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Phytochemistry, Phenolic Contents and Free Radical Scavenging of *Bauhinia thoningii* kurtz and *Ricinus communis* L Extracts as Possible Contribution to Medicinal Effects

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Authors' contributions

This work was carried out in collaboration between all authors. Author OMO wrote the protocol and first draft of manuscript; also managed literature searches. Author AMO designed the study. Author OAA managed the analyses/literature searches of the study and took part in the write up. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Current research places premium on isolation bioactive constituents and antioxidants activities of plants. However, the chemical constituents and antioxidant potential of many local medicinal plants are still unknown. This study sought to assay the phytochemistry and *in vitro* antioxidant potential of *Bauhinia thoningii* (leaf) and *Ricinus communis* (root) as a likely justification of scientifically proved and folklore uses.
Study Design: Air-dried parts of the plants were extracted in solvents and evaporated *in-vacuo*. The extracts were analysed for secondary metabolites.
Place and Duration of Study: Sample: Department of Veterinary Physiology, Biochemistry and

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Pharmacology, University of Ibadan, Ibadan, Nigeria (FHI 108864). The confirmatory identification of the plants was done at Forestry Research Institute of Nigeria (FRIN) with voucher deposited, between 2012 and 2013.
Methodology: Standard procedures were used to carry out phytochemical analyses of aqueous and methanol extracts of the plants. In vitro antioxidant effects of the plants were tested by ferrous reducing antioxidant property (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability. The total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method.
Results: Anthraquinone, phenols and cardenolide were detected in all the plants. The FRAP values of *Ricinus communis* and *Bauhinia thoningii* were 915.3 µmol/g and 620.4 µmol/g while their DPPH scavenging ability was 93.6% and 71.4%. *R. communis* which showed higher phenolic, 90.33 mg/g, content as against 62.14 mg/g for *B. thoningii*.
Conclusion: Results from this study showed a direct relationship between the total phenol constituents and antioxidant activities of the plants. The high phenolic content and the observed radical scavenging activities could be some of the factors responsible for medicinal activities. This may also be clue to the likely mechanism of action of the plants in managing the disease conditions.

Keywords: Ricinus communis; Bauhinia thoningii; phytochemisty; antioxidant; radical; phenol.

1. INTRODUCTION

The recent upsurge in the study of medicinal plants is partly attributed to the discovery that extracts from plants contain active principles. These active principles have been linked to chemical compounds known as secondary plant products. Some secondary products inhibit bacterial or fungal pathogens. The antibacterial activity of the plants has been advanced as reason for self medication by animals in the wild. Such plants have been shown to be rich in phytochemicals like tannins and alkaloids [1].

The presence of the secondary metabolites has also been shown to be the reason for the observed therapeutic effects in traditional medicine. In addition to active ingredients, plants contain minerals, vitamins, volatile oils and other substances that are important in supporting a particular herb's medicinal properties. These secondary metabolites have been shown to be responsible for the radical scavenging capacity of medicinal plants. Hence these bioactive compounds are important especially as an anti-cancer, anti-inflammation, antimicrobial and antioxidant [2,3].

Herbal antioxidants prevent cell and tissue damage as they act as scavenger of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process [4]. Free radicals mainly act by attacking the unsaturated fatty acids in the biomembranes which causes membrane peroxidation, decrease in membrane fluidity and reduction of enzyme and receptor activity and other processes which finally triggers cell death [5].

Bauhinia thoningii kurz is a small tree with leaves shaped like a cow's hoof. It has been shown to have hypoglycaemic activity in experimental diabetic rats [6]. It has also been described as a blood purifier and diuretic. Its folklore uses include blood cleanser and a leaf decoction is used internally and externally for elephantiasis and snake bite. The leaves contain a well-known antibacterial chemical called astragalin [7]. Decoctions of the leaves was claimed to have fever-repelling and expectoral properties. Infusion of leaves and bark are used against worms, dysentery, diarrhoea and malaria [8].

