Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

In vitro antitrypanosomal activity, antioxidant property and phytochemical constituents of aqueous extracts of nine Nigerian medicinal plants

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PEER REVIEW

ABSTRACT

Objective: To study the *in vitro* antitrypanosomal activity, antioxidant property and phytochemical constituents of aqueous extracts of nine Nigerian medicinal plants.
Methods: *In vitro* antitrypanosomal activity test was carried out on aqueous extracts of dried leaves of *Acacia albida* (*A. albida*), *Artemisia absinthium*, *Bryophyllum pinnatum*, *Gongronema latifolium*, *Holarrhena floribunda*, *Leptadenia hastata*, *Pericopsis laxiflora* (*P. laxiflora*) and dried stem barks of *A. albida* and *P. laxiflora*. The phytochemical constituents and composition of the extracts and the *in vitro* antioxidant activity of the extracts were subsequently measured using the α,α−diphenyl−β−picryl−hydrazyl (DPPH) radical scavenging assay, Ferric reducing antioxidant power (FRAP) assay, thiobarbituric acid (TBA) activity assay and H_2O_2 radical scavenging activity assay.
Results: From the study, it was discovered that the stem bark extracts of *A. albida* and *P. laxiflora* were most active against both *Trypanosoma evansi* and *Trypanosoma congolense*. There was complete cessation of motility in both trypanosomes within 5 min at 40 mg/mL of the stem bark extract of *A. albida* and complete cessation of motility within 25 min and 40 min at 40 mg/mL with *P. laxiflora* stem bark extract for *Trypanosoma congolense* and *Trypanosoma evansi*, respectively. Quantitative analysis of the phytochemical constituents of the aqueous extracts of the plant parts such as alkaloids, saponins, flavonoids and phenols revealed that the stem barks of *A. albida*, *P. laxiflora* and leaves of *Leptadenia hastata* contained relatively high amount of all the phytochemicals quantified. The stem bark extracts of *A. albida*, *P. laxiflora* and leaves of *Gongronema latifolium* possess more scavenging capacity when compared to other extracts in relation to vitamin C, the reference antioxidant.
Conclusions: This study provides scientific evidence for the use of *A. albida*, and *P. laxiflora* for the treatment of trypanosomosis and diseases associated with oxidative stress.

KEYWORDS
Aqueous, Trypanosomiasis, Oxidants, Phytochemicals, Medicinal plants

1. Introduction

Tsetse flies are vectors of the Human African Trypanosomosis (HAT or sleeping sickness) and African Animal Trypanosomosis (AAT or nagana)[1]. Although over 30 species and sub−species of tsetse are described in the genus *Glossina* and most of which can transmit trypanosomes, only 8−10 tsetse species are of medical and agricultural importance[1]. Trypanosomosis is widely distributed in Africa, Asia and Latin America[2]. In

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Africa, trypanosomosis is restricted to 37 sub−Saharan African countries; however, its distribution extends to more than 10 million square kilometres of the African continent[3]. The major characteristics of the disease include fever, severe anaemia, cachexia, oedema and reproductive disorders leading to death if untreated[4]. African animal trypanosomes are the main parasitological constraints to livestock production in many sub−Saharan African countries infested with tsetse flies[5]. African trypanosomiasis is responsible for 3 million livestock death and the death of about 55 000 people annually[6,7]. Annual loses have been estimated to amount to over 500 million US dollars in meat, milk, lost in traction power, control programme and in annual administration of about 35 million doses of trypanocidal drugs in Africa[8]. Food and Agriculture Organization of the United Nations (FAO) report indicates that trypanosome−induced annual losses in cattle production alone were estimated in the range of 1.0−1.2 billion US dollars in sub−Saharan Africa[4]. The rapid increase in drug resistance, drug−counterfeiting, unpleasant side effects of most trypanocidal drugs, vectors resistance to insecticides and affordability of the chemotherapeutic and chemoprophylactic agents have brought about increased incidence of the disease and therefore, increased requirement for efficacious therapeutic agents[9,10].

