

Full Length Research Paper

Nutritional composition and antioxidant activities of *Curculigo pilosa* (Hypoxidaceae) rhizome

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Curculigo pilosa (CP) rhizome traditionally used in the manufacture of infant food and sorghum beer in West Africa was assessed for its nutritional composition, mineral content as well as antioxidant activities. Results from the proximate analysis showed that the rhizome contains crude fibre (34.76%), carbohydrate (34.09%) and moderate energy value (188.77 Kcal/100 g). Crude protein, crude fat, ash and moisture constituted 11.01, 0.93, 5.16 and 12.28%, respectively of the rhizome weight. Among the minerals, Fe content (36.14±0.04) was the most abundant. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) activities were found to be high when compared to rutin and butylated hydroxytoluene (BHT). Moderate inhibition of nitric oxide (NO) radicals and reductive capability in phosphomolybdenum assay was also recorded. Phenolic, flavonoids and proanthocyanidin contents were 65.17, 23.17 and 4.23 mg/g of dry plant material, respectively. The study suggests that rhizome of CP may be a good dietary source of nutrients and natural antioxidant.

Key words: *Curculigo pilosa*, proximate composition, mineral contents, antioxidant activity.

INTRODUCTION

The genus *Curculigo* belongs to the family Hypoxidaceae and consists of approximately 20 species of exclusively tropical origin (Kocyan, 2007). The members of the family are small to medium herbs, with grass-like leaves and an invisible stem, modified into a corm or a rhizome. The rhizomes of *Curculigo pilosa* (CP) Schum and Thom, was the first African species to be described of the *Curculigo* genus (Palazzino et al., 2000). The vernacular name of the plant among the Yoruba speaking people of Western Nigeria is 'epakun'. The rhizomes of this plant possess medicinal properties and are used as food. It is traditionally used in the manufacture of infant food and sorghum beer in West Africa. The presence of high amyolytic activity in extracts of CP explains its traditional use in the preparation of easily digestible infant food and

in the traditional method for the preparation of sorghum beer (Dicko et al., 1999).

Extract of CP has been reported to show a dose-dependent vasoconstricting effect on rabbit aorta (Palazzino et al., 2000), antimicrobial (Adebayo-Tayo et al., 2010) and anti-candida activities (Gbadamosi and Egunyomi, 2010). Two benzylbenzoate diglucosides, piloside A and piloside B, and a glucosyl-fused norlignan, pilosidine, nyasicoside, curculigine, curculigoside and pilosidine were reported isolated from the rhizome of this plant (Palazzino et al., 2000). The aim of this study was to investigate the nutritional parameters and antioxidant properties of CP. An understanding of the overall composition and constituents of the rhizome is required to optimize its potential as a source of food and medicine.

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Abbreviations: DPPH, 2,2-Diphenyl-1-picrylhydrazyl; BHT, butylated hydroxyl toluene; FRAP, ferric reducing antioxidant power; CP, *Curculigo pilosa*; NO, nitric oxide.

MATERIALS AND METHODS

Plant material

Rhizomes of CP were bought from the market and authenticated by

Mr B.O. Daramola of the Herbarium unit of Botany Department, University of Lagos, Lagos, Nigeria. The rhizomes were thoroughly rinsed under tap water to remove adhering dirt, before being cut into pieces and air dried. Dried samples were ground into powder (>0.5 mm) using a grinding machine (Christy and Norrison, Chelmsford-England, 8000 RPM, 8** Lab Mill, 50158) and was kept in air tight container for further study.

Preparation of extract and phytochemical screening

100 g powdered sample was weighed and soaked with 300 ml of ethanol for 48 h. The extracts were filtered using Whatmann no 1 filter paper. This process was repeated twice for complete extraction. The filtrate was combined and concentrated below 40°C using a rotary evaporator (Buchi Rotavapor R-215, Flawil, Switzerland). The reduced extract was stored at -20°C. Phytochemical screening was performed using standard procedures (Harbone, 1998).

Proximate analysis

Moisture content

Moisture content was measured based on sample weight-loss after oven drying at 110°C for 2 h.

Ash determination

The ash content was determined gravimetrically following the method of AOAC International (2005). Duplicate samples were incinerated in a muffle furnace (Thermodyn Type 1400 Furnace, Dubouque, USA) at 560°C until constant weight was reached.

