

Phytochemical screening and antioxidant activity of extracts from *Bauhinia rufescens* Lam. and *Physalis angulata* Linn., used in the treatment of ascariasis in Chad

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Abstract Ascariasis is an infection that primarily affects children living in low-income areas, particularly due to a lack of sanitation facilities, high medication costs, and sociocultural practices. The objective of this study was to identify the phytochemical families and evaluate the in vitro antioxidant activity of *Physalis angulata* and *Bauhinia rufescens*. The phytochemical analysis revealed a wide variety of bioactive compounds (polyphenols, alkaloids, flavonoids, tannins, terpenoids, and saponins). The total polyphenol content was determined using the Folin-Ciocalteu method, while antioxidant activity was assessed using the DPPH radical scavenging assay. Inhibitory activity was determined using a colorimetric method. The highest levels were observed for total polyphenols, at 76.51 ± 0.36 mgEqAG/gDM (milligrams of gallic acid equivalent per gram of dry matter) in the methanol extract, followed by alkaloids, 68.35 ± 0.41 mgEqQui/gDM (milligrams of quinine per gram of dry matter) for *B. rufescens* leaves, and 72.52 ± 0.22 mgEqAG/gDM and 68.35 ± 0.41 mgEqQui/gDM, for total polyphenols and alkaloids, respectively, in *P. angulata*. The leaves of *B. rufescens* exhibited DPPH radical scavenging activity, indicating concentration-dependent antioxidant activity, with inhibition rates of 79.65% for the methanolic extract (ME), 62.05% for the aqueous extract (AE), and 32.89% for the hexane extract (HE). A dose-dependent effect was observed for BHT, with concentrations ranging from 50 to 200 μ g/mL in the ABTS^{•+} assay. Butylhydroxytoluene (BHT) scavenged 90.25% of hydroxyl radicals at a maximum concentration of 250 μ g/mL. Furthermore, hydroxyl radical scavenging activity (HRSA) testing showed that the methanolic extract was most effective at reducing ferric ions to ferrous ions. This study highlights the potential of oxidative activity as a natural source of bioactive molecules that may contribute to the treatment of parasitic infections.

Keywords: medicinal plants, geohelminths, anthelmintics, parasitic infections, secondary metabolites, Chad

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1. Introduction

Infections caused by soil-transmitted helminths remain among the most widespread neglected tropical diseases, disproportionately affecting populations in sub-Saharan Africa, Southeast Asia, and Latin America [1]. *Ascaris lumbricoides* infects approximately 800 million people worldwide, leading to chronic malnutrition, anemia, stunted growth, and cognitive developmental disorders in children [2]. Also known as intestinal worms—

multicellular parasites that live in the human intestines—geohelminths are organisms transmitted when people ingest food or water contaminated with parasite eggs or larvae found in the soil [3]. Factors contributing to its development and transmission include: low socioeconomic status, poor hygiene, overcrowding, fecal contamination, heat, and humidity. Severe forms of the disease that cause morbidity and mortality are primarily intestinal obstruction—especially in children—and the migration of adult worms into the biliopancreatic ducts [2]. Limited access to health care and inadequate sanitation infrastructure further exacerbate the burden of disease,

underscoring the urgent need for sustainable and locally accessible anthelmintic drugs, in line with the WHO's 2030 Roadmap for Neglected Tropical Diseases [4].

The World Health Organization (2022) estimates that 80% of the population in developing countries relies on traditional medicine [5]. According to [6], it is not only cultural reasons but also the prohibitive costs of pharmaceutical products, the lack of access to health care infrastructure, and the shortage of qualified health care professionals that drive Africans to turn to traditional medicine. In the Chadian context, traditional treatments for ascariasis caused by *B. rufescens* and *P. angulata* are among several plant species used by local communities, which explains our research team's interest in the scientific evaluation of these medicinal plants. Native to Africa, where it is found in the Sahelian to Sudano-Guinean climates, *B. rufescens* is a bushy, heavily branched shrub or small tree that can grow up to eight meters tall and thrives in all types of soil [7]. The plant's forage value lies in its leafy branches and fresh or dried pods. Its many medicinal uses include its anthelmintic properties and its reputation for treating diabetes, diarrhea, dysentery, gout, leprosy, and malaria [8,9]. *P. angulata*, on the other hand, is an annual, glabrous, ruderal, and segetal herb that grows erect and can reach 80–100 cm in height. It grows sporadically in ruderal areas, in marshy zones, and near food crops [10]. In Africa and beyond, the leaves, fruits, stems, and roots of *P. angulata* are used to treat various ailments (diabetes, malaria, fever, gonorrhea, jaundice, nephritis, sleeping sickness, tumor prevention, schistosomiasis, liver diseases, postpartum hemorrhages and infections, skin conditions, and ear infections [11]. The antioxidant properties of the ethanolic extract and its fractions derived from *B. rufescens* leaves harvested in Chad were investigated, leading to the isolation of compounds; however, studies on *P. angulata* are scarce. The objective of this study is to examine the secondary metabolites contained in the aqueous, hexane, and methanol extracts of *B. rufescens* and *P. angulata* and to determine the antioxidant activity of these same extracts.