Oxidative stress, which is an imbalance between free radical−generating and free radical−scavenging activity has been long implicated in the etiology of various diseases like cancer, diabetes, cerebrovascular disease, autoimmune disorders, ageing *etc*[11,12]. Chronic infection of *Trypanosoma evansi* (*T. evansi*) in camels has been suggested to be associated with a state of oxidative process[13]. This necessitated the use of antioxidants, agents which scavenge the free radicals and prevent the damage they cause, in the treatment of some of these diseases[12,14]. Umar *et al.*[15] reported that the combined administration of antioxidant vitamins ameliorates anemia and organ damage during *Trypanosoma brucei* (*T. brucei*) infection of rats. Indeed some of these antioxidants are from natural sources while some others are synthetic in origin. However, synthetic antioxidants have been suggested to have toxic effects like damage and mutagenesis[11,12]. In addition, synthetic antioxidants show low solubility and moderate antioxidant activity[12]. This necessitated the use of plants, which are generally cheap, available and less toxic, as potential antitrypanosomal agents[9].

The antitrypanosomal activities of most plants have also been known to be as a result of the phytochemicals present in them[16]. Some of these phytochemicals such as flavonoids and phenols are also known to have antioxidant properties[11,16]. The phytochemicals exert beneficial physiological responses and serve as efficient therapeutic agents. Therefore, there is need to evaluate the phytochemical, antioxidant and antitrypanosomal properties of some Nigeria plants simultaneously.

2. Materials and methods

2.1. Chemicals and reagents

Thiobarbituric acid, trichloroacetic acid, oleic acid, 1,1−diphenyl−2−picrylhydrazyl (DPPH), gallic acid and Folin−Ciocalteu phenol reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, glacial acetic acid, ethanol, diethyl ether were purchased from Merck (Merck KGaA, Dermstadt, Germany). All other chemicals were of analytical grade.

2.2. Plant collection and identification

Fresh leaves and stem barks of some plants used locally for medicinal purposes were collected from Ibuzo area in Delta State, Samaru area and Guga forest reserve in Zaria, Kaduna State. They were authenticated at the herbarium unit of the Department of Biological Sciences, A.B.U, Zaria, Kaduna State where voucher specimens were deposited.

2.3. Laboratory animals

Healthy female albino rats were obtained from the Department of Pharmacology, Ahmadu Bello University, Zaria. The animals were acclimatized and maintained on a commercial pelleted poultry growers mash (ECWA, feed, Jos, Plateau State.) and drinking water *ad libitum* for two weeks before commencement of the experiment.

2.4. Test parasite

The *T. evansi* and *T. congolense* parasites were donated by Mr. N. Habila of the Department of Biochemistry, ABU, Zaria. They were maintained in the laboratory by intraperitoneal sub−passage in healthy female albino rats. In passaging, 1×10^3 parasites were introduced intraperitoneally into rats in 0.1−0.2 mL blood/phosphate buffered saline glucose solution. Passaging was considered necessary when parasitemia was in the range of 16−32 parasites per field (usually 5−7 d post infection).

2.5. Plant preparation

Fresh leaves of *Acacia albida* (*A. albida*), *Artemisia absinthium* (*A. absinthium*), *Bryophyllum pinnatum* (*B. pinnatum*), *Gongronema latifolium* (*G. latifolium*), *Holarrhena floribunda* (*H. floribunda*), *Leptadenia hastata* (*L. hastata*), *Pericopsis laxiflora* (*P. laxiflora*) and stem

barks of *A. albida* and *P. laxiflora* were shade–dried for two weeks. The dried leaves and stem barks of the plants were ground into fine powder. Finely ground leaves and stem bark powder of plants (100 g) were macerated in 1 000 mL of cold distilled water and allowed to stand for twenty four hours with intermittent shaking. The plant suspensions were sieved with muslin cloth and finally filtered with Whatman No. 4 filter paper. The filtrate was concentrated to dryness on a water bath for 72 h at 40 °C. The dried crude plant extracts were stored at 4 °C until use.