The castor oil plant, *Ricinus communis* L, is a flowering plant in the family Euphorbiaceae. Its seed is the castor oil bean which is the source of castor oil used as laxative, purgative and demulcent [9]. Inhibition of protein synthesis has been postulated as the mechanism of its insecticide and fungicide potential. Hypothetically, the same mechanism has been postulated in the probe of anticancer potential of the plant [10].

Due to carcinogenic probability, synthetic antioxidants are not preferred to natural antioxidants [5]. In the light of this, current research places premium on antioxidants of plant origin. Without the bioactive plant constituents, the plant loses its value of being medicinal; however, the chemical constituents of many local medicinal plants are still unknown. This study

sought to assay the phytochemistry and *in vitro* antioxidant potential of *Bauhinia thoningii* (leaf) and *Ricinus communis* (root) as a likely justification of scientifically proved and folklore uses.

2. METHODOLOGY

2.1 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-s-triazine (TPTZ), ascorbic acid and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Loius, USA), Folin-Ciocalteu, gallic acid and sodium carbonate from Sigma-Aldrich (Steinheim, Germany). All other chemicals used were of analytical grade.

2.2 Plant Materials Collection

Bauhinia thoningii was collected from the Department of Wild life and Fisheries, University of Ibadan (FHI 108866). *Ricinus communis* was collected from the Department of Agricultural Economics, University of Ibadan, Ibadan, Nigeria (FHI 108864). The confirmatory identification of the plants was done at Forestry research institute (FRIN) with voucher deposited.

2.2.1 Preparation of extracts

A portion (400 g) each of air-dried (at room temperature) parts of the plants was soaked in two different solvents-water and methanol. The one soaked in water was shaken vigorously and filtered after 24 hr using whatman (No 1) filter paper; and concentrated *in-vacuo* to give the aqueous extract. The aqueous extract was stored in the refrigerator until ready for use.

The plant soaked in methanol was stirred regularly and filtered after 72 hrs using Whatman (No 1) filter paper; and concentrated *in-vacuo* to give the methanol extract. This was also stored in the refrigerator until ready for use.

2.3 Qualitative Phytochemical Analysis

Standard procedures as described by Ajaiyeoba et al. [11] were used to screen the powdered air-dried plants for the presence of secondary metabolites. In brief, Dragendoff, Wagner and Mayer reagents were used to test for alkaloids. Frothing test for saponin, ferric chloride test for tannin, ferric chloride/chloroform test for anthraquinone and Keddle/Keller-Killiani test for cardenolides.

2.4 Evaluation of Total Antioxidant Potential (FRAP assay)

A modified method of Benzie & Strain as described by Adedapo et al. [12] was adopted for the FRAP assay. The stock solutions used included 300 mM acetate buffer pH 3.6, 10 Mm 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$. The fresh solution was prepared by mixing 25ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $FeCl_3.6H_2O$. The temperature of the solution was raised to 37°C before using it. Plant extracts were allowed to react with the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 539 nm. The results are expressed in µM Fe (II)/g dry mass and compared with that of ascorbic acid.

2.5 Evaluation of DPPH Radical Scavenging Activity

The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined as described by Adedapo et al. [12]. A solution of 0.135 Mm DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol (methanolic extract) or distilled water (aqueous extract) containing 0.04-0.1 mg of the extract. The reaction mixture was shaken thoroughly and left to stand in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. The ability to scavenge DPPH radical was calculated from the following equation:

DPPH radical scavenging activity (%) =

$[(Abs_{control}-Abs_{sample})]/ (Abs_{control}) \times 100$

$Abs_{control}$= absorbance of DPPH radical + methanol

Abs_{sample}= absorbance of DPPH radical + sample extract/standard

2.6 Evaluation of Total Phenolics

The total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method as described by [13]. An aliquot of the

extracts was mixed with 5 ml of 10% Folin-ciocalteu reagent and 4 ml (75 g/l) sodium carbonate. The tubes were shaken vigorously and allowed to stand for 30 min at 40°C for colour development. The absorbance was measured at 765 nm with gallic acid as standard.

