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Full Length Research Paper

Phenolics and antioxidant activity of *Tridax procumbens* Linn.

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Traditional medicinal plant, *Tridax procumbens* was analyzed for reducing power ability as an antioxidant using the 1,1-diphenyl-2- picrylhydrazyl (DPPH) assay and for total phenolics using the Folin-Ciocalteu method. The results of the analysis show that *T. procumbens* has a percentage antioxidant activity (AA %) of 96.70 which was observed to be higher than those of gallic (92.92%) and ascorbic acids (94.81%) used as standards. The reductive potential determination shows that *T. procumbens* has a very significant reductive potential of 0.89 nm at the same concentration with gallic acid whose reductive potential was 0.99 nm. The total phenolic determination shows that *T. procumbens* has a phenolic content of 12 mg/g GAE. The results of this analysis revealed the fact that plants are rich sources of natural antioxidant.

Key words: Antioxidant activity, DPPH, Folin-Ciocalteu, medicinal plants, *Tridax procumbens*.

INTRODUCTION

Antioxidants prevent the damage done to cells by free radicals-molecules that are released during the normal metabolic process of oxidation. Some of these free radicals include reactive oxygen free radicals species (ROS), reactive hydroxyl radicals ($OH^{\cdot}$), the superoxide anion radical ($O_2^{\cdot}$), hydrogen peroxides (H_2O_2) and peroxyl ($ROO^{\cdot}$) which generates metabolic products that attack lipids in cell membranes or DNA. These are associated with several types of biological damage, DNA damage, carcinogenesis and cellular degeneration related to aging and also contribute to heart disease and arthritis (Hou et al., 2003).

Antioxidants protect unsaturated fats in the body from oxidation by peroxides and other free radicals. Anti-oxidants that inhibit enzyme-catalyzed oxidation include agents that bind free oxygen (reducing agents), such as ascorbic acid (vitamin C), and agents that inactivate the enzymes, such as citric acid and sulfites (Encyclopedia Britannica, 2009). Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer has often resulted in the isolation of principles with antitumor activity (Afolabi et al., 2007).

Studies around the world have identified many new plant constituents with antioxidant activity, among these are the polyphenols (Kahkonen et al., 1999). The anti-oxidant activity of polyphenols has been reported to be mainly due to their redox properties, which can play an important role in neutralizing free radical and quenching oxygen or decomposing peroxides. Polyphenols of plant origin like catechins exert anticarcinogenic, antimutagenic and cardioprotective effects, which is attributed to their free radical scavenging activity (Karou et al., 2005). In Nigeria, *Tridax procumbens* is traditionally used in the treatment of fever, typhoid fever, cough, asthma, epilepsy and diarrhea (Mann et al. (2003). Salahdeen et al. (2004) studied the effect of *T. procumbens* on high blood pressure and heart rate on rats, the effect of *T. procumbens* on liver antioxidant defense system during lipopolysaccharide-induced hepatitis in D-galactosamine sensitized rats was studied by Ravikumar et al. (2005). Ikewuchi et al. (2009) studied the elemental composition of *T. procumbens.* In this paper we report our finding on the total phenol and free radical scavenging activity of *T. procumbens,* a medicinal plant that finds wide application in traditional medicinal practices in Northern Nigeria.

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Table 1. The percentage antioxidant activity of extracts from *T. procumbens.*

	Concentration (µg/ml)				
Sample/standard	10	25	50	125	250
Ascorbic acid	71.52	81.23	85.02	93.66	94.81
Gallic acid	66.25	75.22	81.81	91.60	92.92
T. procumbens	82.13	90.78	93.16	94.56	96.70

MATERIALS AND METHODS

Plant material

The plant, *T. procumbens* (Asteraceae), was collected in Zaria, Kaduna state of Nigeria in June, 2008. It was identified at the Herbarium of the Biological Science Department, Ahmadu Bello University Zaria, Nigeria. The plant was air dried and pulverized into coarse powder.

Extraction

The air dried and pulverized plant material (500 g) was exhaustively extracted with ethanol (1000 ml, BDH) using a soxhlet extractor for 24 h. The extract was concentrated *in vacuo* using a rota vapor.

DPPH free radical scavenging activity

The 1,1-diphenyl -2-picrylhydrazyl (DPPH, Sigma-Aldrich) scavenging activity was carried out according to the method described by Mensor et al. (2001). Different concentrations of the test sample and standard (gallic and ascorbic acids (Fluka)) were prepared; 250, 125, 50, 25 and 10 g/ml, respectively. DPPH solution (1.0 ml, 0.3M) was added to 2.5 ml solution of plant extract and standard after 20 min incubation period at room temperature in the dark, the absorbance of the resulting mixture was measured at 518 nm. The percentage Antioxidant Activity (AA%) was calculated using the expression below:

AA% = 100 - [(Abs sample/nAbs control) × 100]

The absorbance of the control (nAbs) was prepared by adding methanol (1.0 ml) to the extract solution (2.5 ml) without DPPH, while the positive control was prepared by adding 1.0 ml of DPPH solutions to 2.5 ml of ascorbic and gallic acids.

Reducing power ability of the plant

This was determined according to the method of Qyaizu (2006). The plant extract (1.0 ml, 250 g/ml) was mixed with 2.3 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide ($K_3[Fe(CN)_6]$) (BDH). The mixture was incubated at 37°C for 20 min. 10% Trichloroacetic acid (2.5 ml, Merck) was added to the mixture and centrifuged for 10 min at 1000 rpm, the supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. After standing for 10 min, the absorbance was measured at 700 nm.

