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Phytochemical constituents and free radical scavenging activity of fresh and dried samples of *Curculigo pilosa* (Schum. & Thonn.) Engl. (Hypoxidaceae)

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Abstract
Curculigo pilosa (Schum. & Thonn) Engl. is a tropical African flowering plant belonging to the family Hypoxidaceae. It is a highly valued medicinal plant used in traditional medicine to treat constipation, impotence, limb limpness, arthritis, knee joints, and watery diarrhea. It is also used as a potent immunomodulator and aphrodisiac. The aim of this study is to evaluate the phytochemical constituents and free radical scavenging activity of the fresh and dried samples of *C. pilosa*. Fresh plant samples were purchased from herbal market at Iyana-Iba market in Ojo local government area. Some of the samples were oven dried at 30 ºC to get the dried sample. Phytochemical analysis was carried out using standard laboratory procedures while ferric reducing power, nitric oxide and DPPH scavenging assays were used to evaluate the antioxidant activity of the samples. Both dried and fresh samples contained phenols, flavonoids, saponins, alkaloids and cardiac glycosides. Tannins was present in the extracts except the aqueous extract of the dried sample while terpenoids was present only in the acetone extract of the fresh and dried sample but absent in the aqueous extract of both samples. Fresh sample contained the highest amount of phytochemicals in both extracts. The amount of phenols in aqueous and acetone extracts of the fresh sample was 68.51 mg g-1 and 80.94 mg g-1 respectively. Fresh samples exhibited higher scavenging activity than the dried sample. Fresh sample of *C. pilosa* contained more phytochemicals and exhibited higher free radical scavenging activity than the dried sample.

Keywords: *Curculigo pilosa*, medicinal plants, phytochemicals, free scavenging activity, fresh sample, dried sample

Introduction
Curculigo pilosa (Schum. & Thonn.) Engl. is a tropical African flowering plant belonging to the family Hypoxidaceae. It is one of the members of genus *Curculigo* which includes *C. orchioides* Gaertn, *C. capitulata* (Lour) O. Ktze and *C. pilosa* (Schumach. & Thonn.) Engl. *C. pilosa* is an herbaceous plant with stout, erect rhizomes bearing a cluster of grass-like leaves to 60 cm long and flower shoots to 20 cm. It is widely distributed from Senegal to W Cameroons and Madagascar [1]. It is very important in traditional medicine and is used for the treatment of impotence, limb limpness, arthritis, knee joints, and watery diarrhea. It is a potent immunomodulator and aphrodisiac in the Ayurvedic medical system and is also used for the treatment of hemorrhoids, asthma, jaundice, colic and gonorrhea in traditional Chinese and India medicine [2]. *C. pilosa* was reported to be used in the management of obesity in Southwestern Nigeria [3]. In Northern Nigeria, it is used as purgative [4-5]. In Congo (Brazzaville) as a remedy for hernia [6] and In Central African Republic the root reduced to a pulp is applied topically to swellings held to be of fetish origin by porcupines [6]. The foliage is said to be eaten by herbivorous animals in Sudan [7]. The presence of high amylolytic activity in the extracts of *C. pilosa* is responsible for its traditional use in the preparation of easily digestible infant food and in the traditional method for the preparation of sorghum beer [8]. *C. pilosa* had been reported to possess antimicrobial [9] anti-candida [10] and antioxidant activities [11]. *C. pilosa* was reported to contain two benzylbenzoate diglucosides; piloside A and piloside B. It was also reported that norlignan, pilosidine, nyasicoside,

curculigine, curculigoside and pilosidine were isolated from its rhizome [12]. The aim of this study is to compare the phytochemical constituents and free radical scavenging activities of aqueous and acetone extracts of fresh and dried samples of *C. pilosa*.

Materials and Methods

Plant material

Fresh rhizomes of *C. pilosa* were purchased from the herb sellers at Iyana-iba market, Ojo Local Government Area, Ojo, Lagos. They were taken to Department of Botany, Lagos State University for authentication and identification. The voucher specimen was deposited in Lagos State University herbarium for reference purpose. Dried samples were prepared by oven dried the rhizomes at 30°C and ground into powder which was later stored in airtight containers prior to extraction.

Preparation of aqueous extract

The aqueous extract was prepared by soaking 200g of the powdered plant sample into 1000ml of distilled water in beakers and covered for 24 h. The mixture was filtered properly using Whatman's paper No. 1 and the filtrate was freezing dried using freezer drier (RTV 4104 USA).

Preparation of acetone extract

The acetone extract was prepared by soaking 200g of powdered plant sample into 1000ml of acetone using Sohxlet extractor and was further evaporated using rotary evaporator (Laborota 4000- efficient, Heidolph, Germany).

Phytochemical screening

Qualitative analysis

This was carried out according to the procedures of Harbone [13] as follows:

Test for tannins

About 0.5 g of the sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for phlobatannins

0.5g extract of each plant sample was boiled with 2 ml of 1% aqueous hydrochloric acid for 10 minutes. Deposition of a red precipitate indicates the presence of phlobatannins.

Test for saponin

About 2 g of the sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and then the formation of emulsion was observed.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4.

