NOTE:

Evaluation of Total Flavonoid, Total Phenolic Contents, and Antioxidant Activity of Strychnobiflavone

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Abstract: *This work evaluates the antioxidant activity of strychnobiflavone due to the increasing demand of the antioxidant agents day by day. Various in vitro antioxidants assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were used to investigate the antioxidant activity of strychnobiflavone. The results of both DPPH and ABTS show that strychnobiflavone increase the scavenging activity in a concentration-dependent manner due to the phenolic and flavonoid contents. This study revealed that strychnobiflavone is one of the promising and an effective compound for the antioxidant agent.*

Keywords: *antioxidant activity; total flavonoid; strychnobiflavone*

■ INTRODUCTION

The quality of drugs and their impact on human health is regarded as one of the most attractive research interests [1]. For thousands of years, mankind is using plant sources to either alleviate or cure illnesses [2]. Most of the developing countries are investigating and struggling to enhance the quality of their foods. For this purpose, people use different compounds and herbal drugs in their everyday foods as prevention from any diseases [3-5]. Approximately 5–7% of inhaled oxygen, or even more, is converted into reactive oxygen species (ROS) such as O^{2-}, H_2O_2 and •HO [6-7]. These reactive oxygen species are the side products of mitochondrial complex reaction, which can cause Fenton reaction in the body and generally result in degradation of proteins, lipids peroxidation, and oxidation of DNA [8]. It causes many diseases such as cancer, diabetes, atherosclerosis and many chronic diseases [9-12].

Antioxidants are defined as a free radical scavenger that protects us from various diseases like ischemia, anemia, asthma, Parkinson's diseases, inflammation, to name a few [13-14]. They can be used to quench the free radicals in our bodies. Antioxidant compounds in food play an important role as a health-protecting factor. The primary source of natural antioxidants is whole grains, fruits, and vegetables. There are also some known compounds like vitamin C, carotenes, polyphenols, and flavonoids used as antioxidants [15-19].

Many studies are investigating different kinds of fruits, vegetables, and plant extracts as an antioxidant for reducing the risk of the diseases caused by free radicals [20-22]. Strychnobiflavone was previously used as an anti-leishmanial and anti-aging [23-24]. The structure of strychnobiflavone contains phenolic and flavonoid functional groups. Phenols and flavonoids are recently demonstrated as good antioxidants [25-27]. In this paper, the antioxidant activity of Strychnobiflavone hydroethanolic (SHE) was evaluated and compared to vitamin C (ascorbic acid) as one of the most commonly used antioxidants.

■ EXPERIMENTAL SECTION

Materials

Strychnobiflavone (purchased from Sigma-Aldrich), ethanol, water, quercetin, DPPH, ABTS, Folin-Ciocalteau's reagent, gallic acid, potassium persulfate, sodium carbonate, ascorbic acid.

Procedure

Determination of total phenolic contents

The total phenolic contents of SHE was evaluated

using the Folin-Ciocalteau's reagent as a previously described method [28] with slight modification. The sample was mixed with Folin-Ciocalteau's reagent (100 µL) and distilled water (900 µL) and kept for 3 min. Sodium carbonate (200 µL, 15%) was then added. The volume was adjusted to 3 mL with distilled water. The absorbance was measured at 700 nm using 96 well plates. A standard curve was prepared using gallic acid with a concentration range from 150 to 1000 µg/mL.

Determination of total flavonoid contents

The flavonoid contents were determined as previously described [29] with slight modification. One milliliter of 2% aluminium chloride in ethanol was mixed with 1 mL of SHE with various concentrations (62.5 µg/mL to 1 mg/mL). The absorbance was measured at 415 nm after kept for 15 min. The total flavonoid contents were determined using a standard curve of quercetin (0 to 256 µg/mL).

DPPH radical scavenging activity assay

The free radical scavenging activity of SHE was determined using 2,2-diphenyl-1-picrylhydrazyl free radical. Samples with various concentration (ranging from 200 µg/mL to 1 mg/mL) were treated against 0.2 mM DPPH radical using 96 well plates. Ascorbic acid was used as a positive control with the range of concentration of 16 µg/mL to 1 mg/mL. The absorbance was measured at 517 nm.

ABTS radical scavenging activity assay

A mixture of 7 mM ABTS and 2.45 mM potassium persulfate was prepared. It was then mixed in distilled water with a ratio of 1:1 and kept in the dark for 16 h at 4 °C. The sample was diluted in ethanol at the ratio of 1:30, and the absorbance is fixed to 0.7–0.8. With 96 well plates, 20 µL of SHE and 180 µL of ABTS solutions were added to each well. Ascorbic acid was used as a positive control. The absorbance was measured at 700 nm.

