http://dx.doi.org/10.4314/jpb.v8i1.4
Vol. 8 no. 1, pp. 22-26 (March 2011)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Free radical scavenging activity of phenylpropanoid and iridoid esters glycosides from *Stereospermum kunthianum*

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Received 4th November 2010; Accepted 15th February 2011

Abstract
Relative antioxidant activities of the crude methanolic extract of *Stereospermum kunthianum*, two phenylpropanoid glycosides and one iridoid glycosides called Stereospermiside 1, Stereospermin 2, Stereostin 3 previously isolated and characterized from the plant were studied. The free radical scavenging activity was determined by the 2, 2-diphenylpicrahydrazil radical (DPPH) using a spectrophotometer and the absorbance measured at 517nm. The result of the study revealed a significant antioxidant activity of the phenylpropanoid ester glycosides 1, 2 and 3. This work lends support to the antioxidant property of the *S. kunthianum*.

Keywords: Phenylpropanoid glycosides; Iridoid; Antioixdants; DPPH

INTRODUCTION

Antioxidants are chemical constituents which play a significant physiological role in free radicals scavenging property. They help in the prevention of major disease conditions in the organisms (Mazza, 1998, Surai, 2002). Free radicals are believed to play a role in more than sixty different health conditions, including the ageing process, cancer, and atherosclerosis. Reducing exposure to free radicals and increasing intake of antioxidant nutrients has the potential to reduce the risk of free radical-related health problems. Free radicals are chemical species produced in the organism during chemical reactions and metabolic process (Coudert *et al.*, 1999). These are very unstable and reactive species towards endogenous molecules (DNA, proteins, Lipids, carbohydrates) implicated in numerous infections: heart failure, ageing, neurodegenerative disorders, atherosclerosis, cancer, diabetes mellitus, cataract and a plethora of diseases (Coudert *et al.*, 1999). Antioxidants compounds block the oxidation processes that produce free radicals causing the various pathological conditions (Ames, 1983; Block, 1992). The body is therefore under constant attack from these free radicals resulting from the normal physiological activities of the metabolic processes (Surai, 2002).

One important method used for the determination is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH). The molecule of 2, 2-diphenyl-2-picrylhydrazyl is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole,

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ISSN 0189-8442

so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm (Molyneux and Songklanakarin 2004). The importance of antioxidants in the body cannot be overemphasized. The need to look inwards for substances capable of stopping the initiation and or the propagation of reactions (oxidation) leading up to the production of these free radicals is ongoing. Majority of the free radical scavengers are the phytochemicals in medicinal plants. Conventional antioxidants improve animal performance during conditions characterized by increased tissue oxidants levels such as infections and stress (Nockel, 1996). Plants based antioxidants exert their effects by enhancing the level of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase or by lowering level of lipid peroxides in the blood or liver (Tseng *et al*., 1997). Recently, there has been an increased concern about synthetic antioxidants due partly to their possible toxicity against DNA. This may explain the interest in examining plants extracts, compounds from plants as source of cheaper, affordable, available and effective antioxidants and the growing need in nutriceuticals.

In the preliminary phytochemical study of *S. kunthianum*, we have also reported the anti-inflammatory, analgesic and anti diarrheal properties of the plant (Ching *et al*., 2008, Ching *et al.*, 2010. Recently, we reported the isolation and characterization of cinnamic acid ester glycosides and their anti-inflammatory activities also evaluated using different experimental animal models.

A detailed literature review showed that the free radical scavenging activity and antioxidant of these compounds from *S. kunthianum* has not yet been evaluated. Herein, the antioxidant activity of the phenylpropanoid and iridoid esters glycosides from the plant using an in vitro qualitative DPPH method was analyzed and reported.

EXPERIMENTAL

Plant material. The fresh stem bark of *S. kunthianum* was collected in March, 2006 in Ogun state Nigeria. Botanical identification and authentication was done by Mr. Usang Felix of the Forest Research Institute of Nigeria, Ibadan. A voucher specimen (No. FHI 107277) was deposited in the same institute (FRIN).

Extraction and isolation. The shade dried stem bark (500 g) was exhaustively extracted with MeOH (4 x 5 L x 48 h) at room temperature. The extract was evaporated to dryness to yield a residue (80 g).

The isolation of the chemical constituents was done using bioactivity guided isolation technique (Falodun *et al*., 2009).