2. Materials and Methods

2.1.1. Plant Material

The leaves of *B. rufescens* and the whole plant of *P. angulata* were collected in southern Chad, specifically in Sarh in the Moyen Chari province. After harvesting, the samples were sorted, rinsed with distilled water, crushed, and dried in the Biology Laboratory at the University of Sarh (Chad), away from direct sunlight and at room temperature ($25^{\circ}\text{C} \pm 2$) for three weeks. Subsequently, three solvents were used, following the protocols described in [12]. This was followed by a phytochemical study and an assessment of the in vitro antioxidant activity of the extracts.

2.1.2. Methods

2.1.2.1. Extraction

2.1.2.1.1. Aqueous Extraction

Aqueous extraction was performed using the decoction

method. The process begins by heating 50 mL of distilled water to a boil, to which 50 g of plant powder is added. The mixture is kept at a boil for approximately 10 minutes and then stored for 24 hours at 4°C before being filtered through Whatman No. 4 filter paper. The resulting filtrate is stored in the dark at a temperature of 4°C [13].

2.1.2.1.2. Methanolic Extraction

Methanolic extractions were carried out in three steps: maceration, filtration, and evaporation. 100 g of powdered plant parts were used. The extracts were obtained by macerating (72 hours) 100 g of plant material in an 80% methanol–water mixture. The macerate was then filtered through Whatman No. 1 filter paper. The filtrate is then evaporated to dryness using a rotary evaporator. The dry residues were transferred to sterilized vials and stored at 4°C [14].

2.1.2.1.3. Hexane Extraction

Hexane extraction was performed according to the method described in [15]. Fifty grams of dry, fine powder was dissolved in 150 mL of hexane in a blender. The mixture was homogenized by vigorous blending for 10 minutes. The resulting homogenate is filtered twice through cotton wool and once through 3-mm Whatman paper. The first filtrate obtained is designated hexane filtrate 1. To the residues, 150 mL of hexane is added; after 10 minutes of stirring followed by filtration, hexane filtrate 2 is obtained. Repeating the same procedure yields hexane filtrate 3. These three filtrates are then combined and evaporated in a "Venticel" oven at 55°C for 24 hours. This series of operations resulted in an extract known as the hexane extract.

2.1.2.2. Phytochemical Screening

2.1.2.2.1. Qualitative Phytochemical Screening

The identification of the major phytochemical families derived from our two plants is based on visual observation of color or the formation of a precipitate following the addition of a specific reagent. Thus, tannins and polyphenols were identified using the Stiasny reaction followed by the ferric chloride reaction [16], flavonoids using the Shinoda test [16], saponins using the foam test after shaking the extracts [17], triterpenoids and steroids using the Liebermann-Burchard test [18], alkaloids using the Dragendorff tests [18], carbohydrates using the Molisch test [19], and free quinones [20].

2.1.2.2.2. Quantitative Phytochemistry

The quantification of phytochemical compounds was performed using a spectrophotometric method, with reference to calibration curves established using a series of standard solutions containing the reference standards for each compound.

❖ Determination of Total Phenolic Compound Content

The total polyphenol content (soluble and insoluble) was determined using the colorimetric method with Folin-Ciocalteu phenol, following the protocol described in [21]. The polyphenol content is thus expressed in milligrams of gallic acid equivalent per gram of dry matter

(mgEqGA/gDM).

✓ Terpenoid Assay

The terpenoid assay is performed according to the Liebermann-Burchard method [22], and concentrations are determined using a calibration curve established with lupeol [21].

✓ Determination of Total Alkaloids

Total alkaloids are determined using the Dragendorff method [18]. Alkaloid concentrations are determined using a calibration curve established with quinine (0–100 µg/mL) [23].

✓ Determination of Flavonoid Content

The flavonoid concentrations in the extracts were determined using a calibration curve established with quercetin [23]. The results are expressed in milligrams of quercetin equivalent per gram of dry matter (mgEqQuer/gDM) using a calibration curve.

✓ Saponin Content

Saponin content is determined using a calibration curve established with glucose, and the result is expressed in milligrams of glucose equivalent per gram of dry matter (mgEqGal/gMS) using a calibration curve [20].

✓ Tannin Content

The tannin content was determined according to the protocol described by [25]. A 50-mL volume of each extract at a concentration of 2 mg/mL was added to 1,500 µL of a 4% vanillin solution in methanol. The resulting mixture was vigorously shaken, and 750 µL of concentrated hydrochloric acid was added. The mixture was allowed to stand and react at room temperature for 20 minutes. Absorbance was measured at a wavelength of 550 nm against a blank consisting of a 4% vanillin solution in methanol. The tests were performed three times for each sample. The tannin contents of the extracts were determined using the calibration curve, $Y = 0.0306X + 0.0231$, with $R^2 = 0.9929$.

2.1.2.3. Determination of In Vitro Antioxidant Activity

✓ Antioxidant activity is assessed using four methods:

✓ DPPH Assay (1,1-diphenyl-2-picrylhydrazyl)

Assessment of antioxidant activity using the DPPH method. DPPH, 1,1-diphenyl-2-picrylhydrazyl, a violet-colored free radical, is reduced to a yellow compound in the presence of antioxidants [26].