2.6. Determination of parasitaemia

Parasitaemia was monitored in blood obtained from the tail, pre–sterilized with methylated spirit using the wet blood film. Here, the droplet of blood (about 2μL) is placed on a clean microscope slide and covered with a coverslip (22 mm×22 mm). The blood is examined microscopically at ×400 total magnification with phase–contrast microscopy. Approximately 30 fields are examined. Trypanosomes can be recognized by their movement among the red blood cells. The number of parasites was determined using the "rapid matching" method of Herbert and Lumsden[17]. Briefly, the method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline glucose (PBSG, pH 7.2). Logarithm values of these counts obtained by matching with the table of Herbert and Lumsden[17] were converted to antilog to provide absolute number of trypanosomes per millilitre of blood.

2.7. In vitro antitrypanosomal activity test

In vitro antitrypanosomal activity was performed according to method described by Atawodi *et al.*[18] with slight modification. *In vitro* trypanocidal activity was performed in duplicates in 96 well micro titer plates (Flow laboratories Inc., McLean, Virginia 22101, USA). Blood (10 μL) containing about 126–130 parasites per field, after dilution with PBSG in a ratio of 4:1, was mixed with 10 μL of extract solution of 80.0 mg/mL, 40.0 mg/mL and 20.0 mg/mL to produce effective test concentrations of 40 mg/mL, 20 mg/mL and 10 mg/mL, respectively. To ensure that the effect monitored was that of the extract alone, the untreated blood in PBSG was monitored as well. Reference tests were also performed with two concentrations (40 mg/mL and 20mg/mL) of Samoricide® plus (1.05 g diminazene diaceturate+1.31 g antipyrine+1 mg vitamin B$_{12}$)–a commercial trypanocidal drug. Under this *in vitro* system adopted, parasites survived for about 4 h when no extract was present. Cessation or drop in motility of the parasites in extract–treated blood, compared to that of parasite–loaded control blood without extract was taken as a measure of trypanocidal activity.

2.8. Phytochemical screening of the extracts

The fresh aqueous crude extracts were qualitatively screened for the following constituents[19]: flavonoids, saponins, alkaloids, terpenoids, phenols and cardiac glycoside. The qualitative results have been rated from '+' for faint to '+++' for dense turbidity.

2.9. Phytochemical compositions of the extracts

Based on the positive results obtained for some of the phytochemicals, the phytochemical composition of the extracts were determined.

2.9.1. Determination of total phenolic content

Total phenolics were assessed by the Folin–Ciocalteu method[20]. A volume of 20 μL sample (0.1 g/mL) was mixed with 1.5 mL distilled water and then 100 μL of diluted Folin–Ciocalteu reagent (1:2 v/v, in distilled water). After that, 300 μL of 20% sodium carbonate were added. The final mixture was shaken thoroughly and incubated at room temperature in dark place for 2 h. Thereafter, the absorbance of the samples was measured at 765 nm. Gallic acid (0–500 mg/mL) was used for calibration of a standard curve. The results are expressed as gallic acid equivalents (GAE)/g dry weight of the plant tissue. Triplicate measurements were taken and the mean values were calculated and statistically analysed.

2.9.2. Estimation of flavonoid content

The Naili *et al.*[20] method was used to determine flavonoid content. The plant sample (10 g) was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solutions were filtered through Whatman filter paper No. 1 (125 mm). The filtrates was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

$$\% \text{ Flavonoids} = \frac{\text{Weight of flavonoids} \times 100}{\text{Weight of extract taken}}$$

2.9.3. Estimation of alkaloid content

The Naili *et al.*[20] method was used to determine the alkaloid content. The extracts (5 g) were weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and they were covered and allowed to stand for 4 h. The mixtures were filtered and the extracts were concentrated on a waterbath to one–quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to each of the extracts until the precipitation was complete. The whole solutions were allowed to settle and the precipitates was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloids, which was dried at 60 °C for 30 min and weighed.

$$\% \text{ Alkaloids} = \frac{(W1-W2)\times 100}{W}$$

Where W1=Weight of the filter paper plus precipitate, W_2 =Weight of empty filter paper, W=Weight of the extracts taken.