Antioxidant activity determination. The DPPH radical scavenging activities of the test samples were evaluated. Initially, 0.1 ml of the samples at a concentration of 10, 50 and 100 μg/ml was mixed with 1 ml of 0.2 mM DPPH (dissolved in methanol). The reaction mixture was incubated for 20 min at 28 ºC under dark. The control contained all reagents without the sample while methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm using a spectrophotometer. The scavenging DPPH radical activity (%) of the tested sample was calculated as (1 – absorbance of sample / absorbance of control) × 100. The DPPH radical scavenging activity of caffeic acid was also assayed for comparison.

Statistical analysis. All experiments were conducted in 5 determinations and statistical analysis was done using the Statistical Package for Social Science (SPSS) programme. Results are expressed as a mean

of five determinations ± SD

RESULTS AND DISCUSSION

Phenylpropanoid glycosides are a group of natural phenolic compounds mainly extracted from herbal medicines. Quite a number of them have been reported to have multi-pharmacological activities of anti oxidation, xanthine oxidase inhibitory activity and anti tumor (Li *et al.*, 1997). The spectrophotometric method makes use of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical and its specific absorbance properties. The absorbance decreases when the radical is reduced by antioxidants.

The result of the phytochemical screening is shown in Table 1. The phytochemical screening of the plant revealed the presence of copious amount of tannins, phenolics and flavonoids. These phytocompounds have been implicated in the high antioxidant activity of many plant extracts.

The effect of compound **2** (Stereospermiside) (fig. 2) was found to be more active than the iridoid glycoside (Sterostin). Caffeic acid used in this case as a positive control gave relatively significant antioxidant activity. Compound **3** (Sterospermin) showed the highest free radical scavenging activity against a free radical (DPPH) than those of 1 and 2. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). Polyphenolic antioxidants from plants acts by free radical scavenging, singlet oxygen quenching, chelating of transitional metals such as iron, reducing agents and activator of anti oxidative defense enzyme systems to suppress radical damage in biological systems (Kozlowska and Zielinski, 2000; Usoh *et al.*, 2005). Phenylpropanoid glycosides have been shown to possess antioxidant and free radical scavenging properties (Gao *et al.*, 1999, Lee *et al.*, 2004 Tominaga *et al.*, 2005, Jo *et al.*, 2006), anti-lipid peroxidation (Xiong *et al.*, 1996) and inhibition of LDL oxidation (Seidel *at al.*, 2000) due to the presence of phenolic moieties (Xiong *et al.*, 1996).

