AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

In Vitro Antioxidant Activity and Free Radical Scavenging Potential of Hydroalcoholic Extract of *Gutenbergia Nigritana* Benth.

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ABSTRACT

This study was carried out to determine the polyphenolic contents and antioxidant activity of hydroalcoholic extract of *Gutenbergia nigritana* leaves using *in vitro* models. The ability of the extract to scavenge free radicals such as 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) was investigated. The ferric reducing antioxidant power, total phenols and flavonoids contents of the extract were also determined using spectrophotometric methods. The result showed that the extract scavenge the radicals in a concentration dependent manner. The Free radical scavenging potentials of the extract was found to be proportional to its polyphenolic contents. Our findings revealed that the leaves of *Gutenbergia nigritana* contain biologically active constituents and therefore may be used as a potent antioxidant for the management of radical related diseases.

Keywords: *Gutenbergia nigritana*, free radicals, antioxidants, scavenging activity.

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Received 20 April 2013, Accepted 02 May 2013

Please cite this article in press as: Aluko BT. *et al.*, *In Vitro* Antioxidant Activity and Free Radical Scavenging Potential of Hydroalcoholic Extract of *Gutenbergia Nigritana* Benth..American Journal of PharmTech Research 2013.

INTRODUCTION

Reactive oxygen species (ROS) are generated as by-products of oxygen reduction in biological systems. They play an important role in the regulation of normal metabolic processes and immune function including cell growth, energy production and the synthesis of nucleic acids hormones and proteins [1]. However, they become deleterious when they are not eliminated by endogenous systems [2]. These ROS such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) nitric oxide (NO) and organic hydroperoxide (ROOH) are the underlying cause of oxidative stress which have been implicated in the pathogenesis of human degenerative diseases such as: atherosclerosis, sickle cell anaemia, myocardial infarction, heart failure and Parkinson disease [3]. This is due to their ability to react with electron donors thereby damaging proteins, lipids and DNA [4]. The role of antioxidants whether synthetic or natural in scavenging free radicals and promoting their decomposition has been documented [5]. A number of synthetic antioxidants have been used over the years as food additives. These includes: butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and propyl gallate [6]. Unfortunately, the ingestion these antioxidants have been reported toxic to human beings [7]. Therefore the dire need of the hour is to replace these synthetic antioxidants with natural ones from plant preparations in order to alleviate the extent of deterioration by ROS. The antioxidant properties of herbal preparations have been attributed to their phytoconstituents . These bioactive compounds such as phenolics and flavonoids are known to decompose the formation of peroxides [8].

Gutenbergia nigritana Benth is an annual herb that belongs to the family of *Asteraceae.* This family of plants is native to tropical and subtropical regions of Central America, eastern Brazil and west Africa[9]. The leaves of *G. nigritana* are used in south – western Nigeria for the treatment of hypertension and asthma. It is also the major constituent of a decoction used for the treatment of anemia. Our survey of literature revealed that the medicinal potentials of this plant have not been scientifically documented. The aim of this study therefore, was to evaluate the polyphenolic contents and the antiradical properties of its leaves in an attempt to establish its potential medicinal properties.

MATERIALS AND METHODS

Collection and identification of plant sample

The leaves *Gutenbergia nigritana* of were collected from a local farmland in Ikere Ekiti South-Western Nigeria in the month of July, 2012. The plant was authenticated by Mr Omotayo (herbarium curator) at the Department of Plant Science, University of Ado Ekiti, Nigeria where

the voucher specimen (Aluko 12a) was deposited.

Reagents

The reagents used in this study includes: gallic acid, quercetin, Folin- Ciocalteu reagent, 2,2 diphenyl-2-picrylhydrazyl (DPPH), potassium persulfate, hydrogen peroxide, sodium nitroprusside, sulphanilic acid, naphthylenediamine dichloride, ferric chloride, thiobabituric acid, trichloroacetic acid, potassium ferricyanide, ascorbic acid and butylated hydroxyl toluene. All other solvents used were of analytical grade.

Sample extraction

The leaves were air dried for 10 days and then pulverized into fine powder using an electric blender. Fifty grams of the powdered sample was extracted with 250 ml of 70% ethanol. The solvent was removed by filtration using a Buchner funnel with Whatman's No 1 filter paper. The filtrate was concentrated to dryness under vacuum in a rotary evaporator thereafter; the extract was collected in clean bottles and left opened in a laboratory fume hood for complete evaporation of residual solvent. The percentage yield for the extract was 4.4 % w/w.