2.9.4. Estimation of saponin content

The method of Obadoni and Ochuko was used to determine the saponin content[21]. Each extract (20 g) was put into a conical flask and 100 mL of 20% aqueous ethanol was added. The mixtures were heated over a hot water bath for 4 h with continuous stirring at about 55 °C. The mixtures were filtered and the residues re–extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90 °C. The concentrates were transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and they were shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. A volume of 60 mL of n–butanol was added. The combined n–butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solutions were heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponins content was calculated as percentage.

$$\% \text{ Saponins} = \frac{(\text{Weight of filter paper+residue})-(\text{Weight of filter paper alone})\times 100}{\text{Weight of extract taken}}$$

2.10. Measurement of in vitro antioxidant activity

2.10.1. Thiobarbituric acid (TBA) test

The TBA test was conducted according to the method of Ottolenghi[22]. Samples (4 mg) were dissolved on 4 mL of absolute ethanol to make 1 mg/mL solution. Each sample was then mixed with 4 mL of oleic acid solution (2.52%), 0.02 mol/L of phosphate buffer (8 mL) and 4 mL of distilled water to make it up to 20 mL. The solution was incubated at 40 °C in the dark. Ascorbic acid was used as standard antioxidant (positive control) while distilled water was used as blank (negative control). To 1 mL of the samples above 2 mL of trichloroacetic acid (20%) and 2 mL of TBA (0.1 mol/ L) solution were added. This mixture was then placed in a boiling water bath at 100 °C for 10 min. After cooling, it was centrifuged at 1006 ×g for 20 min and absorbance of the supernatant was then measured at 532 nm using UV–Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K).

2.10.2. Reducing antioxidant power

The reducing antioxidant power of the extracts was determined using the method of Oyaizu[23]. Different concentrations of the extracts (10–100 μg/mL) in 1 mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1006×g. The supernatant (2.5 mL) was then mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance measured at 700 nm against a blank using UV–Vis spectrophotometer (Jenway 6100, Dunmow, Essex, UK). Ascorbic acid was used as control. Increased absorbance of the reaction mixture indicates increase in reducing power.

2.10.3. DPPH radical–scavenging activity assay

The DPPH radical scavenging activity was determined using the method of Ak and Gülçin[24]. Each extract (4 mg) was weighed and added to 4 mL of methanol. Varying concentrations (10–100 μg/mL) were prepared from each sample solution using methanol as solvent and 500 μL (0.1 mmol/L) DPPH solution (in methanol) was added giving a total of 2.5 mL per sample concentration. Varying concentrations of the standard, vitamin C, were also prepared at similar concentrations while the blank (control) was prepared using methanol and DPPH without the extract. The mixtures were shaken vigorously and left to stand for 30 min in the dark at 37 °C, and the absorbance was then measured at 517 nm against a blank. Lower absorbance of the mixture indicated higher free radical scavenging activity. The percentage inhibition was calculated as

$$\% \text{ Inhibition} = (A_0 - A_1)/A_0 \times 100$$

where A_0 was the absorbance of the blank, A_1 was the absorbance in the presence of the extract or the standard, vitamin C.

2.10.4. Hydrogen peroxide (H_2O_2) scavenging activity

The ability of the plant parts to scavenge hydrogen peroxide was determined according to the method of Talaz et al[25]. A solution of hydrogen peroxide (40 mmol/L) was prepared in phosphate buffer (pH 7.4, 0.2 mol/L). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer (Jenway 6100, Dunmow, Essex, UK). Extracts (10–100 μg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mmol/L). Absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of the extracts and standard compound (ascorbic acid) was calculated using the following equation:

$$\text{Percent scavenged } [H_2O_2] = \left[\frac{A_0 - A_1}{A_0} \right] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample or ascorbic acid.

3. Results

The *in vitro* anti–trypanosomal activities of the nine aqueous extracts on *T. evansi* show that *A. albida* leaves and stem bark, and *P. laxiflora* stem bark caused complete cessation of *T. evansi* motility (Table 1). *A. albida* leaves at 40 mg/mL caused a cessation of motility within 60 min; the stem bark of *A. albida* at 40 and 20 mg/mL concentrations caused cessation of motility within 5 and 60 min respectively and *P. laxiflora* stem bark at 40 mg/mL caused cessation of *T. evansi* motility at 40 min. All other plant extracts showed only either drastic reduction in *T. evansi* motility after 60 min or slight reduction in motility or no noticeable reduction in motility at all the concentrations tested.

