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Full Length Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *SOLANUM ERIANTHUM*

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ABSTRACT
The antioxidant and free radical scavenging ability of the extract was assessed against 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and hydrogen peroxide (H_2O_2). The ability of the extract to reduce ferric ions to its ferrous form was also evaluated. The total phenols, flavonoids, tannins and saponin contents of the extract was also determined to assess its corresponding effect on the antioxidant potential of this plant. The activity of the extract against the free radicals was concentration dependent. The result revealed a linear correlation between the extract and its polyphenolic contents. This study provides evidence that the leaves of *Solanum erianthum* contain phytoconstituents which may serve as natural antioxidants.

Keywords: Bioaccumulation, Heavy metals, *Amaranthus sp.* L, Katsian metropolis and Wastewater

INTRODUCTION

Reactive oxygen-free radicals (ROS) have been implicated in the etiology many diseases and in aging process (Renuka *et al.,* 2012). These free radicals, which cause tissue damage via oxidative stress, are generated by aerobic respiration, inflammation, and lipid peroxidation (Miliauskas *et al.,* 2004). Examples of free radicals include: superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH) nitric oxide (NO) and organic hydroperoxide (ROOH). The importance of these free radicals in metabolic processes has been documented (Flora, 2007). However, their accumulation in biological systems has been shown to pose serious adverse effects (Aluko *et al.,* 2013). This has been attributed to their ability to react with electron donors thereby damaging vital macromolecules such as proteins, lipids and DNA. The deleterious effects of these radicals are neutralized by synthetic or natural antioxidants (Oyedemi *et al.,* 2010). Herbs are potential sources of phytoconstituents with potent antioxidant potentials. These constituents known as phytochemicals are nonnutritive compounds that contribute to flavor and colour (Shirmila and Radhamany, 2013). Many phytochemicals such as alkyl sulfide (found in onions and garlic), carotenoids (from carrots), and flavonoids (present in fruits and vegetables) are known to have potent antioxidant activities (Craig, 1999).

Solanum erianthum popularly known as 'Potato tree' is a fast growing shrub that belongs to the Family of *Solanaceae* (Schmelzer and Gurib-Fakim, 2008). This family of plant has cosmopolitan distribution and is widely found in tropical and subtropical regions of West Africa, North America and Northern South America (Subramanian and Venugopal, 2013). Several species of *Solanum* are known to possess pharmacological activities including antimycotic, antiviral, molluscicidal, antimalarial, teratogenic and cytotoxic properties (Huang *et al.,* 2009). The decoction of the leaves of *S. erianthum* is used by herbalists in Nigeria for the treatment of leprosy, hemorrhoids and malaria (Makinde *et al.,* 1988).There is dearth of scientific information on the medicinal applications of *S. erianthum.* The primary objective of the present investigation is to evaluate the antioxidant property of the leaves of this plant

MATERIALS AND METHOD

Collection and identification of plant sample

The leaves *Solanum erianthum* were obtained from a local farmland in Ikere Ekiti South-Western Nigeria in the month of July, 2012. The plant was authenticated by the herbarium curator (Mr Omotayo) at the Department of Plant Science, University of Ado Ekiti, Nigeria where the voucher specimen (Aluko / 12b) was deposited.

Reagents

The reagents used were purchased from Sigma Chemical Co. (St. Louis, MO, USA). These 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 ferric cyanide, ascorbic acid and butylated hydroxyl toluene. All other solvents used were of analytical grade.

Sample extraction

The leaves were air dried under shade for 10 days. It was then pulverized into fine powder using an electric blender. Fifty grams of the powdered sample was extracted with 250 ml of 70% ethanol. The extract was filtered using Whatman's No 1 filter paper and concentrated to dryness under vacuo. The extract was then collected in clean bottles and left opened in a laboratory fume hood for complete evaporation of residual solvent.

