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Evaluation of Antioxidant Property of the Methanolic Leaf Extracts of *Trema guineensis* (Tg) and *Cassia sieberiana* (Cs)

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Abstract

Oxidative stress leads to degenerative diseases, such as cancer, diabetics and heart diseases. Antioxidants are known to act as a defense mechanism that protects against oxidative damage caused by free radicals produced in the body. Medicinal plants are preferably used to manage degenerative diseases in many countries. This study was conducted to determine the antioxidant activity of methanol leaf extract of Tg and Cassia s (Cs) using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and 2,2-azinobis-(3 – ethylbenzothiazoline -6 –sulphonate (ABTS) assay. Butylatedhydroxytoluene and Rutin which were respectively used as standards, displayed 159.27% and 92.02% inhibition. The % antioxidant activity of the leaf extracts for DPPH Assay was in this order: (50 mg/ml) Tg ssss(97.38%)> Cs (95.02%); (40 mg/ml) Tg (96.99%) > Cs (94.59%); (30 mg/ml) Tg (96.26%) > Cs (91.54%); (20 mg/ml) Tg (95.51%) >Cs (88.63%);(10 mg/ml) Tg(94.95%) > Cs(86.73%). The % antioxidant activity of leaves extract using ABTS Assay at varying concentrations is as follows: (50 mg/ml) Tg (33.36%) > Cs (27.03%); (40 mg/ml) Tg (19.32%) > Cs (17.90%); (30 mg/ml) Tg (16.95%) > Cs (10.46%); (20 mg/ml) Tg (15.31%) > Cs (5.92%); (10 mg/ml) Tg (13.88%) > Cs (2.75%) respectively. Tg had the most dominant activity. This indicates that they can be used as natural antioxidant for the remedy of degenerative diseases.

Keywords: Antioxidant activity, , ABTS, BHT, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), , Rutin, Methanolic extracts

Introduction

Oxidative stress (OS) is the imbalance between cellular production of reactive oxygen species (ROS) and the ability of cells to scavenge them. OS has been inculpated as a prospective contributor to the pathogenesis of several diseases, such as cancer, diabetes and heart disease. ROS cause the damage of many cellular components including lipids, proteins and nucleic acids, such as DNA leading to subsequent cellular death by modes of mortality [1]. The damage can become more widespread due to weakened cellular antioxidant defense systems. All biological systems have antioxidant defense mechanism that safeguards against oxidative damages and repairs enzymes to remove damaged molecules. However, this natural antioxidant mechanism can be inadequate, hence dietary intake of antioxidant compounds is

paramount. Eating of fruits and vegetables is known to reduce the risk of several diseases, such as cancer, cardiovascular diseases and stroke caused by free radicals [2] and such health benefits are mainly enforced due to the presence of phytochemicals, such as polyphenols, carotenoids vitamins, E and C. Although ~~the~~ phenolic compounds are frequently found in both edible and non edible herbs, cereals, fruits, vegetables, oils, spices and other plant materials [3], scientific information on antioxidant properties of some native plants, restricted to certain regions and known only by local populations, is still rather rare. Therefore, the assessment of such properties remains a fascinating and rewarding task, particularly to find new promising sources of natural antioxidants for superfood and drugs [4].

Trema guineensis (family Ulmaceae). It is a flowering shrub distributed in Africa, South of the Sahara, Madagascar and tropical Asia. is a pioneer specie that can grow on poor soil and can be used to regenerate forest areas [5]. The tree has various uses as herbal medicine in a wide range of cultures. The leaves and bark are used to treat cough, sorethroat, toothache, gonorrhea, malaria, insomnia and female infertility [6].

Cassia sieberiana (family: Fabaceae). It is a perennial tree with a low branching crown, native of African, but widely spread in Indian and tropical Africa. It is extensively used for treatment and prevention of numerous diseases, such as degenerative diseases caused by oxidants like diabetes and hypertension. It is also used to remedy malaria and convulsion [7]. Also, liquid obtained after soaking the roots in water, is used for bath to cure tiredness. A decoction of the bark, leaves or root is used for the treatment of dysentery, diarrhea and vomiting. The twigs are used for remedying sleeping sickness [8].

The aim and objective of this study is to evaluate the antioxidant activity of two medicinal plant leaf from Bida, Nigeria.

