Comparative antioxidant activity of different parts of *Bauhinia purpurea* L.
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Kaniz F Urmi¹, Samina Mostafa², Gulshanara Begum¹, Tamannatul Ifa¹, Kaiser Hamid²*

¹Department of Pharmacy, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh.
²Department of Pharmacy, East West University, Dhaka, Bangladesh.

*Correspondence: kaiserpharm_1134@yahoo.com

Abstract
The present study was undertaken to explore as well as to compare the antioxidant activity of the different plant parts of Bauhinia purpurea L. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and nitric oxide (NO) scavenging capacity were measured to determine the antioxidant activity of both leaves and bark of the plant. Solvent–solvent partitioning was accomplished to obtain extracts of different polarities as n-hexane, ethyl acetate, and methanol extract. All the extracts exhibited potent antioxidant activity in terms of DPPH and NO scavenging capacity. In case of DPPH radical scavenging, ethyl acetate extract of bark was found to have highest activity with IC₅₀ value of 1.08 µg/mL followed by n-hexane extract of bark and leaves with IC₅₀ values of 2.40 and 3.07 µg/mL, respectively. The IC₅₀ value of standard ascorbic acid was 33.77 µg/mL. In case of NO scavenging activity, the ethyl acetate extract of leaves showed highest activity with IC₅₀ values of 1.04 µg/mL followed by n-hexane and ethyl acetate extract of bark having IC₅₀ values of 1.92 and 2.04 µg/mL, respectively. The IC₅₀ value of standard ascorbic acid was 71.06 µg/mL.

Keywords: Bauhinia purpurea; DPPH; nitric oxide; antioxidant activity.

Introduction
Reactive oxygen species (ROS) are generated as a by product of biological reaction and from exogenous factors. Some of them are important in cell metabolism including energy production, phagocytosis, and intercellular signaling (Ottolenghi, 1959). But ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions, and metabolic processes have a wide variety of pathological effects such as DNA damage, carcinogenesis, and various degenerative disorders such as cardiovascular diseases, aging, and neuro-degenerative diseases (Gyamfi et al., 1999; Osawa, 1994; Noda et al., 1997). Therefore, a potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical mediated cellular damage and diseases (Ahmad et al., 1998).

From recent studies, it has been found that a number of plant products including polyphenols, terpenes, and various plant extracts exerted an antioxidant action (Zhoc and Zheng, 1991; Quinn and Tang, 1996; Seymour et al., 1996; Prasad et al., 1996). Considerable amount of data have been generated on antioxidant properties of food plants around the globe (Cav et al., 1996; Kaur and Kapoor, 2002). However, traditionally used medicinal plants warrant such screening.

Bauhinia purpurea L. belonging to the family Fabaceae, locally known as Kanchan, Rakta Kanchan etc., is native of Southern Asia, Southeast Asia, Taiwan, and China. The root, stem, bark, and leaf of B. purpurea are also reported to be used in the treatment of jaundice, leprosy, cough, pain, fever, ulcers, stomach cancer, rheumatism, convulsions, delirium, and septicaemia (Chopra et al., 1956; Asolkar et al., 2000; Parrota, 2001; Kirthikar and Basu, 2001; Janardhanan et al., 2003). In India, the root of B. purpurea is used for the treatment of diarrhea, ulcer, boils, and abscesses (Kirthikar and Basu, 2001), whereas in Pakistan, the fresh and dried flower buds of B. purpurea are used as a food material, while the leaves, stems, and roots are widely used to treat infections, pain, diabetes, jaundice, leprosy, and cough (Morais et al., 2005). The antioxidant activity has been previously reported by Shajiselvin et al. (2011) and Chew et al. (2011). But to the best of our knowledge, the comparison in antioxidant activity of different
parts has not been conducted before. The purpose of this study was to reinvestigate as well as to compare the antioxidant activity between leaves and bark of *B. purpurea* L.

**Materials and Methods**

**Plant materials**
The fresh leaf and bark of the *B. purpurea* plant was collected from the area of Savar in Jahangirnagar University during February 2011. The *B. purpurea* plant was taxonomically identified by the National Herbarium. The accession number of *B. purpurea* voucher specimen is 35516.