Determination of total phenolics

The total phenolics in the extract were determined using Folin-Ciocalteu method as described by Kujala et al. (2000). To each sample solution (1.0 ml) and the standard (gallic acid) was added 5 ml of Folin-Ciocalteu (Sigma-Aldrich) and 4 ml Sodium carbonate (7% w/v) and shaken. The solution was allowed to stand for 30 min in the dark at room temperature, after which absorbance was measured at 765 nm using a spectrophotometer. The amount of total phenolics was expressed as gallic acid equivalent (GAE) in milligram per gram dry plant extract using the expression;

C = c x V/m

Statistical analysis

All determination were replicated three times and the results expressed as mean ± SD

RESULTS AND DISCUSSION

The results of the DPPH radical scavenging activity of *T. procumbens* (Table 1) shows that it possesses very high percentage antioxidant activity, 96.70% at a concentration of 250 g/ml. It shows a reductive potential of 0.89 nm (Figure 1). The results of the determination of total phenol (Table 2) expressed as gallic acid equivalent (GAE) shows that *T. procumbens* have a high phenolic content of 12 mg/g GAE. DPPH is a relatively stable Nitrogen centered free radical that easily accepts an electron or hydrogen, it react with suitable reducing agents as a results of which the electrons become paired off and the solution losses color depending on the number of electrons taken up (Blois, 2001). The results shows that *T. procumbens* extracts may have hydrogen donors thus scavenging the free radical DPPH, with high AA% of 96.70% at 250 g/ml which was observed to be higher than even those of the standards (ascorbic and gallic acids) at a concentration of 250 µg/ml used. The results of the reductive potential of the plant extract and that of gallic acid standard showed that, *T. procumbens* possesses a high reductive potential (0.89 nm) as compared to the standard (0.99 nm). The high reductive potential indicates that the plant have redox properties which allows them to act as reducing agents, hydrogen donors or oxygen quenchers (Rice-Evans et al., 1998). The results of the determination of total phenol expressed as gallic acid equivalent (GAE) show a high phenolic content of 12 mg/g GAE. The result of the analysis shows that there is a relationship between the phenol content of medicinal plants and antioxidant activity. This findings support earlier reports that plant metabolites like flavonoids, tannins, catechins and other phenolic

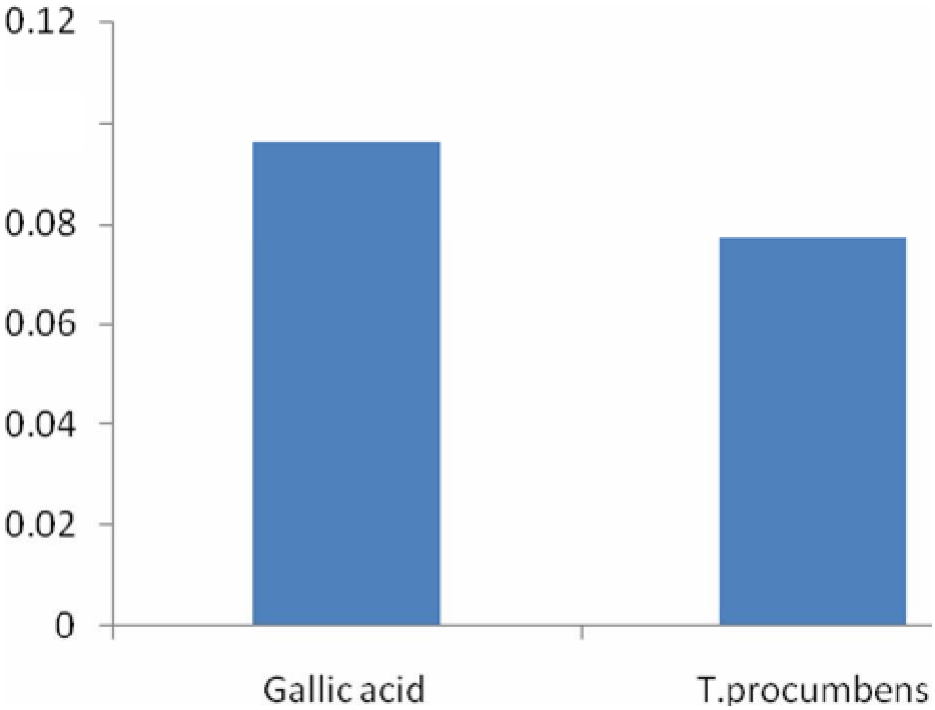

Figure 1. Reducing potential of *T. procumbens* and gallic acid standard.

Table 2. The determination of total phenolics of extracts of *T. Procumbens.*

Sample/Standard	Concentration (mg/g dry plant)	Mean absorbance
Gallic acid	0.02	0.168 ± 0.06
	0.05	0.346 ± 0.05
	0.10	0.614 ± 0.04
	0.15	0.955 ± 0.02
	0.20	1.248 ± 0.00
T. procumbens	0.12*	0.740 ± 0.05

compounds possesses antioxidant activity (Rice-Evans et al., 1995) and have played a preventive role in the development of cancer, heart and age related diseases. They have also been reported to be chemo-preventive agents by lowering cholesterol and repairing damage cells (Kahkonen et al., 1999).

Conclusion

The study concludes that *T. procumbens,* has antioxidant activity which was established to correspond to the amount of total phenolic content of the plant samples. Hence the plant is a potential source of natural antioxidant which could be useful in physiological and pathological medicine, and of great interest to food manufacturing industries.

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