■ RESULTS AND DISCUSSION

Total Phenolic Contents

The total phenolic contents in strychnobiflavone were determined using the spectrophotometric method. The standard curve was obtained from different concentrations of gallic acid. The standard curve of gallic acid was compared to the SHE sample. As indicated in Table 1, the gallic acid equivalency (GAE) increases with the increase in the concentration of SHE. It indicated that SHE has a phenolic functional group in its structure, which beneficial for scavenging and stabilizing free radicals. At the concentration of 62.5 µg/mL, the GAE is 29.77. It reaches 126.106 at 1000 µg/mL. It shows that the GAE value increases with the increase in concentration; hence, it is concentration-dependent.

Total Flavonoid Contents

The total flavonoid contents of SHE is also determined using spectrophotometric method. The total flavonoid contents were estimated by the standard curve of quercetin in Fig. 2. From Table 2, the quercetin equivalency (QE) increases with the increase in SHE concentration. At a concentration of 1000 µg/mL, the QE value is 269.32, almost twice larger than that of 125 µg/mL. Therefore, the flavonoid contents increase in a dose-dependent manner. SHE has more flavonoid contents than phenolic contents (Table 1).

Table 1. Gallic acid equivalent (GAE) of the total phenolic contents in strychnobiflavone

Concentration (µg/mL)	Absorbance of SHE	Gallic acid equivalent (mg)
62.5	0.08122	29.770213
125	0.1214	38.3191489
250	0.2103	57.234043
500	0.3123	78.9361702
1000	0.5340	126.106383

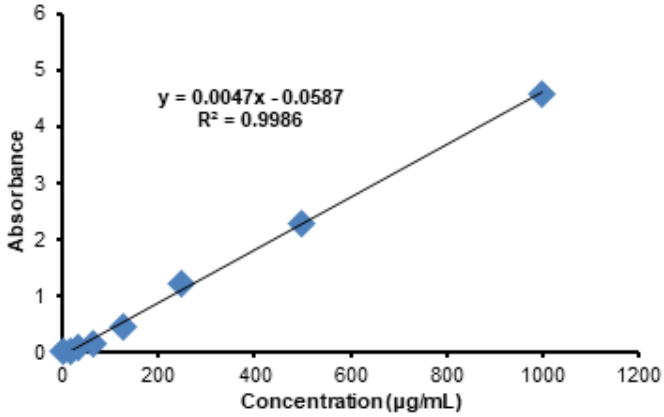

Fig 1. Standard curve of gallic acid

Table 2. Quercetin equivalent (QE) of the total flavonoid contents in strychnobiflavone

Concentration (µg/mL)	Absorbance of SHE	Quercetin equivalent (mg)
62.5	0.0657	132.3548
125	0.1329	154.0323
250	0.2203	182.2258
500	0.3002	208
1000	0.4903	269.3226

Result for DPPH Assay

The antioxidant activity of SHE was determined from the scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyle radical, as shown in Fig. 3. Ascorbic acid (Vit. C) is used as a positive control. The scavenging activity of SHE increases in dose-dependent manners. The SHE has the phenolic and flavonoid content to scavenge the free radical of DPPH. Therefore the more concentration of SHE, the higher scavenging activity will be obtained. Even though SHE shows less activity than the ascorbic acid, it can be considered as one of the promising candidates for stabilizing the toxic radical.

Result for ABTS Assay

The scavenging activity of SHE against ABTS radicals was investigated at various concentrations.