Total protein assay

The total protein content was determined by biuret method (Gonall et al., 1949).

Total fat

Fat was determined according to FAO (1986).

Total carbohydrate

Carbohydrate content was determined according to AOAC (2003).

Crude fibre

Crude fibre was estimated by acid-base digestion with 1.25% H₂SO₄ (W/V) and 1.25% NaOH (W/V) solution (AOAC, 1990). Gross energy value was calculated by multiplying protein, fat, and carbohydrate contents with factors of 4, 9 and 4, respectively.

Mineral analysis of *Curculigo pilosa*

Mineral contents were determined using atomic absorption spectrophotometer (Analyst 200, Perkin Elmer, Waltham, MA, USA) after digestion in mixed acids (nitric acid:perchloric acid = 4:1) and completing to volume (50 ml) with distilled water.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Antioxidant activity was determined with the DPPH radical scavenging method as described by Liyana-pathirana and Shahidi (2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with five different concentration of the extract, ranging from 10-50 µg/ml. The reaction mixture was shaken thoroughly and left on the bench at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm using a spectrophotometer (UNICO 2100). Rutin and butylated hydroxytoluene (BHT) were used as reference. The ability to scavenge DPPH radical was calculated by the following equation.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Where, absorbance of control is the absorbance of DPPH radical plus methanol.

Ferric reducing antioxidant power (FRAP) assay

A modified method of Benzie and Stain (1996) was adopted for the FRAP assay. The stock solutions include 300 mM acetate buffer 3.1 g C₂H₃NaO₂ · 3H₂O (sodium acetate) and 16 ml C₂H₄O₂ (acetic acid) pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl (hydrochloric acid) and 20 mM FeCl₃ · 6H₂O solution. 0.1 ml of the plant extracts were allowed to react with 3 ml of FRAP solution. The absorbance of the mixture was measured spectrophotometrically at 593 nm using a spectrophotometer (UNICO 2100). The standard curve was linear between 100 and 500 mM FeSO₄. Results are expressed in mM Fe (II)/g dry mass and compared with that of rutin and BHT

Nitric oxide (NO) scavenging assay

NO radical inhibition can be estimated by the use of Griess illosvoy reaction (Badami et al., 2005). The reaction mixture 3 ml containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and sample extract (10 to 50 µg/ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture was mixed with 1 ml of sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) and allowed to stand for about 10 min for complete diazotization. Then 1 ml of naphthylethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 min. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solution. Rutin and BHT were used as standards.

Phosphomolybdenum method

The principle of the assay is based on the reduction of molybdate (vi) to molybdate (v) by the sample being analyzed and the subsequent formation of green phosphate molybdate (v) complex at acidic pH (Prieto et al., 1999). 0.3 ml of ethanolic extract of the sample was combined with 3 ml of the reagent solution which contain 0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The test tubes were covered with foil paper and incubated at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against blank. The blank contained 3 ml of reagent solution and 0.3 ml of ethanol and incubated under the same conditions as the rest of the sample. The results were expressed as equivalents of ascorbic acid µmol/g of sample.

Determination of total phenolics

The total phenol content in the extract was determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). 0.5 ml (0.1 mg/ml) of the extract was mixed with 2.5 ml Folin-ciocalteu reagent previously diluted with water 1.10% and 2 ml (75 g/l) sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for colour development. Absorbance was then measured at 765 nm using spectrophotometer. The final concentration of the extract was 0.1 mg/ml. Total phenolic contents were expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 14.02x + 0.3198$, $R^2 = 0.9783$, where x is the absorbance and y is the tannic acid equivalent (mg/g).

Determination of total flavonoids

Total flavonoids were estimated using the method of (Ordenez et al., 2006). To 1 ml of sample, 1 ml of 2% $AlCl_3$ ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow colour indicated the presence of flavonoids. Total flavonoids contents were calculated as quercetin (mg/ml) using the following equation based on the calibration curve $y = 4.9x + 0.1134$, $R^2 = 0.9655$, where x is the absorbance and y is the quercetin equivalent (mg/ml).