✓ Reductive Method (FRAP)

The principle of this method is based on the ability of an antioxidant to reduce the Fe(III)-2,4,6-tri(2-pyridyl-s-triazine) (TPTZ) complex to a blue Fe(II)-TPTZ complex, as described in [27].

✓ ABTS Cation Radical Assay

The ABTS cation radical was prepared by mixing equal volumes of a 2.45 mM potassium persulfate ($K_2S_2O_8$) solution and a 7 mM ABTS stock solution; the mixture was stored in the dark at room temperature for 16 hours before use [28]. The resulting solution is diluted with ethanol to achieve an absorbance between 0.7 and 0.8 at 734 nm. 990 µL of this freshly prepared solution is added to 10 µL of extract, and the absorbance is measured at 734 nm after 6 minutes for each series of analyses. BHT is used as the positive control.

✓ Hydroxyl Radical Scavenging Capacity

Hydroxyl radical scavenging activity (HRSA) was determined according to the protocol in [29], with some modifications. This activity was assessed using the salicylate method, which is based on the ability of antioxidants to inhibit the formation of hydroxylated products from sodium salicylate.

2.1.2.4. Statistical Analyses

Descriptive statistics were used to determine the parameters of dispersion (mean, standard deviation, outliers, and frequency). Means were compared using Student's t-test and one-way ANOVA in the R software (version 4.5.0). A difference was considered significant when the probability of the comparison was less than the significance threshold (5%) or $p < 0.05$.

3. Results and Discussion

3.1. Results

3.1.1. Phytochemical Screening

3.1.1.1. Qualitative Phytochemistry

The phytochemical classes investigated in the *B. rufescens* and *P. angulata* samples are presented in Table 1 and Table 2, respectively.

Table 1. Phytochemical screening of *B. rufescens* leaves

Meta bolite s	Alkaloids	Cou mari ns	Qui none s	Flav onoi ds	Sap onin s	Ta nin s	Terp énoid s	Su gar s
EH	+	-	+	+	+	+	++	-
EA	+	+	+	+	+	+	++	+
EM	++	++	+	++	+	+	+	+

Note:- : no coloring, ++ : medium color, + : light coloration

Table 2. Phytochemical screening of the whole plant of *P. angulata*

Meta bolite s	Alkaloids	Cou mari ns	Qui none s	Flavo noids	Sap onin s	Ta nin s	Terp énoid s	Su gar s
EH	+	-	+	+	+	+	+	+
EA	++	-	+	+	+	+	++	+
EM	++	+	+	++	+	+	+	+

Note:- : no coloring, ++ : medium color, + : light coloration

Table 1 and Table 2 revealed the presence of all major groups of compounds sought in the methanolic extracts of *B. rufescens* and *P. angulata*. Alkaloids, quinones, flavonoids, saponins, tannins, and terpenoids are also present, at varying levels, in the aqueous and hexane extracts of these plants. Coumarins and sugars were absent from the hexane extract of *B. rufescens*, and coumarins were also found to be absent from the hexane and aqueous extracts of *P. angulata*.

3.1.1.2. Quantitative Phytochemistry

To quantify the major groups of compounds, calibration curves were established and are presented in Figure 1 through 6.

The total alkaloid, total saponin, total terpenoid, total polyphenol, and total tannin contents of the *B. rufescens* leaf extracts are shown in Figure 7.

Figure 8 shows the concentrations of these same major groups of compounds in *P. angulata* extracts.