Table 1

The *in vitro* antitrypanosomal activity of nine aqueous plant extracts on *T. evansi*.

Plants	Plant part	Time of change in motility (min)		
		40 mg/mL	20 mg/mL	10 mg/mL
A. albida	Stem bark	5*	60*	60**
A. albida	Leaves	60*	60**	60***
A. absinthium	Leaves	60**	60***	60−
B. pinnatum	Leaves	60**	60***	60−
G. latifolium	Leaves	60**	60***	60−
H. floribunda	Leaves	60**	60***	60−
L. hastate	Leaves	60**	60***	60−
P. laxiflora	Stem bark	40*	60**	60**
P. laxiflora	Leaves	60***	60−	60−
Samoricide® plus		NT	35*	60**
Control		About 5×10⁸ parasites per millilitre of blood, actively motile for 3 h (Herbert and Lumsden, 1976)		

−: No observable reduction in motility; *: Complete cessation of motility; **: Reduced motility drastically; ***: Motility reduced slightly; NT: Not tested.

The *in vitro* anti–trypanosomal activities of the extracts on *T. congolense* show that *A. albida* and *P. laxiflora* stem barks caused complete cessation of *T. congolense* (Table 2). *A. albida* stem bark at 40 mg/mL concentration caused cessation within 5 min while *P. laxiflora* stem bark at the same concentration caused cessation of motility within 25 min. All other plant extracts showed either drastic reduction in motility after 60 min or slight reduction in motility or no noticeable change in motility at all concentrations tested.

Table 2

The *in vitro* antitrypanosomal activity of nine aqueous plant extracts on *T. congolense*.

Plants	Plant part	Time of change in motility (min)		
		40 mg/mL	20 mg/mL	10 mg/mL
A. albida	Stem bark	5*	60**	60***
A. albida	Leaves	60**	60***	60−
A. absinthium	Leaves	60**	60***	60−
B. pinnatum	Leaves	60**	60***	60−
G. latifolium	Leaves	60**	60***	60−
H. floribunda	Leaves	60***	60−	60−
L. hastate	Leaves	60**	60***	60−
P. laxiflora	Stem bark	25*	60**	60***
P. laxiflora	Leaves	60***	60−	60−
Samoricide® plus		NT	35*	60**
Control		About 5×10⁸ parasites per millilitre of blood, actively motile for 3 h (Herbert and Lumsden, 1976)		

−: No observable reduction in motility; *: Complete cessation of motility; **: Reduced motility drastically; ***: Motility reduced slightly; NT: Not tested.

The result of the phytochemical screening shows that none of the extracts contain terpenoids and cardiac glycosides (Table 3). *A. albida* stem bark, *L. hastata* leaves and *P. laxiflora* stem bark contain phenols, saponins, flavonoids and alkaloids. *A. albida* leaves, however, show the presence of flavonoids and alkaloids only while *A. absinthium* and *B. pinnatum* leaves show the presence of saponins and flavonoids. *G. latifolium* leaves show the presence of flavonoids and alkaloids while *H. floribunda* leaves show the presence of saponins and alkaloids, and *P. laxiflora* leaves show the presence of phenols and alkaloids.

Table 3

Phytochemical screening of the nine aqueous plant extracts.

Plant	Plant part	Phenols	Saponins	Flavonoids	Alkaloids	Terpenoids	Cardiac glycosides
A. albida	Stem bark	+++	+++	+++	+++	−	−
A. albida	Leaves	−	−	+++	+++	−	−
A. absinthium	Leaves	−	+++	++	−	−	−
B. pinnatum	Leaves	−	+++	+++	−	−	−
G. latifolium	Leaves	+	−	++	++	−	−
H. floribunda	Leaves	−	+++	−	+++	−	−
L. hastate	Leaves	+++	+++	+++	+	−	−
P. laxiflora	Stem bark	+++	++	++	++	−	−
P. laxiflora	Leaves	+	−	−	+	−	−

+++: Dense turbidity; ++: Moderate turbidity; +: Faint turbidity; −: Absence of turbidity.