Determination of total proanthocyanidins

Determination of proanthocyanidin was based on the procedure reported by (Sun et al., 1998). A volume of 0.5 ml of extract solution was mixed with 3 ml of 4% vanillin methanol solution and 1.5 ml hydrochloric acid, the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. The extract was evaluated at a final concentration of 0.1 mg/ml. Total proanthocyanidins contents were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve $y = 2.34x - 0.0084$, $R^2 = 0.9663$, where x is the absorbance and y is the catechin equivalent (mg/g).

RESULTS AND DISCUSSION

Proximate and nutritional composition

The results of the proximate analysis of CP rhizomes are shown in Table 1. Moisture content was 12.8%, which fall within the standard range of 0 to 13%. This moisture content range has been reported to be suitable for storage without microorganism degradation of the triglyceride (James, 1995). Water soluble and alcohol extractives of CP were 39.45 and 46.31%, respectively. Extraction yield depends on the extraction method, solvent, time and temperature. The values of total ash, acid insoluble and water insoluble ash determinations were 5.16, 0.78 and 0.41% dried weight, respectively. The total ash is comparable to that reported for some medicinal plants such as *Punica granatum* (5.75%), *Spharanthus hiritus* (6.54%) by Hussain et al. (2009). Ash content is an index of mineral contents in biota.

The extractive potency of CP rhizome in alcohol and water is also shown in Table 1. The values were

significantly different from each other, with alcohol (46.31%) being higher than that of water (39.45%). The percentage crude fat of CP rhizome is 0.93. The importance of fat in food may not be over-emphasized as it contributes to the energy value of foods.

Total carbohydrate (34.09%) calculated by difference was the predominant component in the rhizome of CP. This is expected rhizomes being the storage organ for carbohydrate. This amount is comparable to that of *Curculigo orchoides* (35.78%) reported by Raaman et al. (2009). The very high carbohydrate content of CP rhizome is significant to health. Apart from supply of energy, carbohydrate is also needed in numerous biochemical reactions not directly concerned with energy metabolism (Macdonald, 1999).

Next in terms of content is the crude fibre (34.76%). Fibre is recognized as a useful ingredient for the control of oxidative processes in food products and as a functional food ingredient (Mandalari et al., 2010). Crude fibre decreases the absorption of cholesterol from the gut. It also delays digestion and conversion of starch to sugars an important factor in the management of diabetes. Diet high in fibre may also protect against cardiovascular disease, colorectal cancer and obesity (Cust et al., 2009). The calorific value of CP rhizome is 188.77 Kcal/100 g. This appears minimal and so may contribute less sugar to the blood sugar pool (Okolie et al., 2009).

The crude protein content of CP rhizome was about 11.01%, which is relatively low compared to the carbohydrate but it can contribute to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance of body protein (Pazhanichamy et al., 2010).

The results of the elemental analysis of CP are presented in Table 2. These values are average of 3 independent measurements. The concentrations of the various elements analyzed in this study decreased in the order of $Fe > K > Zn > Na > Mg > Cu > Pb > Mn > Cr > Ni > Ca > Cd$. Among the various elements Cd, Mn, Ni, Cr, Pb, Ca, Na, Mg and Cu are found to be present in trace amount and met well the recommended dietary allowance (NRC/NAS). The differences in the concentration of various elements within the different plants is attributed to the preferential absorbability of a particular plant for the corresponding element and the mineral composition of the soil in which the plant grows and as well as the surrounding climatologically conditions (Rajurkar and Damame, 1998).

Macronutrients compositions especially sodium, potassium and calcium are essential cations abundantly present in plants. The chemical analysis of CP showed that the concentration of Na, K and Ca were 0.84, 5.12 and 0.12 ($\mu\text{g/g}$) dried weight, respectively (Table 2). The content of K was higher in comparison to Na. This is considered to be an advantage from the nutritional point of view. The intake of sodium chloride and diets with a

Table 1. Proximate composition of *Curculigo pilosa* rhizome.

Parameter	Composition (%)
Moisture content	12.28±0.06
Total ash	5.16±0.08
Acid insoluble ash	0.78 ±0.04
Water insoluble ash	0.41± 0.03
Water soluble extractives	39.45±1.19
Alcohol soluble extractives	46.31±0.97
Total carbohydrates	34.09±1.06
Total protein	11.01±0.97
Total fat	0.93±0.07
Crude fibre	34.76 ±0.05
Energy	188.77 Kcal/100 g

Data represent mean ± SEM (g/100g dry weight) of triplicate determinations.