Phtytochemical Screening

Total phenols were estimated as gallic acid equivalents (GAEs) using the method of Wolfe *et al.* (2003). The flavonoids content was determined by method described by Ordonez *et al.* (2006) using quercetin as a reference compound. The determination of tannin was done according to the method of Edeoga *et al.* (2005). The saponin content in the leaves of *S. erianthum* was done using the method of Obadoni and Ochuko, (2001).

DPPH scavenging assay

The scavenging ability of the extract against DPPH radical was determined using the method of Liyana – Pathiranan and Shadidi, (2005) using trolox as standard. A volume of 0.1 ml of the extract and trolox (200 – 1000 µg/ml) was mixed with 2.9 ml of 0.1 mM

DPPH–methanol solution. The mixture was incubated for 30 min at 25 °C in darkness, after which the decrease in absorbance at 517 nm was measured. The scavenging ability of the extract and trolox was calculated as follows:
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 + sample or standard.

Hydrogen peroxide scavenging assay
The method of Hazra *et al*. (2008) was employed for the hydrogen peroxide scavenging assay of the extract of *S. erianthum*. The stock solution was 4 mM hydrogen peroxide prepared in 0.1 M phosphate buffer (pH 7.4). The solution (0.6 ml) 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 follows:
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 + sample or standard.

Determination of ferric reducing power
The method of Oyiazu (1986) was employed for determination of the ferric reducing power of the extract. Two milliliters (2 ml) of the extract and standard was mixed with 2 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2 mL of 1% potassium ferricyanide. The mixture was incubated at 50^0C for 20 min, and then 2mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. Thereafter, 5mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm.

Statistical analysis
All data were expressed as mean ± standard deviation (SD) of three replicates. Statistical analysis was done using analysis of variance (ANOVA). Significant levels were tested at $P < 0.05$.

RESULTS
The phytochemical analysis of the leaves of *S. erianthum* revealed high concentrations of saponins with appreciable amounts of flavonoids , tannins and phenolics as shown in Table 1.

Table 1: The phytochemical constituents of ethanolic extract of *S. erianthum* leaves

Phytochemical constituents	Amount of compound (%)	Extract equivalents
Total phenolics (µg GAE/mg)	0.488 ± 0.04	Gallic acid
Total flavonoids (µg QE/g)	0.188 ± 0 .02	Quercetin
Saponin	9.46 ± 1.00	ND
Tannin	0.80 ± 0.20	ND

Data expressed as means ± SD (n=3). ND: Not detected, GAE and QE are gallic acid and quercetin equivalent respectively.

DPPH radical scavenging assay
Percentage inhibition of DPPH is presented in Figure 1. The percentage inhibition produced by the extract was significantly different (*P <0* .05) from that of trolox, the standard antioxidant. The extract showed concentration-dependent inhibition.

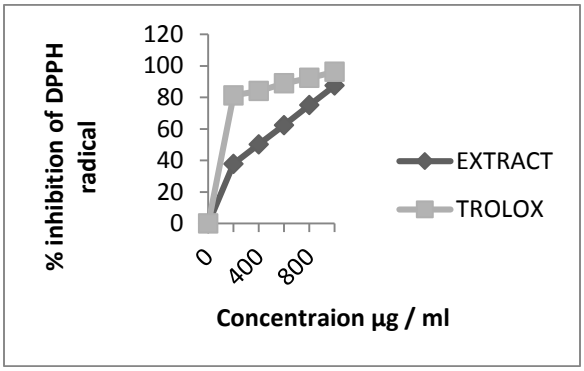

Figure 1: Scavenging effects of ethanolic extract of *S. erianthum* leaves on DPPH radical. The results are means ± SD (n=3).

Hydrogen peroxide scavenging assay
The hydrogen peroxide scavenging activity of ethanolic extract of *S. erianthum* increased in a dose dependent manner (Figure 2). The standard drug (BHT) showed better scavenging activity than the extract.