2.7 Statistical Analysis

The experimental data obtained were expressed as mean±standard error of mean of triplicates. Where applicable, the results were subjected to one way analysis of variance (ANOVA) with significant values set at $P < .05$.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the qualitative phytochemical analysis of the plants under study. Alkaloid was detected in *Ricinus communis* by Drangedoff's. Cardenolide and saponins were present in plants, *Bauhinia thoningii* and *Ricinus communis*. While anthraquinones was present in *Bauhinia thoningii* a trace of it was detected in *Ricinus communis.* The inverse was the case for tannins.

Table 2 shows comparative (aqueous and methanol extract) total antioxidant potential of *Bauhinia thoningii* and *Ricinus communis* compared with that of ascorbic acid (reference agent). The ferrous reducing antioxidant potential (FRAP) of the methanol extract was higher in both plants. *Ricinus communis* showed a higher antioxidant potential 810.10 µmol Fe (II)/g (aqueous) and 915.34 µmol Fe (II)/g (methanol) compared with *Bauhinia thoningii*, 520.35 µmol Fe (II)/g (aqueous) and 620.37 µmol Fe (II)/g (methanol).

3.1.1 Ascorbic Acid-control

DPPH radical scavenging activity of the plants extract was concentration (0.04 mg/ml to 0.1 mg/ml) and extracting solvent (aqueous and methanol) dependent (Table 3). The lowest activity recorded for the plants was *B. thoningii* 11.12% (aqueous), *R. communis* 20.63% (aqueous) at 0.04 mg/ml. The activity increased to 71.43% *B. thoningii* (methanol) and 93.63% *Ricinus communis* (methanol) at a concentration of 0.1 mg/ml.

Total phenolic content, expressed as gallic acid equivalent of the plants under study was also extract concentration and solvent extraction dependent (Table 4). It showed a variation from 15.12 mg/g, 23.23 mg/g (0.04 mg/ml aqueous extract) to 50.14 mg/g, 55.32 mg/g (0.1 mg/ml methanol extract) for *B. thoningii* and *Ricinus communis*, respectively.

Table 1. Qualitative phytochemical analysis of *Bauhinia thoningii* (leaf) and *Ricinus communis* (root)

Plant		Test	
ALKALOIDS	**ALKALOIDS (Dragendoff's)**	**ALKALOIDS (Meyer's)**	**ALKALOIDS (Wagner's)**
Bauhinia thoningii	-	-	-
Ricinus communis	+	-	-
	CARDENOLIDES (Keller-Killiani)	CARDENOLIDES (Keddle)	
Bauhinia thoningii	+	-	
Ricinus communis	+	-	
	ANTHRAQUINONES		
Bauhinia thoningii	+		
Ricinus communis	±		
	SAPONINS		
Bauhinia thoningii	+		
Ricinus communis	+		
	TANNINS		
Bauhinia thoningii	±		
Ricinus communis	+		

+ ------- present , - = ------- absent ± ------- trace

Table 2. **Total antioxidant potential of aqueous and methanol extract of *Bauhinia thoningii* (leaf) and *Ricinus communis* (root)**

Extracts	FRAP (µmol Fe(II)/g) aqueous	FRAP (µmol Fe(II)/g) methanol
Bauhinia thoningii	520.35±10.45	620.37±3.45
Ricinus communis	810.10±2.43	915.34±12.34
Ascorbic acid	1742.11±20.34	-

Legend: FRAP- Ferrous reducing antioxidant potential

Table 3. DPPH radical scavenging activity of aqueous and methanol extract of *Bauhinia thoningii* (leaf) and *Ricinus communis* (root)

Extract	Concentration (mg/ml)	% Inhibition±EM (n=3) aqueous	% Inhibition±SEM (n=3) methanol
Bauhinia thoningii	0.04	11.2±1.43	30.83±2.34
	0.06	20.55±1.72	55.64±0.67
	0.08	39.60±0.17	60.72±1.34
	0.1	46.72±1.14	71.43±3.45
Ricinus communis	0.04	20.63±1.42	88.45±1.34
	0.06	39.02±0.53	92.82±0.33
	0.08	57.73±1.34	93.23±1.23
	0.1	65.64±0.73	93.63±2.34
Ascorbic acid	0.04	70.05±3.45	-
	0.06	90.36±1.87	-
	0.08	100.07±1.87	-
	0.1	100.03±0.45	-