Table 2. Mineral composition of *C. pilosa* rhizome.

Parameter	Composition of dried sample (µg/g)
Cu	0.54± 0.00
Mg	0.40± 0.03
Mn	0.36±0.02
Ca	0.12±0.00
Na	0.84±0.00
Fe	36.14±0.04
Zn	2.44±0.17
Cd	0.09±0.00
Pb	0.39±0.02
Ni	0.15±0.00
Cr	0.41±0.00
K	5.12±0.00

Values are means ± standard deviations of duplicate determinations. All samples were determined in duplicates.

high Na/K ratio has been related to the incidence of hypertension (Chen et al., 2010). Iron is present in higher concentration in CP 36.14 µg/g. This observation is similar to what was reported for *Piliostigma thonningii* (Jimoh and Oladiji, 2005). The authors reported that the level of iron amongst all minerals analyzed was found to be the highest. The remarkably high level of Fe in the rhizome underscores its importance in the preparation of infant food. Iron is a component of haemoglobin, myoglobin and the cytochrome pigments of the respiratory chain of mitochondria. The content of Zn in CP is 3.62 µg/g. Zinc is a trace mineral element important for the normal functioning of the immune system. The concentration of Pb, Cr, and Ni in CP rhizome is 0.52, 0.41, 0.12 (µg/g) dried sample, respectively. These values are lower than the recommended level for toxicity

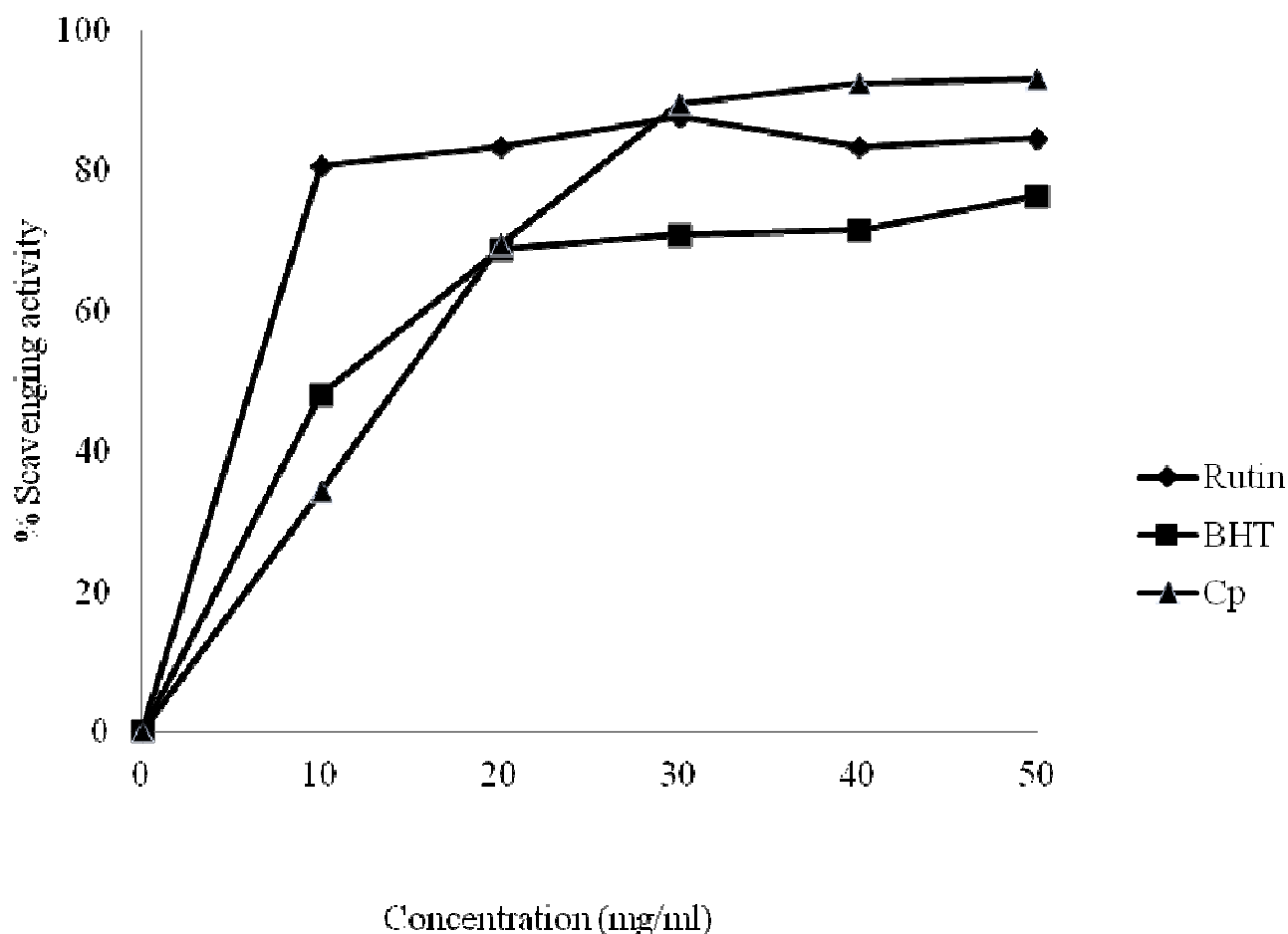
in plants (Hussain et al., 2009).