Based on the phytochemical screening, the phytochemical composition of the nine aqueous extracts was determined (Table 4). The phenol content of *L. hastata* is significantly higher ($P<0.05$) than that of *G. latifolium* and *P. laxiflora* leaves but does not vary significantly ($P>0.05$) from that of *A. albida* stem bark. *B. pinnatum* leaves has significantly ($P<0.05$) the highest concentration of saponins compared to *A. albida* and *P. laxiflora* stem barks, and *H. floribunda* and *L. hastata* leaves. It is not, however, significantly higher ($P>0.05$) than *A. absinthium*. *A. albida* stem bark has the highest amount of flavonoid (23.33%) which is significantly different from that of other extracts while *A. absinthium* leaves has the lowest amount of flavonoid (11.20%). *H. floribunda* leaves has been shown to have the highest amount of alkaloids (12.8%) which is not significantly different ($P>0.05$) from that of *A. albida* stem bark while *L. hastata* has the least amount of alkaloid which is not significantly different ($P>0.05$) from the level in *P. laxiflora* leaves.

Table 4

Phytochemical composition of the nine aqueous plant extracts.

Plant	Plant part	Phenol (mg/mL)	Saponins (%)	Flavonoids (%)	Alkaloids (%)
A. albida	Leaves	NT	NT	18.33±0.12^{cd}	9.60±0.02^c
A. albida	Stem bark	1.45±0.01^b	10.00±0.01^b	23.33±0.25^e	11.20±0.03^d
A. absinthium	Leaves	NT	11.40±0.13^{bc}	11.20±0.03^a	NT
B. pinnatum	Leaves	NT	12.60±0.11^c	17.60±0.17^c	NT
G. latifolium	Leaves	0.73±0.00^a	NT	12.80±0.11^a	8.00±0.01^b
H. floribunda	Leaves	NT	10.40±0.02^b	NT	12.80±0.13^d
L. hastate	Leaves	1.74±0.02^b	9.50±0.03^b	19.20±0.23^d	6.40±0.01^a
P. laxiflora	Leaves	0.80±0.00^a	NT	NT	7.10±0.02^{ab}
P. laxiflora	Stem bark	1.29±0.01^b	7.60±0.02^a	14.40±0.12^b	8.00±0.03^b

Values are triplicate readings of mean±SD. Different superscripts indicate significant difference in values down the column at $P<0.05$. NT: Not tested.

The *in vitro* antioxidant status of the nine extracts was quantified using various methods some of which considered the scavenging potency of the extracts (Figures 1–4). Figure 1 shows that *A. albida* stem bark has the highest Ferric ion reducing capacity of the nine extracts followed by *P. laxiflora* stem bark. Vitamin C, the standard antioxidant, however, has the highest reducing capacity. The reducing capacity of all the extracts increased with increasing concentration of the extracts, which is obtainable in all the various antioxidant methods used. The hydrogen peroxide scavenging activity of the nine extracts showed that vitamin C, the reference antioxidant, still has the highest percentage radical scavenging activity, followed by *P. laxiflora* stem bark and *G. latifolium* while *P. laxiflora* leaves has the least scavenging activity (Figure 2). The free radical scavenging activity using the DPPH shows that apart from vitamin C, *P. laxiflora* stem bark has the highest percentage inhibition compared to other extracts followed by *A. albida* stem bark while *A. albida* leaves have the least percentage inhibition (Figure 3). *G. latifolium* had the highest TBA scavenging activity which was better than the reference antioxidant, vitamin C (Figure 4). *H. floribunda*, *P. laxiflora* leaves and stem bark and *L. hastata* have relatively high percentage of TBA scavenging activity.
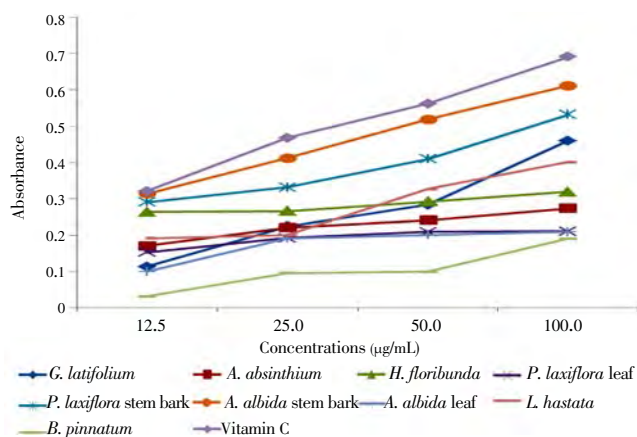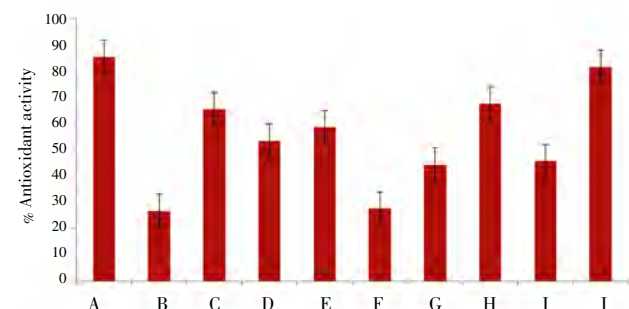