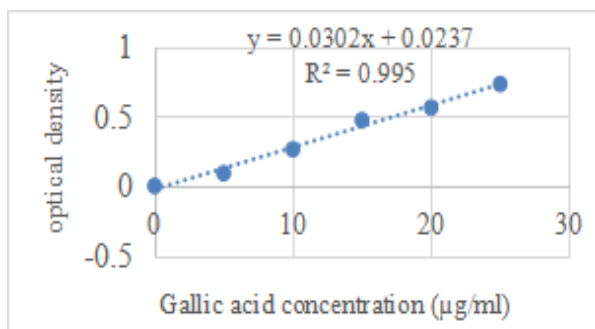


Figure 1. Calibration curve for gallic acid

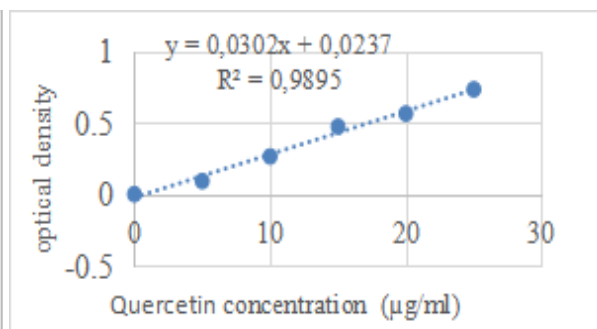


Figure 2. Quercetin calibration curve

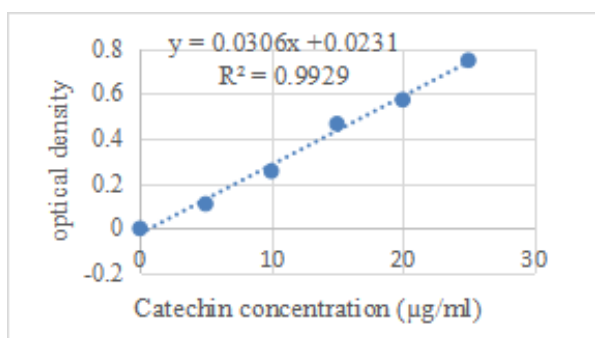


Figure 3. Catechin calibration curve

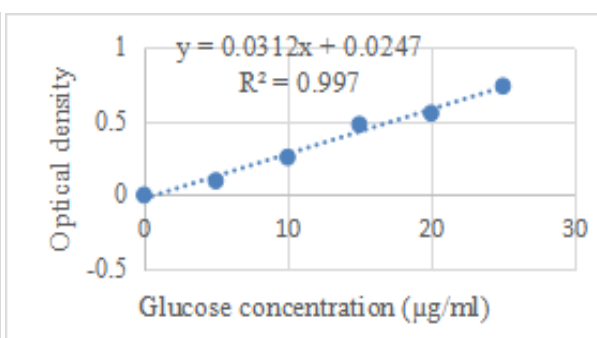


Figure 4. Glucose Calibration Curve

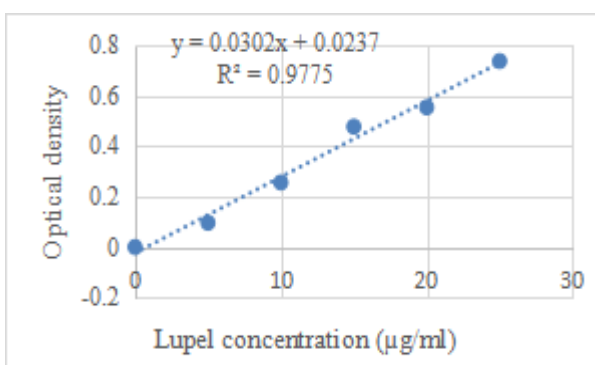


Figure 5. Standard curve for lupeol

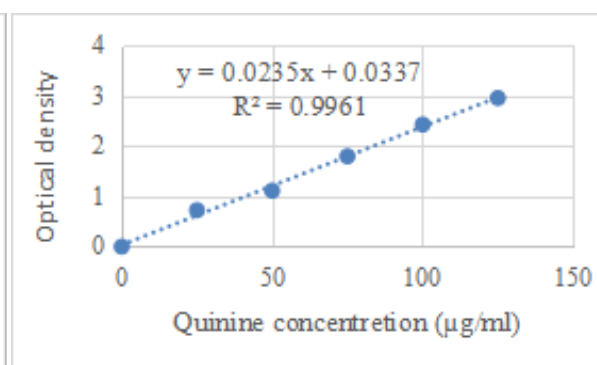


Figure 6. Calibration curve for quinine

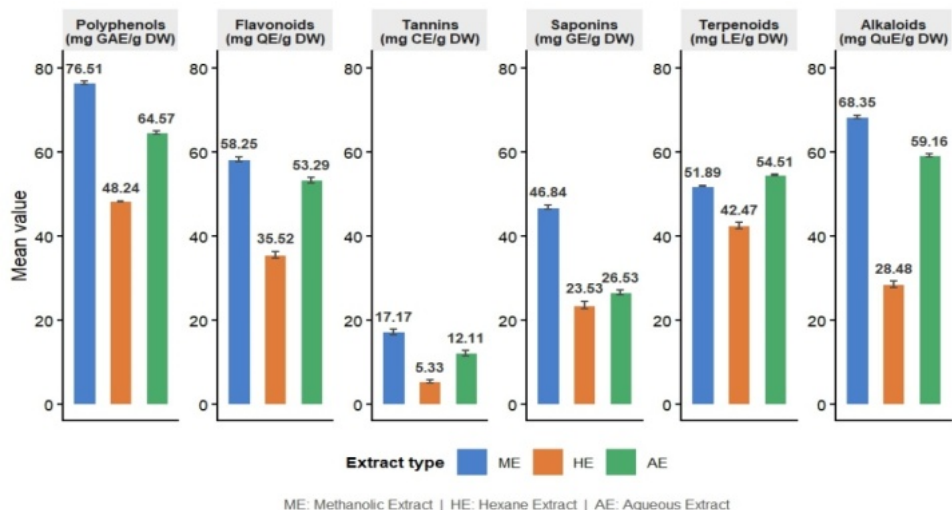


Figure 7. Concentrations of several major groups of secondary metabolites in *B. rufescens*