Determination of total phenolics

The total phenolic content of *Gutenbergia nigritana* leaves was determined using the method of Wolfe *et al*, [10]. The reaction mixture contained 2.5 ml of 10 % (v/v) Folin- Ciocalteu reagent, 2 ml of 7.5 % (w/v) of sodium carbonate and 0.5 ml (1 mg/ml) of the extract. The mixture was mixed together and incubated at 40^{o}C for 30 min after which the absorbance was measured at 765 nm. The total phenolic content was calculated from the equation obtained from the calibration curve of gallic acid and expressed as µg/mg gallic acid equivalent.

Determination of total flavonoids

Total flavonoids content of *Gutenbergia nigritana* leaves was determined by the modified method of Ordonez *et al* [11]. The extract (1 ml in a final concentration of 0.1 mg/ml) was mixed with 1ml of 2 % (w/v) aluminium chloride prepared in ethanol and left in the dark at room temperature for 1 h. A yellow colour was observed which was measured spectrophotometrically at 420 nm. The calibration curve was prepared for quercetin and the total flavonoid content was calculated as µg/mg quercetin equivalent.

DPPH radical scavenging assay

The scavenging ability of the extract against DPPH radical was determined using the method of Liyana – Pathiranan and Shadidi [12]. One millilitre of 0.135mM of DPPH in methanol was mixed with 1 ml of different concentrations of the leaf extract and trolox. The mixture was kept in a dark cupboard for 30 minutes. The absorbance of the resulting solution was measured

spectrophotometrically at 517 nm and the scavenging ability of the extract was calculated as:
DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/(Abs control) ×100
Where Abs control is the absorbance of DPPH radicals + methanol; Abs sample is the absorbance of DPPH radical + extract or standard.

Hydrogen peroxide scavenging assay

The modified method of Hazra *et al*, [13] was employed for the hydrogen peroxide scavenging assay of the Extracts of *Gutenbergia nigritana* leaves. The stock solution contained 4 mM hydrogen peroxide prepared in 0.1 M phosphate buffer (pH 7.4). A volume of 0.6 ml of the solution was added to 2 ml of the extract and standard (200 – 1000 µg/ml) and incubated for 15 min at room temperature. The absorbance was read at 230 nm and the percentage inhibition of hydrogen peroxide was calculated as:
H_2O_2 scavenging activity (%) = [(Abs control – Abs sample)] / (Abs control) ×100
Where Abs control is the absorbance of H_2O_2 radicals; Abs sample is the absorbance of H_2O_2 radical + extract or standard.

Nitric oxide scavenging assay

The nitric oxide scavenging activity of the extract was evaluated by the method of Sakat *et al*, [14] A reaction mixture of two millilitres of 10 mM sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) and 0.5 ml of plant extract or standard (2000 – 1000 µg/ml) was mixed thoroughly. The mixture was incubated for 2.5 h at 25^{o}C and thereafter, 1ml was taken and mixed with 1 ml of Griess reagent (equal volumes of 0.33 % sulphanilic acid prepared in 20 % glacial acetic acid and 0.1 % (w/v) naphthylenediamine dichloride) and incubated at room temperature for 30 min. The absorbance of the chromophore formed was read at 540 nm and the percentage nitric oxide inhibition by the extracts was calculated using the following equation:
NO scavenging activity (%) = [(Abs control – Abs sample)]/(Abs control) ×100
Where Abs control is the absorbance of NO radicals; Abs sample is the absorbance of NO radical + extract or standard.

Determination of reducing power

The reducing power of the extract was determined according to the method described by Warokar *et al*, [15] with some modifications. 0.5 ml of the extracts or standard drug was mixed with 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1 % (w/v) potassium ferricyanide. The mixture was incubated for 20 min at 50^{o}C. After incubation, 1 ml of 10 % (w/v) TCA was added and centrifuged at 3000 rpm for 10 min and the supernatant was decanted. A volume of 1.25 ml

of the solution was allowed to react with 1.25 ml distilled water and 0.25 ml of 0.1 % (w/v) of ferrous chloride for 10 min and the absorbance was read at 700 nm. The observed increase in absorbance which was concentration dependent indicated the ferric reducing ability of the extract.

Statistical analysis

All results were expressed as mean ± standard deviation (SD) of three replicates and were subjected to analysis of variance (ANOVA). Significant levels were tested at $P < 0.05$.