Figure 1. Ferric ion reducing capacity of the nine aqueous plant extracts in comparison with vitamin C.
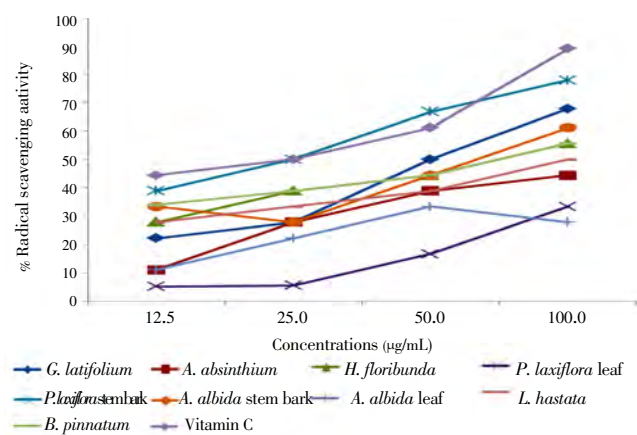
Figure 2. H_2O_2 radical scavenging activity of the nine aqueous plant extracts in comparison with vitamin C.
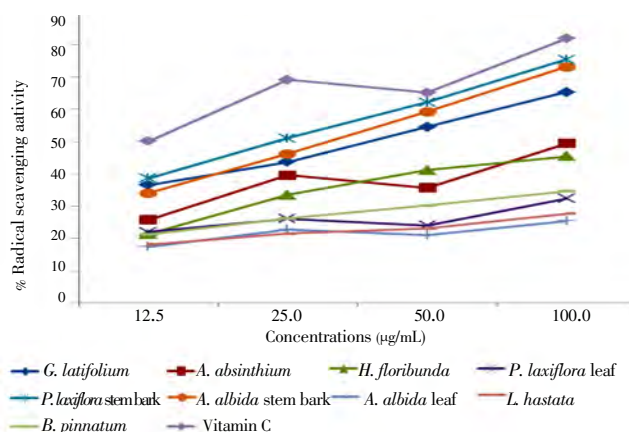
Figure 3. Radical scavenging capacity of the nine aqueous plant extracts in comparison with vitamin C using DPPH

Figure 4. Total antioxidant activity using TBA method on the nine aqueous plant extracts.
A: *G. latifolium*; B: A. absinthium; C: *H. floribunda*; D: *P. laxiflora*; E: *P. laxiflora* stem bark; F: *A. albida* stem bark; G: *A. albida* leaf; H: *L. hastata*; I: *B. pinnatum*; J: Vitamin C.