Test for steroids

2 ml of acetic anhydride was added to 0.5 g the extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test)

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for alkaloids

A 5 mg sample of the extract dissolved in 3 ml of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer's reagent and 1 ml of Dragendroff's reagent were added to 1 ml of the filtrate and turbidity was observed.

Quantitative screening

Estimation of tannins

500 mg sample of the concentrate was dissolved in 50 ml of distilled water, and shake for one hour. A 5 ml aliquot of the filtrate was mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 720 nm within 10 minutes [14].

Estimation of total phenolic compound

0.5g sample of each extract was dissolved in 50 ml of water. 0.5 ml of 0.1 ml of Folin- Ciocalteu reagent (0.5 N) was added, mixed and incubated at room temperature for 15 minutes. After this, 2.5 ml sodium carbonate solution (7.5% w/v) was added and further incubated for 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value [15].

Total flavonoids content estimation

1 ml of sample solution (100µg/ ml) was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol [16].

Free radical scavenging activity

DPPH radical scavenging activity assay

An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/ ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 min the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read

at 517 nm. The scavenging effect was calculated using the expression:

$$\% \text{ inhibition} = [A_0\text{-}A_1] \times 100/A_0$$

Where A_0 is the absorption of the blank sample and A_1 is the absorption of the extract [17].

Nitric oxide scavenging activity assay

A volume of 4 ml sample of plant extract of different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 m Min phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30 °C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylenediamine was measured at 550 nm. Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation:

$$[(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard [18].

Reducing power assay

Various concentrations of the extracts (25 to 100µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 5 ºC for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 16µg/ml) was used as standard [26].

Results and Discussion

Phytochemical screening

The results of the qualitative and quantitative phytochemical screenings of aqueous and acetone extracts of fresh and dried samples of *C. pilosa* are presented in Tables 1 and 2 respectively.

Table 1: Qualitative analysis of acetone and aqueous extracts of *C. pilosa* rhizomes

Phytochemicals	Acetone extract (Fresh sample)	Acetone extract (Dried sample)	Aqueous extract (Fresh sample)	Aqueous extract (Dried sample)
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Tannins	+	+	+	-
Cardiac Glycoside	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	-	-
Anthraquinones	_	_	-	-
Steroids	_	_	-	-

Table 2: Quantitative analysis of phytochemical constituents of different extracts of *Curculigo pilosa* rhizomes (mg g^{-1})

Plant extract	Flavonoids	Phenols	Saponins	Tannin	Reducing sugar	Alkaloids	Terpenoids
Aqueous Dried	17.91±0.02	52.46±0.45	25.67±0.51	30.87±0.13	22.27±0.44	18.61±0.52	18.61±0.22
Acetone Dried	24.49± 0.13	56.78±0.17	42.75±0.11	28.35±0.85	27.89±0.54	24.58±0.3	23.73±0.25
Aqueous Fresh	30.08±0.06	68.51±0.30	35.12±0.56	36.53±0.70	26.32±0.32	10.48±0.80	26.72±0.23
Acetone Fresh	56.86±0.15	80.94±0.47	52.34±0.12	37.24±0.40	30.34±0.17	3.53±0.12	33.47±0.66

Values are mean of three replicates (n=3)