RESULTS AND DISCUSSION

The antioxidative properties of herbal preparations have been attributed to their polyphenolic constituents. They are capable of neutralizing free radicals, chelating metal catalysts and inhibiting the activity of oxidizing enzymes in biological systems [16]. These properties confer health beneficial potentials on phenolic compounds. The findings of our study revealed high concentrations of total phenolics and flavonoids in *G. nigritana* leaves (Table 1).

Table 1: Polyphenolic contents of hydroalcoholic extract of *G. nigritana* leaves.

Total phenolics (µg GAE/mg)	Total flavonoids (µg QE/g)
0.488 ± 0.04	0.188 ± 0 .02

Data expressed as means ± SD (n=3). GAE and QE are gallic acid and quercetin equivalent respectively.

The antioxidant potential of plant extracts has been closely related to the presence of phenolic compounds [12, 17]. Phenols and flavonoids have been reported to reduce the risk of cardiovascular disease, cancer, urinary tract diseases and metabolic syndrome through their ability to donate hydrogen atom [18]. The appreciable level of polyphenols in the extract of the leaves suggests that it might be used for the treatment of radical related problems such as diabetes and cardiovascular problems.

DPPH radical scavenging assay

DPPH assay is the most widely used method to establish the antioxidant activity of natural products. It is a stable radical that produces violet color in ethanol solution. This color changes to either yellow or colorless due to the antioxidant potential of test samples. The hydroalcoholic extract of *G. nigritana* leaves exhibited the inhibition of DPPH radical in a concentration related manner though lower when compared with trolox (Figure 1). The inhibition of DPPH radical by the extract could be linked to polyphenolic compounds which are capable of donating electrons to quench free radicals.

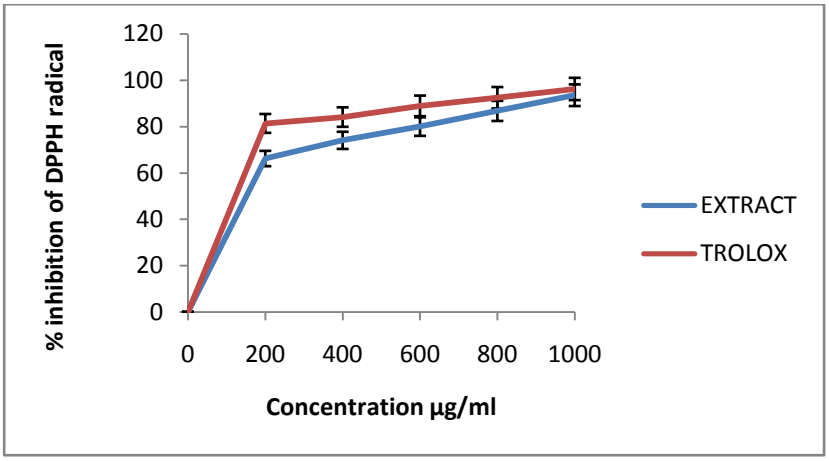

Figure 1: **Scavenging effects of hydroalcoholic extract of *G. nigritana* leaves on DPPH radical. The results are means ± SD (n=3).**

Hydrogen peroxide scavenging assay

Hydrogen peroxide is a pro-oxidant that is capable of crossing the membranes to oxidize a number of biological compounds. It can give rise to hydroxyl radical by interacting with cellular components and thus, cause tissue damage that may eventually lead to cell necrosis [19]. In our study, the hydrogen peroxide scavenging activity of *G. nigritana* hydroalcoholic extract and BHT increased in a dose dependent manner (Figure 2). At a concentration of 1000µg/ml the percentage inhibition on H_2O_2 radical was 71.31% and 94.55% respectively. Although BHT showed better scavenging activity, the inhibition of H_2O_2 by the extract can be attributed to the proton donating potential its phenolic contents.

