FREE RADICAL SCAVENGING POTENTIAL OF METHANOL EXTRACT OF *SMILAX ROXBURGHIANA*

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Summary

The aim of the present study was to investigate free radical scavenging activity of methanolic stem bark extract of *Smilax roxburghiana*. Different fractions i.e. n-hexane, carbontetrachloride and dichloromethane of *Smilax roxburghiana* stem bark were investigated for their antioxidant activity by utilizing DPPH method. Tert-butyl-1-hydroxytoluene (BHT) was used as reference standard. In this investigation, the dichloromethane soluble fraction of *Smilax roxburghiana* showed the highest free radical scavenging activity with IC_{50} value 18.00 µg/ml. Carbon tetrachloride soluble fraction exhibited moderate antioxidant potential having IC_{50} value 58 µg/ml. The n-hexane fraction showed mild free radical scavenging with the IC_{50} value of 290 µg/ml. Considering IC_{50} value of reference standard (25 µg/ml) dichloromethane soluble fraction of *Smilax roxburghiana* showed excellent antioxidant activity. This finding suggests further investigation to isolate the active chemical(s) from dichloromethane soluble fraction which may responsible for such antioxidant activity.

Key words: Free radical, *Smilax roxburghiana*, DPPH method, BHT, IC_{50} value

Introduction

Over the past decade, there has been a resurgence of interest in the investigation of natural materials as a source of potential drug substance. This is because people are more concern about the side effects of several synthetic drugs, their developing resistance towards infectious diseases, higher cost of treatment and inadequate supply. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. Plant derived medicines are cheap & readily available in remote rural areas. Therefore they are getting more interest to the poorer people in developing countries. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. More than 30% of the entire plant species, at one time or other was used for medicinal purposes[1].

Now-a-days scientists are trying to treat many cellular and metabolic diseases like diabetes, obesity and cancer etc. by using plant or plant extracts. It was found that in human body reactive oxygen species (ROS), are continuously generated leading to oxidative damage to cellular components such as proteins, lipids and DNA. This damage ultimately leads to different degenerative processes such as ageing, cardiovascular diseases, cancer, Alzheimer's disease and other neurodegenerative diseases[2,3,4,5]. Generally the generated ROS are detoxified by the antioxidants present in the body. But over production of ROS and/or inadequate antioxidant defense leads to these unwanted damage to our body. Several studies have shown that plant derived antioxidant scavenge free radicals and modulate oxidative stress-related degenerative effects[2,6].

Tert-butyl-1-hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) etc. are widely used synthetic antioxidants. They are used as food additives to increase shelf life of lipid containing products. Unfortunately their use in food stuff has some controversy because some (BHT, BHA) are known to have not only toxic and carcinogenic effect to humans but have abnormal effects on enzyme systems[7,8]. As a result the use of these synthetic antioxidants in foods is discouraged because of their toxicity and carcinogenicity[9,10]. Therefore, researchers are concentrating on the extraction, identification and application of natural antioxidants as well as methodology development for their evaluation.

Bangladesh is rich in medicinally important flora, & drugs of herbal origin have been used in traditional systems of medicines since ancient time. Several studies have been done to find out the hidden medicinal potentials of these plants. The antioxidant potential of six indigenous plants- *Aegle marmelos Corr.*(Local Name- Bel), *Abroma augusta Linn* (Local Name- Ulot Kambal), *Lagerstroemia speciosa* (Local Name-Jarul), *Cassia fistula* (Local Name-Bador Lathi), *Anthocephalus chinensis* (Local Name- Kadam), *Syzygium cumini* Skeel (Local Name- Jam), had been shown by Laizuman *et al.,* 2009[11]. The present investigation aims to quantitatively estimate the antioxidant potential in the extracts of stem bark *Smilax roxburghiana*.

Smilax roxburghiana (Family Smilacaceae) locally known as Kumarilata is used for certain skin diseases, including psoriasis, rheumatoid arthritis, gout, enteritis, urinary tract infections, skin ulcers etc. Afroze *et al.* (2004)[12] found twenty-one chemical constituents isolated from n-hexane extract of whole *Smilax roxburghiana* plant that showed toxicity towards the brine shrimp *Artemia salina*, and showed significant piscicidal activity. However the present investigation presents the first report on the antioxidant potential of extracts from the stem bark of *Smilax roxburghiana.*

Materials and Methods

Plant material

The investigated plant *Smilax roxburghiana* was collected from Manikgonj, in November 2009. The plant was dried in shade and then stem bark part was separated from other parts. The separated part was ground in coarse powder using high capacity grinding machine. The powders were then preserved in air tight containers.

Extraction and fractionations

Coarsely powdered stem bark of *Smilax roxburghiana* was extracted with methanol by cold extraction process. The crude extracts were then filtered and the solvent were removed until solid/semisolid mass were produced. Then the crude extract was dissolved in 10% water in

methanol (100 ml) and partitioned between n-hexane, carbon tetrachloride, and dichloromethane fractions.

Chemicals and drugs

All chemicals and drugs were obtained commercially and were of analytical grade. DPPH (1,1-diphenyl-2-picrylhydrazyl), tert-butyl-1-hydroxytoluene (BHT) purchased from Sigma-Aldrich Co. LLC. Hexane, carbon tetrachloride, and dichloromethane were acquired from Fisher Scientific Korea Ltd. (Seoul, Republic of Korea).

Designing of the experiment

Different fractions of methanol extract of *Smilax roxburghiana* were subjected to free radical scavenging activity by the method of Brand-Williams *et al.* (1995)[13]. Here, tert-butyl-1-hydroxytoluene (BHT) was used as reference standard.