Materials and Methods

Sample Collection and Preparation

Fresh leaves of *Trema guineensis* and *Cassia sieberiana* were separately collected with the assistance of a herbalist from Patssukashi Forest, along Bida-Bussu road, Bida, Niger State, Nigeria. They were identified and authenticated by the taxonomy section of the Department of Plant Biology, Ahmadu Bello University, Zaria. The samples were washed under running tap water to remove soil and other dirt, air dried at room temperature for four weeks and ground into powder using an electric grinder.

A 150 g portion of the powdered leaves of each sample was cold macerated in 600 cm^3 of methanol for 72 h and filtered with Whatman filter paper No 1. Each filtrate was concentrated and further over a water bath at 35°C. Each obtained extract was transferred into labelled and weighed sample

bottles. Extracts were preserved in a refrigerator for future use [9].

Evaluation of antioxidant activity

DPPH assay

The DPPH method was used to assess the total antioxidant capacity of each extract The DPPH scavenging activity of each extract was determined using Butylatedhydroxytoluene (BHT) and methanol as standard and blank solvent, respectively. Stock solutions 25 mg/ml were prepared in methanol from each extract. Working solutions (10, 20, 30,40, and 50 mg/ml) were prepared from these stock solutions. DPPH solution (0.96 mM) was also prepared in the same solvent. Thereafter, 1 ml of DPPH solution was mixed with each working solution followed by incubation for 30 min at room temperature. Control was prepared by adding 1 ml DPPH to 4 ml methanol. The absorbance of samples and the control were measured at 517 nm using Rayleigh UV/Vis-2601 spectrophotometer [10]. The DPPH scavenging activity of each extract was measured as percent inhibition using the following equation:

Inhibition (%) = [Absorbance (Control) − Absorbance (test sample)/Absorbance (Control)] × 100 (1)

ABTS assay

In the ABTS radical scavenging assay (an electron transfer-based assay), the 2,2- azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation, having a dark blue color, is reduced by an antioxidant into colorless ABTS, which can be measured spectrophotometrically. The $ABTS^+$ scavenging activity of each extract was determined according to the method described by [11]. $ABTS^+$ was generated by reacting 7 mM $ABTS^+$ aqueous solution and 2.45 mM potassium persulphate (1:1), kept in the dark at room temperature for 16 h before use. $ABTS^+$ solution was diluted with methanol to obtain an absorbance of 0.700 at 734 nm. 5 ml of plant extract at concentrations (10, 20, 30, 40 and 50 mg/ml) was added to 3.995 ml of diluted $ABTS^+$ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was carried out in each assay. The assay was done in triplicates. The percentage inhibition of absorbance at 734 nm was calculated using the formula,

$ABTS^+$ scavenging effect (%) = ((AB –AA)/AB) ×100

Results

The percentage antioxidant activity of each plant extract was assessed by DPPH free radical assay and ABTS radical assay. BHT and rutin were used as standards.

DPPH Assay

In this method, BHT which was used as the standard gave the highest antioxidant potential (159.27%). The present study revealed that the leaf extract of *Trema guineensis* gave a better radical

scavenging activity of 97.38% as compared to leaf extract of *Cassia sieberiana* as shown in Table 1.

ABTS Assay: In ABTS Assay, rutin served as the standard and manifested (92.02%) maximum inhibition, while extract Tg and Cs displayed inhibitions of (33.36%) and (27.02%) as shown in Table 2.

Table 1: Percentage DPPH inhibition of leaf extracts

Concentration (mg/ml)	BHT	Cs	Tg
50	159.27 ±0.28^{a}	95.09 ± 0.03^{c}	97.38 ± 0.20^{b}
40	145.85 ±0.00^{a}	94.59 ± 0.03^{c}	96.99 ± 0.03^{b}
30	140.42 ±0.09^{a}	91.54 ± 0.11^{b}	96.26 ± 0.36^{b}
20	132.74 ±0.13^{a}	88.63 ± 0.17^{d}	95.51 ± 0.11^{b}
10	88.49 ± 0.20^{c}	86.73 ± 0.06^{d}	94.95 ± 0.11^{a}

Key: Means ± standard errors on the same row with different superscript (a b and c) are significantly different from each other ($p \leq 0.05$). Tg: *Trema guineensis* leaf extract;
Cs: *Cassia sieberiana* leaf extract; BHT: Butylated hydroxytoluenes.