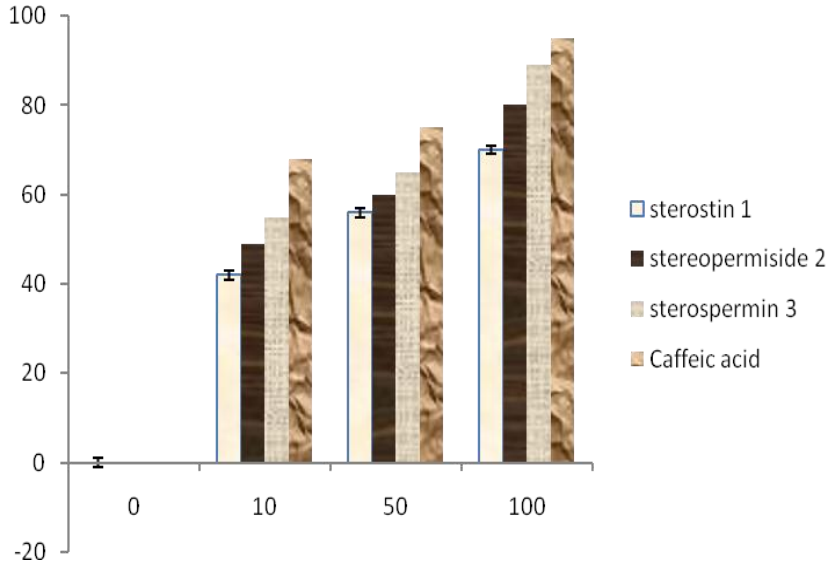

Fig.1. DPPH of phenylpropanoid glycosides with DPPH

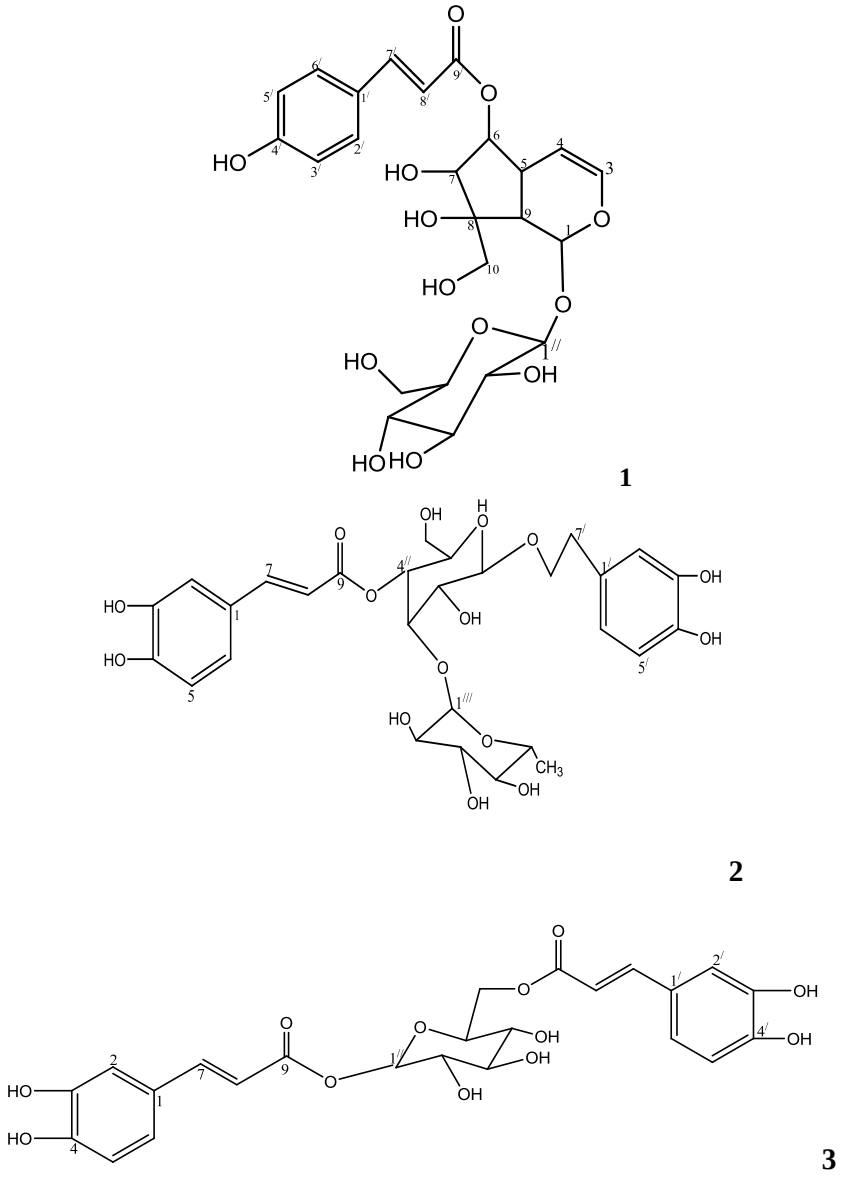

Fig. 2 : Iridoid and phenylpropanoid ester glycoside

The iridoid glycoside compound 1 (Sterostin) (Fig. 2) also showed marked antioxidant activity and displayed strong free radical scavenging property at a concentration of (Fig.1). It has been reported that iridoid glycosides possess free radical scavenging and xanthine oxidase (XO) activity (Ismailogu *et al.*, 2002; Chai-Ming *et al.*, 2000). This highly amplified XO inhibition activity might partly contribute to the result of superoxide anion scavenging activity (Falodun *et al.*, 2009). These findings together with those of previous studies, suggest that *S. kunthianum* possesses abundant phenylpropanoid glycosides, and this plant could be a potential therapeutic substance in the treatment of oxidative stress related human infections and diseases.

Conclusion

These data strongly suggest that the compounds possess a great potential as antioxidant for various applications.

ACKNOWLEDGEMNT

Special thanks to Chinese Academy of Science (CAS) and Third World Academy of Science (TWAS) for the award (Visiting Scholar) to carryout research in South China Botanical Gardens.

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