2.0 ml of a methanol solution of the extract at different concentrations (500 to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 minutes reaction period at room temperature in dark place the absorbance was measured against at 517 nm against methanol as blank by UV spectrophotometer. Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \; X \; 100$$

Where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test material). BHT was used as positive control. To minimize personal error, the average of five absorbance value were taken. Then percentages of inhibition values were plotted against different concentrations (500 to 0.977 µg/ml). From the graph, the IC_{50} value was obtained.

Results

In this investigation, the dichloromethane soluble fraction of *Smilax roxburghiana* showed the highest free radical scavenging activity with IC_{50} value 18.00 µg/ml. Carbon tetrachloride soluble fraction exhibited moderate antioxidant potential having IC_{50} value 58 µg/ml. n-hexane of the methanol extract of *Smilax roxburghiana* showed mild free radical scavenging with the IC_{50} value of 290 µg/ml. These values are quite comparable to that of the value obtained for tert-butyl-1-hydroxytoluene (IC_{50} value 25 µg/ml) used as standard. The IC_{50} values were calculated and are depicted in (Table 1-5).

Discussion

ROS is formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress ROS levels can increase dramatically, which can result in significant damage to cell structures[14]. Hence antioxidants are employed to scavenge the excess ROS.

In this study the effect on the free radical scavenging ability was determined through the DPPH assay because it is one of the most effective, reactive, reliable, simple and reproducible in vitro method for evaluating this important activity of single compounds as well as plant extracts[15,16,17,18]. DPPH itself is a stable nitrogen-centered free radical. The color of ethanolic DPPH solution changes from purple to yellow, due to the formation of diphenylpicrylhydrazine a stable diamagnetic

molecule, upon reduction by either the process of hydrogen radical or electron-donation[19]. The antioxidant potential was assayed from colored methanol solution of DPPH radical by the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer.

The free radical scavenging property of antioxidant is believed to be due to either their ability to be oxidized themselves or providing proton to the reactive species. At recent time antioxidant property of herbal plant is evaluating extensively and many phytoconstituents have been reported to exhibit free radical scavenging property. The chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility with human body.

The present investigation provides a comprehensive profile of the antioxidant activity of extracts of an important medicinal plant, *Smilax roxburghiana.* Though different plants from Genus *Smilax* have been reported to possess antioxidant properties, the present investigation represents the first report on the antioxidant potential of *Smilax roxburghiana*. Our data shows significant antioxidant potential exists especially in the dichloromethane soluble fraction. This indicates the potential of the extracts as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits.

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Table 1: Antioxidant activity of tert-butyl-1-hydroxytoluene (BHT)

Concentration (µg/ml)	Absorbance of extract	Absorbance of blank	% inhibition
500	0.033±0.001	0.365	90.96
250	0.037±0.0006		89.87
125	0.051±0.001		85.96
62.5	0.085±0.001		76.58
31.25	0.156±0.002		57.15
15.625	0.232±0.06		36.44
7.813	0.287±0.053		21.48
3.906	0.319±0.066		12.66
1.953	0.330±0.1		9.59
0.977	0.343±0.076		6.08

Values in the table are presented as mean ± SEM ($N = 5$)

Table 2: Antioxidant activity of n-hexane soluble fraction of *Smilax roxburghiana*

Concentration (µg/ml)	Absorbance of extract	Absorbance of blank	% inhibition
500	0.157±0.001	0.365	56.99
250	0.188±0.001		48.49
125	0.211±0.001		42.19
62.5	0.225±0.001		38.41
31.25	0.251±0.001		31.23
15.625	0.301±0.031		17.53
7.813	0.344±0.038		5.75
3.906	0.348±0.040		4.71
1.953	0.351±0.052		3.84
0.977	0.358±0.044		1.92

Values in the table are presented as mean ± SEM ($N = 5$)

Table 3: Antioxidant activity of carbon-tetrachloride soluble fraction of *Smilax roxburghiana*

Concentration (µg/ml)	Absorbance of extract	Absorbance of blank	% inhibition
500	0.089±0.001	0.365	75.62
250	0.121±0.001		66.85
125	0.138±0.001		62.19
62.5	0.169±0.001		53.70
31.25	0.215±0.001		41.10
15.625	0.222±0.022		39.18
7.813	0.249±0.014		31.78
3.906	0.284±0.028		22.19
1.953	0.304±0.055		16.71
0.977	0.321±0.043		12.05

Values in the table are presented as mean ± SEM ($N = 5$)

Table 4: Antioxidant activity of dichloromethane soluble fraction of *Smilax roxburghiana*

Concentration (µg/ml)	Absorbance of extract	Absorbance of blank	% inhibition
500	0.021±0.0003	0.365	94.21
250	0.033±0.001		91.07
125	0.050±0.001		86.41
62.5	0.093±0.002		74.41
31.25	0.138±0.001		62.08
15.625	0.191±0.04		47.73
7.813	0.227±0.036		37.86
3.906	0.284±0.059		22.19
1.953	0.304±0.086		16.71
0.977	0.321±0.075		12.05

Values in the table are presented as mean ± SEM ($N = 5$)

Table 5: Scavenging of free radical by different extract of *Smilax roxburghiana* and tert-butyl-1-hydroxytoluene in DPPH method

Sample	IC50 (µg/ml)
tert-butyl-1-hydroxytoluene (standard)	25
n-hexane soluble partitionate	290
Carbontetrachloride soluble fraction	58
Dichloromethane soluble fraction	18

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