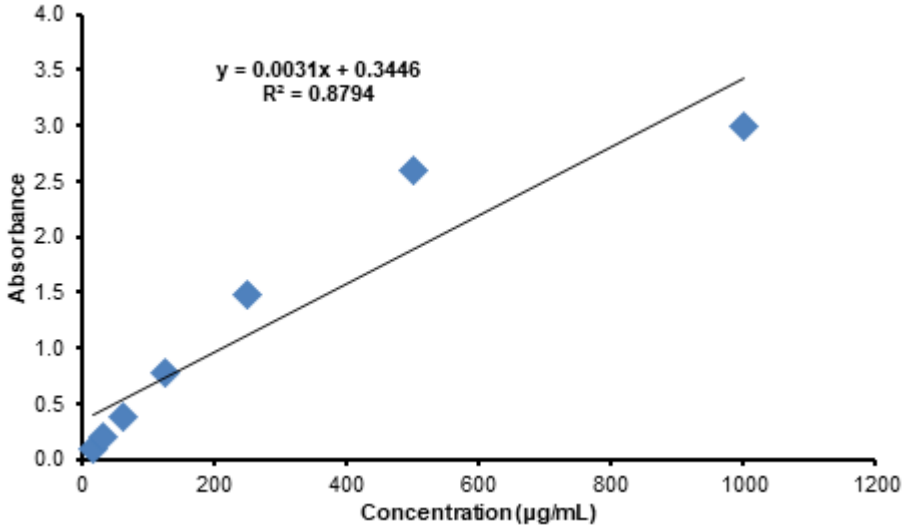

Fig 2. Standard curve of quercetin

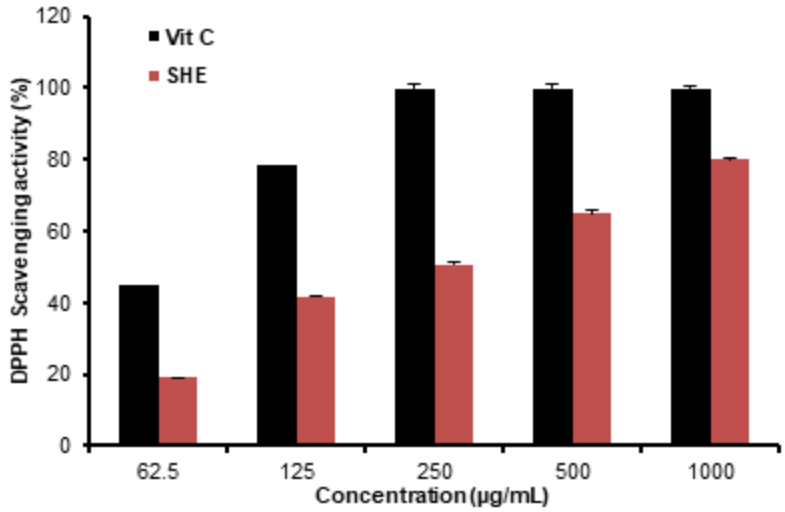

Fig 3. Scavenging activity of SHE against DPPH free radicals at various concentrations. Ascorbic acid was used as a positive control. The SHE scavenge the DPPH radical in a dose-dependent manner

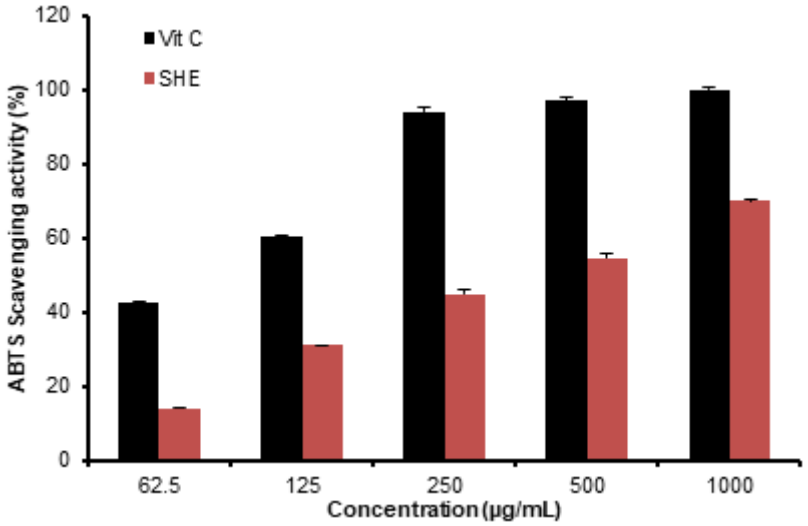

Fig 4. Scavenging activity of SHE against ABTS radicals at various concentrations. Ascorbic acid was used as a positive control. SHE suppresses the free radical in a dose-dependent manner

Ascorbic acid was used as a positive control for comparison. ABTS radical cation was produced in a stable form using potassium persulfate. After a stable absorbance was obtained, the antioxidant SHE is added into the reaction medium, and the antioxidant activity was measured based on the decolorization phenomenon. The scavenging activity of the SHE increases with an increase in concentration. The scavenging activity of SHE showed a slightly higher percentage in DPPH assay (Fig. 4) compared to the ABTS assay. This is because DPPH is soluble in the hydroethanolic system while ABTS is in aqueous. Most organic compounds are less soluble in aqueous media; thus, DPPH is more convenient to be scavenged.

This study confirms the presence of phenolic and flavonoid contents in SHE. The results indicate that the value of total flavonoid is higher than that of total phenolic contents. The SHE shows antioxidant activity on the DPPH and ABTS assays. The scavenging of a stable free-radical molecule like DPPH or ABTS determines the antioxidant capacity of strychnobiflavone. The performance of SHE against DPPH and ABTS radical was compared with the well-known strong antioxidant ascorbic acid. Hence, this work revealed that SHE can be considered as one of the good candidates for an antioxidant that suppresses the free radicals generated by UV radiation.

■ CONCLUSION

The strychnobiflavone hydroethanolic soluble compound was first investigated in term of the phenolic and flavonoid contents. The results showed that SHE has total phenolic contents as well as flavonoid contents which are known for the scavenging of free radicals. We also found that SHE has more flavonoid contents than the phenolic one. Furthermore, the DPPH and ABTS assays were performed to evaluate the antioxidant scavenging activity. Both DPPH and ABTS results confirm the antioxidant activity of SHE for the scavenging of the toxic free radicals.

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