Hex showed no inhibition against the bacterial organisms.

Table 5. Zone of inhibition (mm) produced by the extracts of *P. nigrescens* leaves and bifonazole in plate cultures of various fungi

Samples		**Fungal organisms**			
	Conc .	***A. niger***	***A. flavus***	***C. albican***	***Penicillium spp.***
P-Hex	300 (mg/mL)	-	28.0 ± 0.0	-	-
	150	-	27.8 ± 0.08	-	-
	75	-	27.5 ± 0.05	-	-
Bifonazole	50 (µg/mL)	29.0 ± 2.0	-	-	-
	25	19.8 ± 0.3	-	-	-
	12.5	19.5 ± 0.5	-	-	-
	6.25	15.5 ± 0.5	-	-	-
Control	-	-	-	-	-

Values are represented as mean ± SEM, - = no inhibition

N.B.:P-MtOH, P-EtOH and P-EtOAc showed no inhibition against the fungal organisms.

In this study, we investigated the phytochemical constituents, antioxidant properties and antimicrobial activities of *n*-hexane (P-Hex), ethyl acetate (P-EtOAc), 70% ethanol (P-EtOH) and methanol (P-MtOH) extracts of the leaves of *P. nigrescens*.

Phytochemical investigations revealed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, terpenoids, carbohydrates, reducing sugars and steroids across the four extracts of *P. nigrescens* leaves, while saponins and anthraquinones were absent (Table 1). Saponins and anthraquinones have previously been reported in methanol and hydro-methanol extracts of *P. nigrescens*[31–33]. Our result does not agree with these previous findings; this however may be due to differences in the sites of plant collection sincethe constituents of a plant can be influenced by the environment in which the plant is grown [34].

To investigate the antioxidant properties of the different extracts of *P. nigrescens*, their total phenolic content, total flavonoid content, total antioxidant capacity and reducing power were determined. The extracts were also evaluated for their activities against DPPH radical, nitric oxide and lipid peroxidation.

Plant phenolics including flavonoidsare major groups of compounds acting as primary antioxidants or free radical scavengers, thus phenolic and flavonoid contents are important antioxidant indices. The total phenolic contents of the four extracts were comparable. The highest content (0.432 ± 0.030 mg gallic acid/g extract) was recorded in P-EtOAc, while P-Hex, P-MtOH and P-EtOH had 0.428 ± 0.030,0.401 ± 0.070 and 0.321 ± 0.030 mg gallic acid/g extract, respectively. For the flavonoid contents, the highest (0.064 ± 0.006 mg quercetin/g extract) was found in P-EtOAc, while the least content (0.020 ± 0.003) was recorded in P-Hex.Cumulatively, the four extracts had a total phenolic and flavonoid contents of 1.58 ± 0.10 mg gallic acid/g extract and 0.15 ± 0.016 mg quercetin/g extract. This total phenolic content is similar to those previously reported (1.35 ± 0.02 mg/g extract) in crude aqueous leaf extract of *P. nigrescens*[35], while the total flavonoid content is higher than that (0.01±0.00 mg/g extract) reported in the same study.

The total antioxidant capacity (TAC) of the four extracts mirrors their total phenolic contents; the extracts had comparable TAC values as presented in Table 2. Total antioxidant capacity assay is an antioxidant method that is based on the reduction of Mo(VI) to Mo(V).This assay shows the capacity of the extracts to donate an

electron to stabilizeredox-active transition metal ions that contribute to formation of free radicals.

The extracts further showed ferric reducing powers, which were concentration-dependent as presented in Figure2. It was noticed that P-MtOH, P-EtOH, and P-EtOAc displayed considerable reducing powers comparable to ascorbic acid. The reducing power indicates the ability of the extracts to reduce Fe (III) to Fe (II). The ferric reducing capacity of the extracts, as demonstrated, corroborates the total antioxidant capacity and attests their reducing potential by electron donation.The various extracts of *P. nigrescens* leaves showed reducing powers in the range 0.05–0.68 at the maximum concentration of 100 µg/mL. In a previous study, methanol and aqueous extracts of *P. nigrescens*were reported to have reducing power of ≈0.72 and ≈0.59, respectively, at a concentration of 250 µg/mL [36].

The DPPH radical is a widely used substrate for evaluating the radical scavenging activity of antioxidant substances. The four extracts of *P. nigrescens* leaves showed concentration-dependent scavenging activities in a similar manner to ascorbic acid against DPPH radical as depicted in Figure 1A. A lower IC_{50} value is indicative of a higher DPPH radical-scavenging activity. From the IC_{50} values presented in Table 3, P-EtOAcexhibited a higher radical scavenging activity than the other extracts, which was comparable to that of ascorbic acid.This observed higher radical scavenging activity of P-EtOAccould be correlated to its relatively higher total phenolic and total flavonoid contents.The various extracts of *P. nigrescens* leaves showed DPPH radical scavenging activities in the range of54.27–79.13% at the maximum concentration of 100 µg/mL. These activities were greater than those previously reported in ethanol (≈59% at 200 µg/mL), methanol (≈57% at 250 µg/mL) andaqueous (≈51% at 250 µg/mL) extracts of the leaves [36, 37].