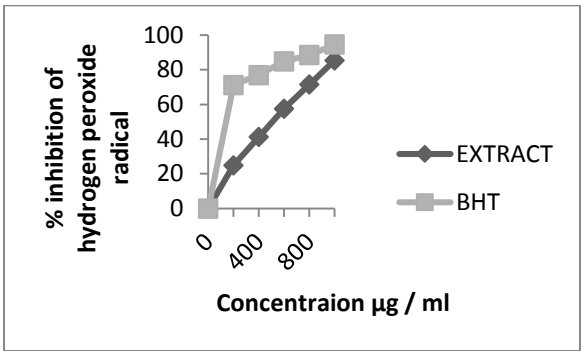

Figure 2: Scavenging activity of ethanolic extract of *S. erianthum* leaves on hydrogen peroxide radical. The results are means ± SD (n=3).

Ferric reducing ability
The reductive ability of the extract occurred in a dose dependent manner. This was evident in the transformation of ferric ions to its ferrous form at 700 nm (Figure 3).

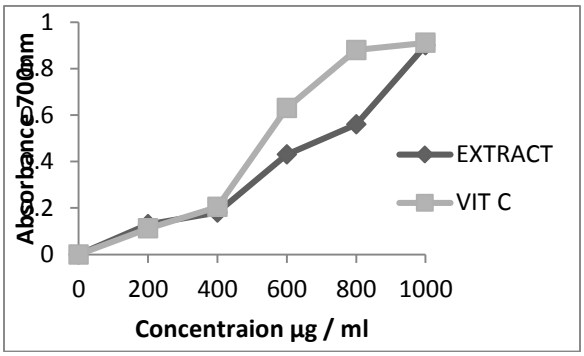

Figure 3: Ferric reducing ability of ethanolic extract of *S. erianthum* leaves. The results are means ± SD (n=3).

DISCUSSION

Phenolic compounds and some of their derivatives are known by their antioxidant and scavenging properties against free radicals. The antioxidant activity of medicinal plants has been linked to their phytochemical compositions (Liu *et al.,* 2008) The presence of phytoconstituents such as phenols, flavonoids, tannins and saponins in the ethanolic extract of *S. erianthum* leaves may be a strong factor contributing to the local usage of this plant for the management of diseases. Total phenols and flavonoids have demonstrated modulatory effects against lipid peroxidation involved in atherogenesis and carcinogenesis (Mbabie *et al.,* 2011). Tannins are polyphenolic compounds that possess anti-diuretic and anti-diarrhoea properties (Okwu, 2004). Saponins have demonstrated potential therapeutic activities as antifungal, antibacterial, and antioxidant agents (Aluko *et al.,* 2012). The polyphenols present in the extract of the leaves can contribute synergistically to its antioxidant potential.

The ethanolic extract of *S. erianthum* inhibited DPPH, indicating its antioxidant activity. The DPPH test provides information on the reactivity of compounds with a stable free radical DPPH that gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger the absorption reduces and the DPPH solution is decolorized as the colour changes from deep violet to light yellow. The degree of reduction in absorbance is reflective of the antioxidant potency of the extract (Barreira *et al.,* 2008).

Hydrogen peroxide is a precursor for the production of hydroxyl radicals in cellular components. Hydroxyl radicals are considered to be one of the rapid initiators of lipid peroxidation process, abstracting hydrogen atoms from polyunsaturated fatty acid, which brings about peroxidic reactions of membrane lipids (Lipinski, 2011). The hydrogen peroxide scavenging activity of ethanolic extract of *S. erianthum* can be attributed to the proton donating potential its phytochemical components.

The reducing power of a compound is related to its electron transfer ability, and may therefore serve as a significant indicator of its potential antioxidant activity (Abbasi *et al.,* 2013). The reductive ability of the extract occurred in a dose dependent manner. This can be attributed to the electron donating ability of the polyphenols present in the extract.

Conclusion

The data obtained from this study implies that the ethanolic extract of *S. erianthum* leaves is rich in antioxidants. It is logical to speculate that the chemical constituents are responsible for the potent antioxidant properties exhibited by this plant. However, the mechanism of action of these compounds should be elucidated

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