Preliminary screening of metabolites shows the presence of tannins, cardiac glycosides, flavonoids and saponins while anthraquinone, alkaloid and phlo-batannins were not detected. The presence of these secondary metabolites could contribute to the medicinal value of the extract. Table 3 presents the total phenolics, flavonoids and proanthocyanidin concentrations obtained in CP extract. The contents of phenolic, flavonoids and proanthocyanidin were 65.17, 23.17 and 4.23 mg/g of dry extract, respectively. The high polyphenolic content of the plant clearly suggests the presence of large number of hydrogen donating groups in the phenolic compounds (Gouthamchandra et al., 2010). This indicates that the polyphenols present in the extracts of leaves could be partly responsible for the beneficial effects.

Table 3. Polyphenolic content and antioxidant activities of *C. pilosa*

Extract	Total phenolic ^a	Total flavonoid ^b	Total proantho-Cyanidin ^c	Phosphomolybdenum assay ^d	FRAP ^e
<i>C. pilosa</i>	65.17±0.01	23.17±0.07	4.23±0.00	7.75±0.02	195.02±0.15
BHT	-	-	-	-	21.83±0.01
Rutin	-	-	-	-	35.05±0.02

Analyses were mean of three replicates ± standard deviations. ^a, Expressed as mg tannic acid/g of dried extract; ^b, expressed as mg quercetin/g of dried extract; ^c, expressed as mg catechin/g of dried extract; ^d, expressed as ascorbic acid μmol/g of sample; ^e, expressed in units of μmol Fe (II)/L.

**Figure 1.** DPPH free radical scavenging activity of *Curculigo pilosa*.

Antioxidant activities

The total antioxidant activity of CP was assessed by DPPH, NO scavenging, FRAP and phosphomolybdenum methods at concentration ranging from 10 to 50 μg/ml compared to BHT and rutin or catechin as the case may be. DPPH radical scavenging activity of CP was estimated by comparing the percentage inhibition of formation of DPPH radicals by the extract and those of rutin and BHT (Figure 1). The inhibition was concentration-

dependent, increasing from 10-50 μg/ml. At 50 μg/ml, the scavenging activity for rutin, BHT and CP were 84.56 and 76.33 and 93.05%, respectively. The ethanol extract of CP showed a dose dependent scavenging activity NO radicals with percentage inhibition of 53.24% at 50 μg/ml concentration compared to catechin (63.24%) (Figure 2).

The FRAP assay is developed for direct test of total antioxidant power of a sample. The total antioxidant power (μmol Fe (II)/g) of CP compared to BHT and rutin is shown in Table 3. The value for CP (195.02) is