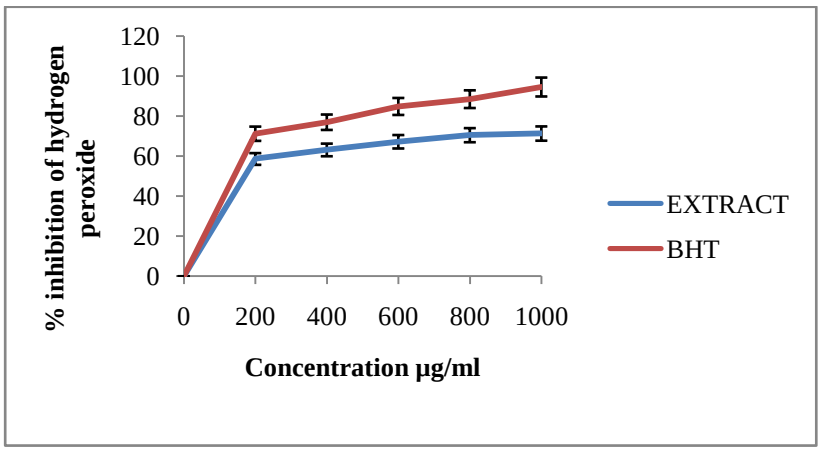

Figure 2: **Scavenging activity of hydroalcoholic extract of *G. nigritana* leaves on hydrogen peroxide radical. The results are means ± SD (n=3).**

Nitric oxide scavenging assay

Nitric oxide (NO) radical is associated with inflammatory conditions such as atherosclerosis, arthritis, ulcerative colitis and carcinomas. It reacts with superoxide radical to generate highly

reactive peroxy nitrate anion [20]. The inhibition of NO radical by hydroalcoholic extract of *G. nigritana* increased with increasing concentration of the extract and the standard (Figure 3). The percentage inhibition of the extract was lower than that of the standard. The inhibitory potentials of the extract against NO radical may be due to their ability to compete with oxygen thereby inhibiting the generation of nitrite.

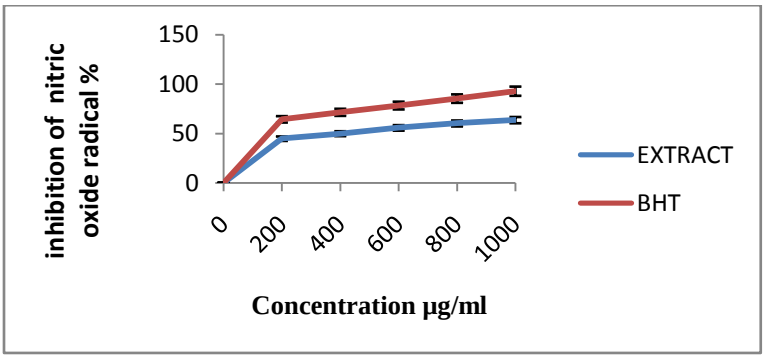

Figure 3: **Inhibitory effects of hydroalcoholic extract of *G. nigritana* leaves on nitric oxide radical. The results are means ± SD (n=3).**

Ferric reducing ability

The reducing capacity of a test compound serves as an indicator of its potential antioxidant activity due to the presence of reductants [21]. The reductive ability of *G. nigritana* extract occurred in a dose dependent formation of Perl's Prussian blue to reduce ferric ions to its ferrous form is evident in the concentration dependent formation of Perl's Prussian blue at 700 nm (Figure 4). Our result revealed that hydroalcoholic extract of *G. nigritana* leaves is an electron donor that can terminate radical chain reaction.

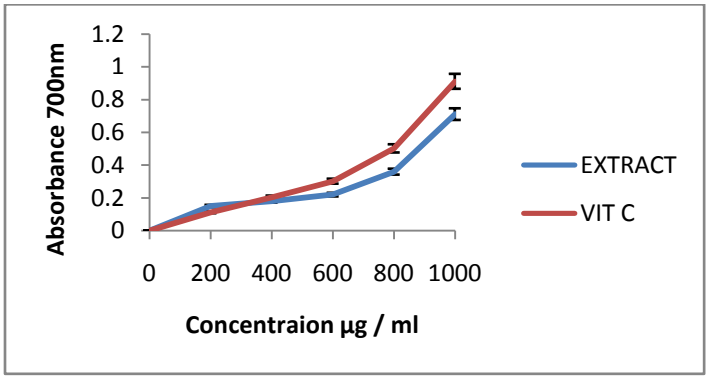

Figure 4: **Ferric reducing ability of hydroalcoholic extract of *G. nigritana* leaves. The results are means ± SD (n=3).**

CONCLUSION

The result obtained from this study demonstrated that the hydroalcoholic extract of *G. nigritana* leaves possess potent antioxidant profile that can be harnessed to alleviate the extent of oxidative

deterioration by free radicals. The antioxidant potential exhibited by the extract is related to its phenolic content. Further studies are necessary to isolate the compounds in this extract and establish their possible pharmacological properties prior to clinical use.

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