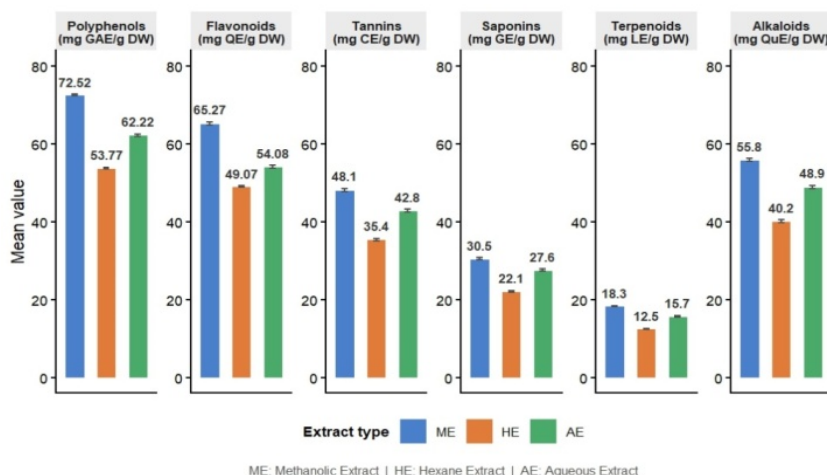


Figure 8. Concentrations of several major groups of secondary metabolites in *P. angulata*

✓ Total polyphenol, total flavonoid, and total condensed tannin contents

Methanolic extracts had the highest levels of total polyphenols, total flavonoids, and total condensed tannins in both plants (*B. rufescens* and *P. angula*) compared to hexane extracts. The concentrations obtained for *B. rufescens* and *P. angulata* were 76.51 ± 0.36 mg AA/g and 72.52 ± 0.22 mg AA/g, respectively, and 58.25 ± 0.63 and 65.27 ± 0.20 mg Quer/g, 17.17 ± 0.64 mg Cat/g and 13.34 ± 0.04 mg Cat/g. The hexane extracts had the lowest values: 48.24 ± 0.15 mg AA/g of total polyphenols, 35.52 ± 0.81 mg Quer/g of total flavonoids and 05.33 ± 0.43 mg Cat/g of condensed tannins for *B. rufescens*, and 53.77 ± 0.25 mg AA/g of total polyphenols, 49.07 ± 0.98 mg Quer/g of total flavonoids, and 06.55 ± 0.85 mg Cat/g of total condensed tannins for *P. angulata*.

✓ Total terpenoid and total saponin contents

The results revealed that the aqueous extract of *B. rufescens* had a significantly higher total terpenoid content (54.51 ± 0.16 mg lupeol/g) compared to the hexane extract (42.47 ± 0.71 mg lupeol/g) of the same plant. In *P. angulata*, the same trend was observed for both the aqueous extract (51.82 ± 0.56 mg lupeol/g) and the methanolic extract (41.47 ± 0.36 mg lupeol/g). When comparing the total terpenoid contents obtained from the two plants, no significant difference was observed regardless of the type of extract.

Total saponin content ranged from 23.53 ± 0.93 mg Glu/g of the hexane extract to 46.84 ± 0.52 mg Glu/g of the methanol extract in *B. rufescens* and from 18.19 ± 0.82 mg Glu/g for the hexane extract to 48.13 ± 0.11 mg Glu/g for the methanol extract in *P. angulata*.

✓ Total alkaloid content

The high alkaloid content of *B. rufescens* leaves is reflected in levels ranging from 28.48 ± 0.80 mg Qui/g in the hexane extract to 68.35 ± 0.41 mg Qui/g in the methanol extract. The whole plant of *P. angulata* contains between 34.3 ± 0.27 mg Qui/g in the hexane extract and 66.5 ± 0.63 mg Qui/g in the aqueous extract.

3.1.2. Antioxidant Capacity

❖ Antiradical Tests

✓ DPPH Assay

The antiradical activity of BHT and the aqueous, hexane, and methanol extracts of *B. rufescens* against

DPPH $\cdot$ is shown in Figure 9. At a concentration of 250 μ g/mL, BHT produced a maximum inhibition of 90.25%. The extracts showed maximum inhibition rates of 79.65% for ME, 62.05% for AE, and 32.89% for HE.

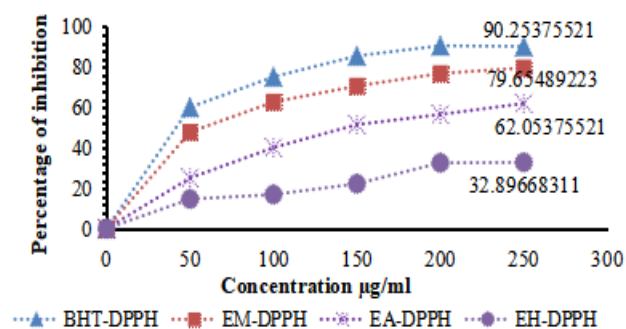


Figure 9. Inhibition of DPPH $\cdot$ by BHT in extracts of *B. rufescens*

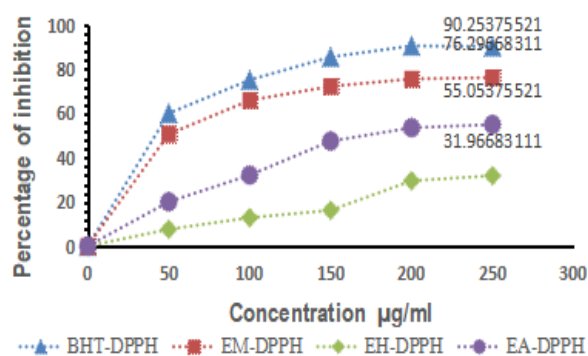


Figure 10. Inhibition of DPPH $\cdot$ by BHT in *P. angulata* extracts

Figure 10 also shows the inhibition percentages of the stable DPPH radical by BHT and by the *P. angulata* extracts. The methanolic extract inhibited the stable DPPH $\cdot$ free radicals by 76.29%, compared to 90.25% for BHT. The aqueous extract (55.05%) and the hexane extract (31.96%) showed the lowest inhibition rates.