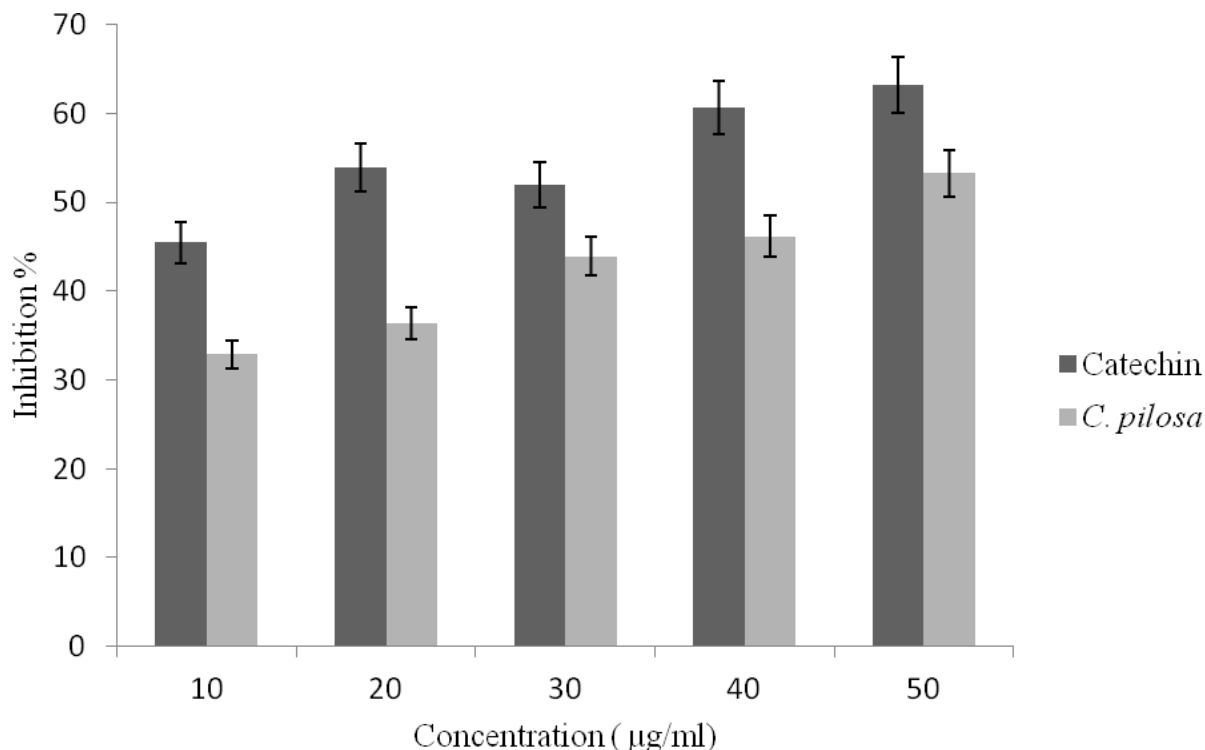


Figure 2. Nitric oxide scavenging activity of *Curculigo pilosa*.

significantly higher than that of rutin (35.05) and BHT (21.83). CP extract showed a higher ability to reduce Fe^{3+} to Fe^{2+} in the assay.

The antioxidant capacity of CP rhizome was also determined by the formation of green phosphomolybdenum complex. The formation of the complex was measured by the intensity of absorbance in extracts at the concentration of 1 mg/ml and expressed relative to that of ascorbic acid. The antioxidant power was 7.75 ± 0.02 µmol AAE/g.

Antioxidants are known to alleviate oxidative stress by scavenging free radicals and protect biological macromolecules from their toxic effect (Ames, 1983). Antioxidants have also gained more importance on account of their positive involvement as health promoters in conditions such as cardiovascular problems, atherosclerosis, and treatment of many forms of cancer and ageing process. In our results, the ethanolic extract of CP rhizome showed higher inhibition of DPPH radicals than BHT and rutin at 50 µg/ml. The FRAP was also higher than that of the standards. The extracts also showed moderate inhibition of NO radicals.

The present findings have certainly indicated that the extracts have the proton donating ability and could serve as free radical inhibitors or scavengers acting possibly as primary antioxidants. The comparatively high scavenging activity of ethanol extract of CP can be correlated to the higher phenolic content. This agrees with reports of many authors (Jimoh et al., 2008; Sofidiya et al., 2009).

Conclusion

The nutritive component as determined in this study justified the traditional use of the rhizome in weaning preparation for infants and could be a suitable alternative for providing necessary nutrients to human judging from the high carbohydrate and dietary fibre content and adequate protein and low lipid content. Moreover, in addition to the bioactive secondary metabolites constituents, the capability of the extract to scavenge different free radicals in different systems indicates that they may be useful therapeutic agents for treating radical-related pathological damage.

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