Free radical scavenging activity

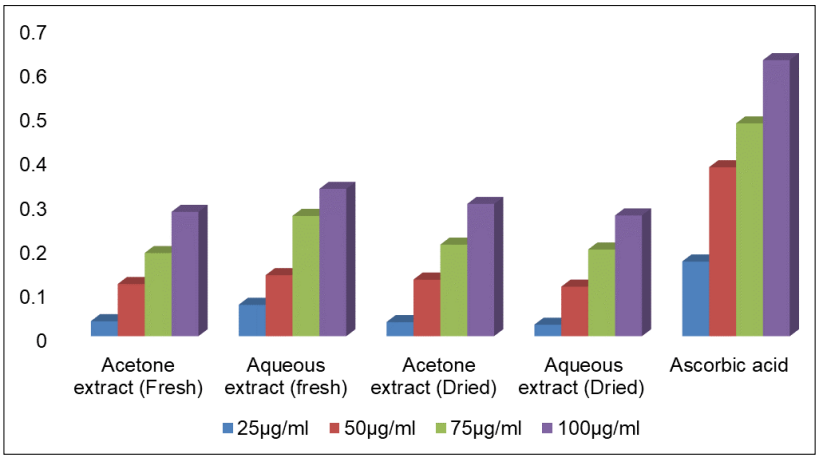

Fig 1: Reducing power activity of aqueous and acetone extracts of *C. pilosa*

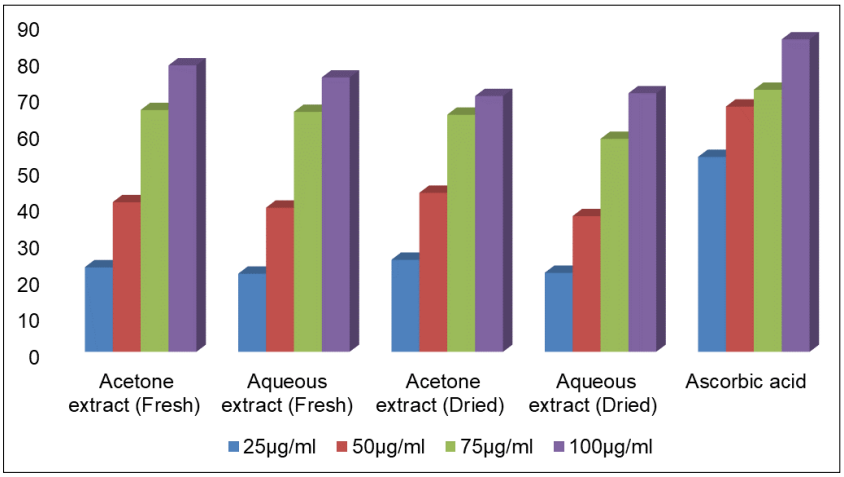

Fig 2: Nitric oxide scavenging activity of aqueous and acetone extracts of *C. pilosa*

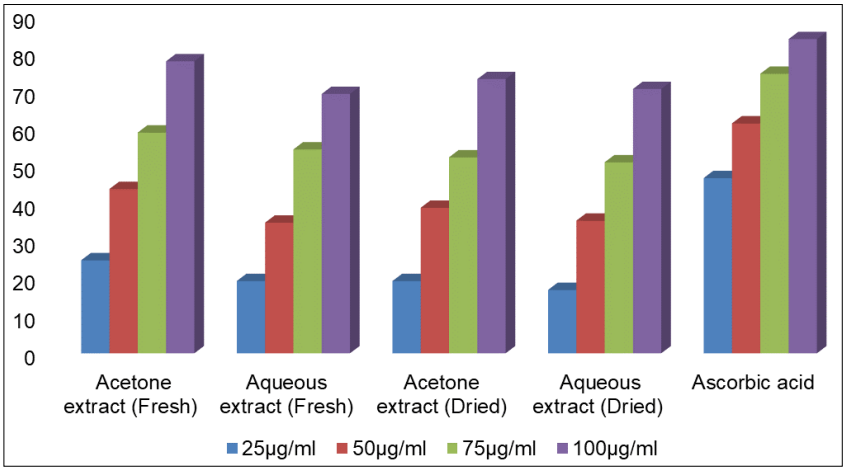

Fig 3: DPPH scavenging activity of aqueous and acetone extracts of *C. pilosa*

Discussion

Both qualitative and quantitative phytochemical screenings revealed the presence of secondary metabolites such as alkaloids, saponins, tannins, phenols, flavonoids, terpenoids and cardiac glycosides which are of significant therapeutic actions. All the phytochemicals tested for were present in all the samples except anthraquinones and steroids. Trace amounts of anthraquinones in the extract of *C. pilosa* has been reported [11]. The amounts of phytochemicals in both extracts of fresh samples are higher than in dried samples except alkaloids that were higher in dried samples than in fresh samples. It has been reported that drying affects the amounts and quality of phytochemicals present in plant samples [20, 21]. The phenolic content in aqueous extract of fresh sample was more than other phytochemicals present in the rhizomes of *C. pilosa*. Phenolic compounds have been reported to possess antioxidant, anticancer, hepatoprotective and cytotoxic ant properties [22]. They protect against the onset of degenerative diseases such as cancer, cardiovascular dysfunctions, diabetes and other inflammatory diseases [23]. Alkaloids have been reported to be sedatives, anti-cancerous, antimicrobials, anti-malarial and insecticidal [17]. Terpenoids are anti-rheumatics, anti-malarial, and detoxifying agents [22].

Both aqueous and acetone extracts of fresh sample exhibited higher reducing power activity than the dried samples. This may be due to the considerable amount of phytochemicals present in the fresh samples compared to the dried samples. At 100µg/ml, aqueous extract of the fresh sample showed higher reducing power activity than acetone extract. This could be as a result of higher amount of phenols present in the aqueous extract of the fresh sample. Phenols have been reported to be antioxidants by donating hydrogen from their hydroxyl groups which reacts with reactive oxygen species [24]. All the extracts and the standard showed dose-dependent reducing power activity. In nitric oxide scavenging assay, acetone extract of fresh sample exhibited the highest antioxidant activity among all the extracts. All the extracts and the standard also showed dose-dependent scavenging activity. Ascorbic acid showed more scavenging power at lower concentrations than the extracts. All the extracts including the standard exhibited dose-dependent DPPH radical scavenging activity. The extracts of both fresh and dried samples showed similar scavenging activity; however, at 100µg/ml, acetone extract of the fresh sample showed more scavenging activity than the remaining extracts.

Conclusion

C. pilosa is a highly valued medicinal plant with loads of phytochemicals with significant therapeutic actions. The plant exhibited considerable free radical scavenging activity comparable to ascorbic acid which is the standard. However, it was discovered from the study that fresh sample contained more phytochemicals and exhibited higher antioxidant activity than the dried sample. Hence, the use of fresh plant samples in traditional medicine is hereby suggested.

Conflicts of Interest

The authors declare no conflict of interest

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