✓ ABTS $\cdot$ assay

The inhibitory effects of BHT and *P. angulata* extracts on ABTS $\cdot$ are shown in Figure 11. A dose-dependent effect was observed for BHT, the hexane extract, the aqueous extract, and the methanol extract at concentrations ranging from 50 to 200 μ g/mL. A plateau

was then reached for each of the four curves. At each of these concentrations, BHT exhibited greater ABTS $\cdot^+$ inhibition than the methanolic extract, followed by the aqueous extract and the hexane extract.

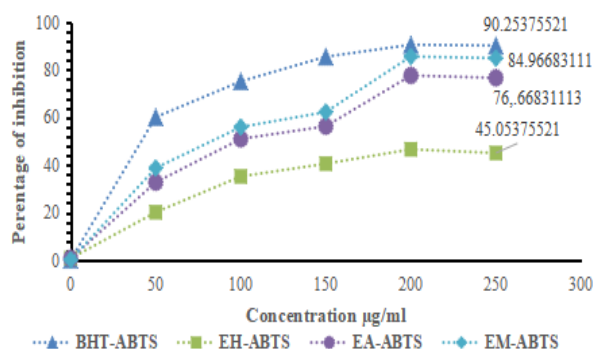


Figure 11. Inhibition of ABTS $\cdot^+$ by BHT and *B. rufescens* extracts

As for the *P. angulata* extracts, the trend remains the same as for those of *B. rufescens*, both in terms of how the curves change with concentration and in terms of their order based on the percentage of inhibition of the ABTS $\cdot^+$ cation radical (Figure 12)

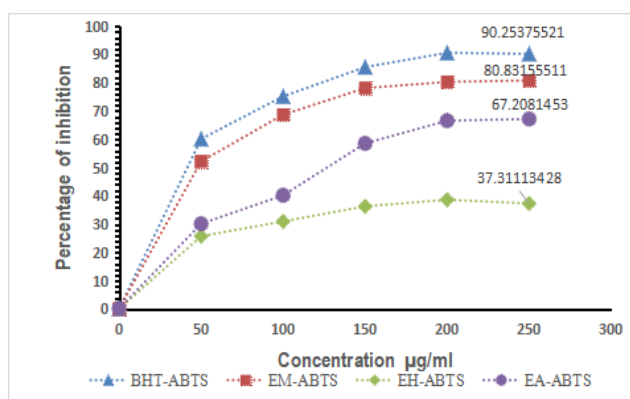


Figure 12. Inhibition of ABTS $\cdot^+$ by BHT and *P. angulata* extracts

✓ HRSA test

B. rufescens extracts and BHT inhibited the $\cdot\text{OH}$ radical, as shown in Figure 13. The $\cdot\text{OH}$ radicals produced during the experiment were inhibited by 92.25% by BHT at a maximum concentration of 250 $\mu\text{g/L}$. At this concentration, the methanolic extract of *B. rufescens* was the most active (76.05%), followed by the aqueous extract (62.89%). The hexane extract exhibited low anti-HRSA activity, failing to achieve 50% inhibition at concentrations of 250 $\mu\text{g/L}$ or less (48.49%).

Figure 14 also shows that the inhibitory activity of BHT's $\cdot\text{OH}$ radicals is consistently superior to that of the plant extracts studied. The aqueous extract of *P. angulata* (76.81%) outperforms the methanolic extract (69.55%) and the hexane extract (55.11%) at a concentration of 250 $\mu\text{g/L}$; BHT, on the other hand, exhibits 92.25% inhibition at the same concentration.