Nitric oxide is an endogenous molecule that modulates several important biochemical processes;however, when in excess it could also mediate various pathological conditions such as inflammation, neurodegenerative disorders and endothelial dysfunctions. At the simulated physiological pH, the four extracts of *P. nigrescens* leaves exhibited good scavenging activities against nitric oxide, which were comparable to that of ascorbic acid (Fig. 1B). This indicates that the extracts could scavenge nitric oxide in biological tissues and prevent nitric-oxide mediated disorders.

Lipid peroxidation is a free radical associated process that occurs in biological systems and it involves the formation and propagation of lipid radicals, which ultimately damage membrane lipids and could result in various pathologies. In the hepatic tissue employed, the extracts of *P. nigrescens* leaves inhibited lipid peroxidation in a concentration-dependent manner as presented in Figure1C. P-EtOH, P-EtOAc, and P-Hex showed considerable inhibitory effects, which were comparable to that of ascorbic acid. It has been reported that increase in lipid peroxidation arising from consumption of alcohol, drug metabolism and exposure to toxic chemicals is one of the various factors that could predispose to liver damage, tissue damage in inflammation, cancer and aging[38]. In view of this, it is an important health stratagem to reduce lipid peroxidation in human. The activities of the extracts of *P. nigrescens* leaves against lipid peroxidation corroborate those against nitric oxide and also suggest that the extracts contain antioxidant molecules that could protect against free-radical induced stress and associated pathologies.

Furthermore, the antibacterial and antifungal activities of the different extracts of *P. nigrescens* leaves against the employed bacteria and fungi assessed by determinations of zone of inhibition are presented in Tables 4 and 5.Levofloxacin, at all the concentrations tested, showed

considerable inhibitions against the growth of *S. aureus*, *B. subtilis* and *P. aeruginosa*. However, levofloxacin as well as the extracts was not active against *E. coli*. This finding is similar to those of previous studies[39-41],reporting that aqueous and ethanol extracts of *P. nigrescens*were not active against*E. coli* at concentrations up to 200mg/kg. This could be due to the formation of slimy protective layer by *E. coli*[39].

Among the extracts, P-EtOH had the highest growth inhibition against *S. aureus* and *B. subtilis* and at the maximum concentration, 300 mg/mL, its activities against these organisms were comparable to those of levofloxacin at a concentration of 6.25 µg/mL. However, P-EtOAc demonstrated a broader spectrum of antibacterial activity; in addition to *S. aureus* and *B. subtilis*, it was active against *P. aeruginosa*. Its activity against this gram-negative bacterium at a concentration of 300 mg/mL was comparable to that of levofloxacin at a concentration of 6.25 µg/mL. P-MtOH was active only against *B. subtilis*, while P-Hex showed no inhibition against all the bacterial organisms.Aqueous extract of *P. nigrescens* has been reported to be active against *S. aureus*, *B. subtilis*, and *P. aeruginosa*[15].

Bifonazole showed considerable inhibition against the growth of the fungus, *A. niger*but it showed no inhibition against *A. flavus*, *C. albican* and *Penicillium spp*. Among the extracts, P-Hex was the only active extract in the antifungal assay; it showed considerable inhibition against the growth of *A. flavus* but was not active against *A. niger*, *C. albican* and *Penicillium spp*.

Although some of the extracts produced similar zones of inhibition as the standard antibiotics against some of the test pathogens, these were at much higher concentrations. This indicates that the extracts exhibited less antimicrobial potency relative to the standard antibiotics used and it could be due to the fact that the extracts are mixtures of various compounds, present at low concentrations, which could be in some sort of antagonistic interactions. It is therefore necessary to further fractionate the extracts and isolate the individual active compounds.

4-Conclusion

This study was done to investigate the phytochemical constituents, antioxidant properties and antimicrobial activities of *n*-hexane, ethyl acetate, 70% ethanol and methanol extracts of the leaves of *P. nigrescens*. The results showed that the aforementionedextracts of *P. nigrescens* leaves contained various phytochemical constituents, including phenolics and flavonoids. The extracts demonstrated good antioxidantactivities through various mechanisms, including scavenging of free radicals, inhibition of lipid peroxidation and reducing effect by electron donation.Furthermore, the ethyl acetateextractshowed antibacterial activities against *S. aureus*, *B. subtilis*, and *P. aeruginosa*, while *n*-hexane extract demonstrated antifungal activity against *A. flavus*. These indicate that further studies on the individual antioxidant and antimicrobial compoundsin the extracts could be considered.

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