**Drying and pulverization**
The fresh leaf and bark of the *B. purpurea* was washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days and finally dried at 45°C for 36 hours in an electric oven. After complete drying, the entire portions were pulverized into a coarse powder with help of a grinding machine and were stored in an airtight container for further use.

**Extraction of plant material**
The powdered 200 g of leaf and bark extract of *B. purpurea* was extracted with three times methanol of their weight in a flat bottom glass container, through occasional shaking and stirring for 7 days. The extracts were then filtered through filter paper. The filtrates were concentrated at 50°C under reduce pressure.

**Solvent–solvent partitioning of methanolic extracts**

**Partitioning with n-hexane**
The concentrated methanolic extract of *B. purpurea* was made slurry with water. The slurry was taken in a separating funnel and few mL of ethyl acetate (100 mL) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The ethyl acetate fraction (lower layer) was collected. The process was repeated three times. The ethyl acetate fractions of different parts of the plants were evaporated using rotary evaporator at 40°C.

**Tests for antioxidant activity**

**1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity**
The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca et al. (2001). Plant extract (0.1 mL) was added to 3 mL of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated from [(A₀ − A₁)/A₀] × 100, where A₀ is the absorbance of the control and A₁ is the absorbance of the extract/standard. The inhibition curves were prepared and IC⁵₀ values were calculated.

**Nitric oxide (NO) scavenging assay**
Nitric oxide (NO) radical scavenging was estimated on the basis of Griess-Ilosvay reaction using method followed by Govindarajan et al. (2003). In this investigation, Griess-Ilosvay reagent was modified by using naphthyl ethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL), and plant extract (5–250 µg/mL) or standard solution (ascorbic acid, 0.5 mL) was incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of naphthyl ethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions.

**Results and Discussion**
All the extracts of both leaves and bark showed potent DPPH radical scavenging activity. Ethyl
acetate extract of bark was found to have highest activity with IC$_{50}$ value of 1.08 µg/mL followed by n-hexane extract of bark and leaves with IC$_{50}$ values of 2.40 and 3.07 µg/mL, respectively (Figures 1 and 2). The IC$_{50}$ value of standard ascorbic acid was 33.77 µg/mL.

In case of NO scavenging activity, the ethyl acetate extract of leaves showed highest activity with IC$_{50}$ values of 1.04 µg/mL followed by n-hexane and ethyl acetate extract of bark having IC$_{50}$ values of 1.92 and 2.04 µg/mL, respectively (Figures 3 and 4). The IC$_{50}$ value of standard ascorbic acid was 71.06 µg/mL.

It has been previously reported that high antioxidant activity of the ethyl acetate extract was observed for the whole plant of *B. purpurea* (Shajiselvin et al., 2011). This result is congruent with the present study where ethyl acetate extract of both leaves and bark showed highest NO and DPPH radical scavenging activity, respectively. Previous studies also confirmed the presence of phenolic compounds, flavonoids, phytosterols, tannins, saponins, and glycosides (Pahwa et al., 2010). The observed antioxidant activity may be due to presence of these phytochemicals particularly polyphenols and phenolic compounds.
Compounds. Polyphenols are able to neutralize free radicals, scavenge singlet and triplet oxygen, and to break down peroxides. Among the diverse phytochemicals synthesized by plants for defensive purposes, mostly are secondary metabolites. Antioxidants are such type of compounds that prevent tissue destruction from excessive free radicals as triplet chlorophyll, singlet oxygen, and hydroxyl radicals are lethal to plants (Chew et al., 2009).

Conclusion

The present study corroborates the antioxidant activity of both bark and leaves of the plant B. purpurea L. However, the findings here are preliminary in nature. The next step would be to isolate pure compounds, elucidate their structure using different spectroscopic techniques, and evaluate their antioxidant activity both in in vitro and in vivo.

Conflict of Interests

None declared.

Authors’ Contributions

All authors contributed equally to this study.

References