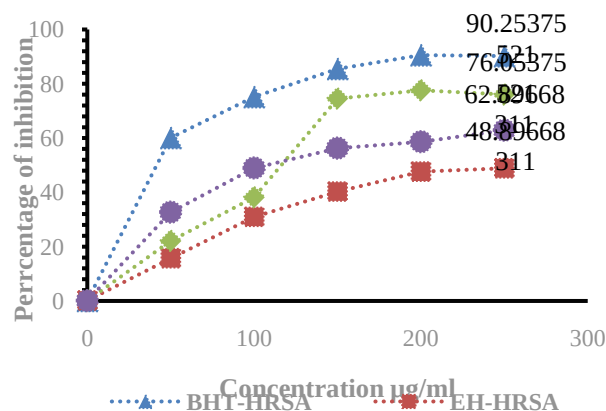


Figure 13. Inhibition of OH $\cdot$ by BHT and *B. rufescens* extracts

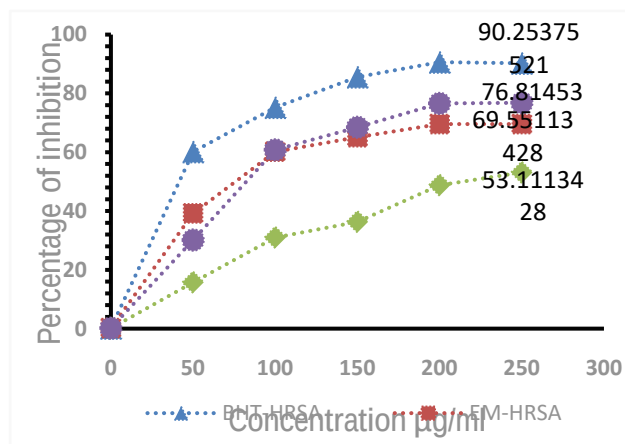


Figure 14. Inhibition of OH $\cdot$ by BHT and *P. angulata* extracts

Table 3. 50% inhibitory concentrations of BHT and extracts against DPPH, ABTS, and HRSA

Control/Sample	IC ₅₀ (µg/mL)		
	DPPH Assay	ABTS $\cdot^+$ Test	HRSA Test
BHT	37 ± 2	37 ± 2	37 ± 2
EH de <i>B. rufescens</i>	120 ± 4	75 ± 2	80 ± 3
EA de <i>B. rufescens</i>	75 ± 2	65 ± 2	50 ± 1
EM de <i>B. rufescens</i>	47 ± 2	52 ± 3	75 ± 2
EH de <i>P. angulata</i>	175 ± 4	47 ± 2	45 ± 3
EA de <i>P. angulata</i>	90 ± 2	95 ± 2	65 ± 1
EM de <i>P. angulata</i>	48 ± 2	42 ± 3	72 ± 2

Legend

ME: Methanolic Extract, AE: Aqueous Extract, and HE: Hexane Extract

The IC₅₀ values obtained from linear regressions based on the DPPH, ABTS, and HRSA test curves are presented in Table 3. In all cases, BHT exhibited IC₅₀ values significantly lower than those of the extracts. The methanolic extracts of *B. rufescens* and *P. angulata* had DPPH $\cdot$ IC₅₀ values of 47 ± 2 $\mu\text{g/mL}$ and 48 ± 2 $\mu\text{g/mL}$, respectively, which were the lowest for each plant. These two extracts also exhibited the lowest IC₅₀ values against ABTS $\cdot^+$: 52 ± 3 $\mu\text{g/mL}$ for the methanolic extract of *B. rufescens* and 42 ± 3 $\mu\text{g/mL}$ for the methanolic extract of

P. angulata. The HRSA assay revealed that the aqueous extract of *B. rufescens* ($IC_{50} = 50 \pm 1 \mu\text{g/mL}$) and the hexane extract of *P. angulata* ($IC_{50} = 45 \pm 3 \mu\text{g/mL}$) had low values for both plants.

✓ Ferric Reducing Antioxidant Power (FRAP) Assay

The iron-reducing powers of the *B. rufescens* and *P. angulata* extracts, expressed in mgEq Vit C/g of extract on a dry weight basis, are presented in Table 4. The hexane, aqueous, and methanol extracts of *B. rufescens* all exhibit antioxidant activity via this mechanism. The aqueous extract thus exhibits a high reducing power compared to the methanolic and hexane extracts. In contrast, for *P. angulata*, the methanolic extract is most effective at reducing ferric ions to ferrous ions. The aqueous extract exhibits greater reducing power than the hexane extract, which, as with *B. rufescens*, has the lowest reducing power.

Table 4. Iron-reducing capacity of extracts from *B. rufescens* and *P. angulata*.

Sample	FRAP (mgEq Vitamin C/gMS)	
	<i>B. rufescens</i>	<i>P. angulata</i>
EH	$53,12 \pm 0,39$	$42,35 \pm 0,52$
EA	$69,58 \pm 0,41$	$59,76 \pm 0,27$
EM	$61,27 \pm 0,15$	$76,89 \pm 0,42$

4. Discussion

Phytochemical screening identified several chemical groups of pharmacological interest in the study of the two plant species collected.

The leaves of *B. rufescens* and the whole plant of *P. angulata* are used by communities in southern Chad to treat parasitic infections. They are primarily used in the form of aqueous decoctions. In addition to these aqueous extracts, other extracts have been prepared using organic solvents, notably n-hexane and methanol. While aqueous extraction remains the simplest and least expensive method, it may be less effective in terms of its ability to extract certain compounds [25].