Table 2: Percentage ABTS inhibition of Leaf Extracts

Concentration (mg/ml)	Rutin	Cs	Tg
50	92.02 ± 0.44^{a}	27.03 ± 1.49^{c}	33.36 ± 2.79^{b}
40	67.07 ±11.18^{a}	17.90 ± 0.15^{b}	19.32 ± 0.74^{b}
30	68.32 ± 7.23^{a}	10.46 ± 0.06^{b}	16.95 ± 0.27^{b}
20	70.02 ± 1.58^{a}	5.92 ± 2.09^{c}	15.31 ± 0.17^{b}
10	52.54 ±11.84^{a}	2.75 ± 0.00^{c}	13.88 ± 0.21^{b}

Key: Means ± standard errors on the same row with different superscript (a,b and c) are significantly different from each other ($p \leq 0.05$). Tg: *Trema guinensis* leaf extract; Cs: *Cassia sieberiana* leaf extract.

Discussion

The results obtained is an indication that leaf extracts of Tg and Cs both exhibited remarkable antioxidant activity in varying concentrations. This could be due to the presence of various phytochemicals as reported by [12 ; 13]. Plants are rich sources of natural antioxidants, the best known are flavonoids, vitamin C and other phenolic compounds. Polyphenols scavenge free radicals and inhibit the oxidative mechanisms that can lead to degenerative diseases [14]. The leaves of the tested medicinal plants can be used as valuable drugs against various free radical induced diseases and should be considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates.

DPPH assay: From the methodological point of view the DPPH method is recommended for its easy mode of operation and accuracy with regard to measuring the antioxidant activity of plant extracts. DPPH radical scavenging activity increases with increasing phenolic components such as flavonoids, tannins, sterols, phenolics and triterpenoids [15]. These phenolic constituents have several hydroxyl groups, containing an o-dihydroxy group which have very strong radical scavenging effect and antioxidant power. Decrease in absorbance shows the more efficient antioxidant activity of the extract in terms of hydrogen atom donating capacity. Again, antioxidants present in the extracts were able to diminish the violet color stable 2, 2-diphenyl-1-picrylhydrazyl radical to the yellow color 2,2-diphenyl-1-picrylhydrazine, probably due to the neutralization of free radicals (DPPH), either by transfer of hydrogen atoms or by transfer of an electron [11]. Present studies revealed that, there were significant difference ($p< 0.05$) in the mean DPPH inhibition at concentration of each extract (50,40,30,20,10 mg/ml) considered between the leaf extracts and the standard (BHT). When 50 mg/ml of each extract was tested, BHT was significantly higher (159.27%) than that of the extracts. DPPH inhibition of the two leaf extracts were significantly different from each other in the decreasing order of Tg (97.38 %) > Cs (95.09 %). At 40 mg/ml of each extract, the order of %Inhibition was BHT (145.85 %) >Tg (96.99 %) > Cs (94.59 %). At 30 mg/ml, the standard was significantly higher than those of extracts and the extracts were not significantly different from each other. At (20 and 10 mg/ml), BHT > Tg > Cs ; Tg > BHT > Cs. As presented in Table 1.

ABTS assay: From the present study, the ABTS inhibition of all the leaf extracts at different concentrations (50, 40, 30, 20, 10 mg/ml) were significantly lower than that of the standard, as shown in table 2. The %inhibition of the standard, for various concentrations of extracts were (92.02, 67.07, 68.32, 72.02 and 52.54 %) respectively. At maximum concentration (50 mg/ml), Tg (33.36 %) ABTS inhibition was significantly higher than that

of Cs (27.03 %). It was also observed from the result, that antioxidant activity of the leaf extracts and standard were dose- dependent. The outcome of this present study is similar to other documentations on the antioxidant activities of the two medicinal plants [7; 12; 13; 16]. Aqeous extract of Tg has reducing power of 24.55 µg/ml [16]. Also the high scavenging activity of ethanol extract Cs stem bark has been reported [13]. This work shows that the methanolic leaf extracts of Tg and Cs are good sources of natural antioxidants, which can be utilized as drugs in pharmaceutical industries.

Conclusion

The antioxidant activity of methanol leaf extracts of Tg and Cs were screened by DPPH free radical assay and ABTS assay in comparison with BHT and Rutin respectively. This research work revealed that the two medicinal plants are rich source of natural antioxidants. Hence, further research could be done to establish its usefulness in food industries or application in medicine and nutrition.

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