4. Discussion

The *in vitro* antitrypanosomal activity of the nine aqueous extract was tested on two different *Trypanosome* spp. *viz*. *T. evansi* and *T. congolense*. Research has shown that different trypanosomes have different sensitivities to antitrypanosomal agents which may be associated with bioavailability and toxicity at the concentrations tested[26]. The cessation or drop in motility of trypanosomes may serve as a measure of anti–trypanosomal potential of a plant extract since according to Freiburghaus *et al.*[27] parasite motility constitute a relatively reliable indicator of viability of most zooflagellate parasites. From the result obtained, stem barks of *A. albida* and *P. laxiflora* were most active against both *Trypanosome* spp. and the antitrypanosomal activities shown by these two plant extracts are comparable to that of the trypanocidal drug used. The variation in the antitrypanosomal activity shown by the different plants and the different plant parts was in consonance with previous report that different plants and plant parts exhibit different antitrypanosomal activities[18]. The antitrypanosomal activity is dependent on the bioactive components, solvents and parts of the plant used[18]. The types of solvent and part of

plants used may account for the variation in phytochemicals present[28]. Indeed several flavonoids and alkaloids have been reported for their antitrypanosomal activities[29]. This may be the reason why *A. albida* and *P. laxiflora* stem barks are more potent antitrypanosomal agents compared to other plant extracts. In contrast, *L. hastata* extract does not have good antitrypanosomal activity despite its relatively high quantities of flavonoids and alkaloids, which may be due to the absence of other chemical constituents in the proper concentration needed to elicit activity. In our phytochemical screening, we could not identify terpenoids and cardiac glycosides, which may be due to the fact that we worked with crude extracts which have a very complex composition and so some compounds may be masked[30]. In addition, reports have shown that little quantity of terpenoids was found in the aqueous extracts of some plants[31].

Some of the candidate plants have already been investigated for their antitrypanosomal activity in other studies. However, there may be difference between varying results on the same plant part depending on the geographical area and the time or season of collection[28]. Our result on *P. laxiflora* is not in agreement with the work of Hoet *et al.*[28], although the disparity may also be accounted for by the test parasites (*Trypanosoma rhodensiense* and *Trypanosoma brucei gambiense*) used. Kerharo and Adam[32] reported a very low concentration of alkaloids in the leaves of *P. laxiflora* which is in accordance to our result. There are reports that alkaloids and phenols are present in the *B. pinnatum*, which is not in agreement with our report[33].

Rarely have researchers compared the antioxidant capacity of plants to their antitrypanosomal activity. Nevertheless, report has shown that chronic infection of *T. evansi* in camels has been suggested to be associated with a state of oxidative process[13]. Our result has shown that *A. albida* and *P. laxiflora* stem barks had the highest antitrypanosomal activities, the most ferric ion reducing capacity, the most hydrogen peroxide radical scavenging activity and the most DPPH radical scavenging capacity, which tended to support the work of Umar *et al.*[15] that reported that the combined administration of antioxidant vitamins ameliorates anemia and organ damage during *T. brucei* infection of rats.

This study provides scientific evidence for the use of the aqueous extracts of *A. albida* and *P. laxiflora* stem barks for the treatment of trypanosomosis and diseases associated with oxidative stress.

Comments

Background

Trypanosomiasis is a disease that has defied satisfactory solution in the tropical countries. Therefore, many efforts geared at finding an effective, safe and cheap source of chemotherapy, including phytotherapy are in progress in many laboratories. This paper is one of such efforts.

Research frontiers

The relationship between phytochemistry, antioxidant and antitrypanosomal effect of some Nigerian medicinal plants.

Related reports

The different antioxidant and antitrypanosomal effects observed with these Nigerian plants is consistent with our earlier results (Atawodi, 2005; Atawodi *et al.*, 2009; Atawodi *et al.*, 2010 Atawodi *et al.*, 2012).

Innovations & breakthroughs

Data relating the phytochemistry, antioxidant effect to the antitrypanosomal property of these Nigerian medicinal plants are hard to find. So this work constitute an important addition to the knowledge in the area.

Applications

Isolation and identification of active components in the active extracts could provide source of effective and safe chemotherapy against trypanosomiasis.

Peer review

This paper is a good contribution to the area of chemotherapy of African trypanosomisis utilizing pharmacologically active phytoconstituents of locally available medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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