Phytochemical analysis of *B. rufescens* extracts revealed that the leaves are rich in alkaloids, coumarins, quinones, flavonoids, saponins, tannins, terpenoids, and sugars. These results are similar to those of studies conducted in Nigeria by [30]. According to [31], the stems are also rich in carbohydrates, flavonoids, saponins, and tannins. The absence of coumarins and sugars in the hexane extract of *B. rufescens* is explained by the same reason. The study by [32] on the leaves and fruits of *P. angulata* harvested in Mubi on the plateaus of Adamawa State (Nigeria) showed that these two plant parts contained, qualitatively, the same major groups of compounds, all of which are present in our samples. The total polyphenol, total flavonoid, and total tannin contents are highest in the methanolic extracts of *B. rufescens* and *P. angulata*. The saponins in *B. rufescens* and *P. angulata*, which are more readily extracted with water than with methanol, appear to have structures that contain sugars attached to sapogenins. The qualitative presence of sugars in these extracts supports this hypothesis. The alkaloids in *B. rufescens* are extracted much more efficiently by methanol than by water or n-hexane. Since n-hexane is a

nonpolar solvent, it cannot effectively dissolve and extract these compounds, which have moderate to high polarity. In contrast, in *P. angulata*, the alkaloids present may be in an ionized form—either as salts of organic acids or bound to other hydrophilic compounds—which facilitates their extraction with water. This is the case with cuscohygrine nitrate, which is found, among other alkaloids, in a total alkaloid content representing 0.6% by weight of the aerial parts [33]. [11] estimated that alkaloids accounted for $2.4 \pm 0.4\%$ by weight of the ethanolic extract of *P. angulata* leaves, but the nature of these compounds was not determined. Terpenoids are present in all three extracts from each plant. The aqueous extracts contain the highest levels compared to the other extracts. This high content suggests the presence of highly oxygenated terpenoids (carboxylated, hydroxylated, or glucosylated). The withanolides isolated from the leaves of *P. angulata* contain not only oxygenated rings (lactone, epoxide) but also numerous OH groups attached to the cyclohexane rings [34]. An antioxidant assay is characterized by a CI_{50} , regardless of the type of assay used. The lower the IC_{50} , the greater the corresponding antioxidant activity. All extracts demonstrated antioxidant activity (Table 3 and Table 4). However, the DPPH·, ABTS·+, and HRSA antioxidant activities remain significantly lower ($p < 0.01$) than those of BHT in all cases. The methanolic extracts of the two plants exhibit the strongest DPPH· and ABTS·+antioxidant activity, which is consistent with their high levels of total polyphenols, total flavonoids, and total condensed tannins. The additional contribution of alkaloids and, above all, terpenoids—of which the aqueous extract of *B. rufescens* is particularly rich—would account for a IC_{50} of $50 \pm 1 \mu\text{g/mL}$, making it the most potent ·OH radical scavenger. Carotenoids, which are present in greater quantities in the leaves than in any other organ of *P. angulata*, could explain the higher ·OH -scavenging activity of the hexane extract of *P. angulata* [35]. Methanolic extracts exhibit the highest iron-reducing activity (FRAP) ($p < 0.01$). The high activity observed may be related to their high levels of polyphenols, flavonoids, and tannins, which contain numerous phenolic groups capable of transferring electrons to ferric ions, thereby reducing them to ferrous ions. Numerous studies have demonstrated a positive correlation between these major groups of compounds and FRAP reducing power [36]. According to [30], the reducing power and DPPH radical scavenging activity of *B. rufescens*—as indicators of the methanolic extract's antioxidant activity—demonstrated the potential of this extract as a source of natural antioxidants. The study in [8] highlighted the antioxidant properties of the ethanolic extract of *B. rufescens* leaves and its fractions collected in central Chad. The active compounds were epicatechin, procyanidin B, and quercetin. *P. angulata* contains not only polyphenols, flavonoids, and tannins, but also alkaloids, terpenoids, and saponins. This chemical analysis and literature review indicate that the phytochemical basis for the ethnomedical use of *B. rufescens* and *P. angulata* lies in their rich content of flavonoids, tannins, and alkaloids. These secondary metabolites have demonstrated antiparasitic activity—particularly against helminths—through various mechanisms [37]. According to [38], flavonoids and tannins have the ability to inhibit the oxidative

phosphorylation of parasites. They can bind to a glycoprotein, collagen, which plays a protective role in the parasite's cuticle. This binding causes damage to the cuticle, followed by the death of the helminth [38]. These pharmacological properties have been noted by several authors, notably [38]; phenols and flavonoids possess anthelmintic activity that can affect the sheath of infective nematode larvae.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this work.

Contributions des auteurs

All authors contributed equally to this study. They made substantial contributions to its design, laboratory procedures, and data collection. They also drafted the manuscript and gave their final approval for its submission to the journal for publication.

Conclusion

Ultimately, the quantitative analysis showed that methanolic extracts generally have higher levels of polyphenols and alkaloids than aqueous and hexane extracts, suggesting that methanol is more effective for extracting these bioactive compounds. Antioxidant activity tests conducted using the DPPH radical scavenging assay revealed a strong ability to neutralize free radicals, indicating that extracts from the leaves of *B. rufescens* and the whole plant of *P. angulata* possess significant antioxidant potential. Furthermore, the antioxidant and iron-chelating activities demonstrated in vitro by all plant extracts may be linked to the presence of phytochemical families. Moving forward, fractionation and isolation studies will be conducted to identify the groups of compounds responsible for this activity.

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