Table 4.Total phenolic content in aqueous and methanol extract of *Bauhinia thoningii* (leaf) and *Ricinus communis* (root)

Extract	Concentration (mg/ml)	Total Phenol(mg/g) GAE (Gallic acid equivalent) (n=3) aqueous	Total Phenol(mg/g) GAE (n=3) methanol
Bauhinia thoningii	0.04	15.12±0.78	25.34±0.76
	0.06	26.14±2.34	35.32±1.34
	0.08	30.12±1.24	52.21±3.23
	0.1	50.14±1.34	62.14±1.34
Ricinus communis	0.04	23.23±1.23	50.15±0.76
	0.06	28.43±0.78	51.81±0.45
	0.08	45.21±0.87	75.23±2.34
	0.1	55.32±1.23	90.33±0.87

3.2 Discussion

In this study the phytochemical/phenolic constituents and antioxidant/free radical scavenging potential of *Bauhinia thoningii* and *Ricinus communis* were analysed. Free radicals are implicated in many neurodegenerative and metabolic disorders like cancer, diabetes mellitus, and atherosclerosis to mention a few [14]. The scavenging activities of antioxidants make them useful in the management of these disease conditions.

The detection of active principles in medicinal plants plays a pivotal role in investigating the beneficial of such plants as well as elucidating the likely mechanisms of action [15]. The active principles may also be instrumental in understanding the likely effects of the plants on the body. Plant phenolics like tannins are a major group of compounds that have been observed to act as primary antioxidants or free radical scavengers [16]. Diseases like diabetes and oxidative stress are interrelated. The presence of phenolics (e.g. tannin) in the plants under study could be factors that enhanced their claimed and investigated beneficial effects for such conditions

[4,17]. A complementary role in managing the conditions [18] has been shown to be played by anthraquinone which was present in the plants. Improved hepatic and muscle glycogen content is desirable in ameliorating diabetic condition. This has been shown to be enhanced remarkable by alkaloid [19] which was present in *R. communis*. This may also be a link in understanding a likely mechanism of action of the plant in managing the condition.

Results from this study showed that there is a direct relationship between the total phenol constituents and antioxidant activities of the plants. For instance, *R. communis* which showed higher phenolic, 90.33 mg/g, content also showed higher scavenging activities 93.63% (DPPH), 915.34 µmol Fe (II)/g (total antioxidant) as against 62.14 mg/g, 71.43%, and 620.37 µmol Fe(II)/g respectively for *B. thoningii*. This corroborates previous studies which established direct correlation between antioxidant activity and total phenolic contents of some plant extracts [20-22]. The phenolic constituents are responsible for the antioxidant activity in absorbing and neutralizing free radicals or preventing decomposition of peroxides. The living system has inherent ability to neutralize harmful effects of reactive oxygen species using antioxidants. These are molecules such as superoxide dismutase (SOD), glutathione peroxidise (GPX) and catalase (CAT). However, a build up of ROS may overwhelm the system leading to damage to the cell structures manifesting in disease conditions. Antioxidants of plant origin, present in the plants under study, are considered to be valuable in mitigating oxidative damage [2].

The concentration dependent antioxidant activity of the plants is in line with the observation of Gordana et al. [23]. The extracting-solvent dependent activity of the plants under study could be due to the amphipathic nature of methanol. Methanol as a solvent has both polar and non-polar ends. In the light of this, it has the potential to dissolve some organic and inorganic constituents. This could be responsible for the observed higher phenolic and antioxidant activities of the methanol extracts over the aqueous extracts.

4. CONCLUSION

In conclusion, since there is a correlation between phenolic content and medicinal activities of plants, the high phenolic content of the plants under study and the observed radical scavenging activities could be some of the factors responsible for their medicinal activities in previous studies [4,17]. The established mode of action of the active components may also give clue to the mechanism of action of the plants in managing the disease conditions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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