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Comparative antioxidant activity of different parts of *Bauhinia purpurea* L.

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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 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. 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. The IC_{50} 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 Herberium. The accession number of *B. purpurea* voucher specimen is 35516.

Drying and pulverization

The fresh leaf and bark of the plant of *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 *n*-hexane (100 mL) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The *n*-hexane fraction (upper layer) was collected. The process was repeated three times. The *n*-hexane fractions of different parts of the plants were evaporated using rotary evaporator at 40°C.

Partitioning with ethyl acetate

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 $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. The inhibition curves were prepared and IC_{50} 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-napthylamine (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

Figure 1: DPPH radical scavenging activity of the leaves of *B. purpurea*.

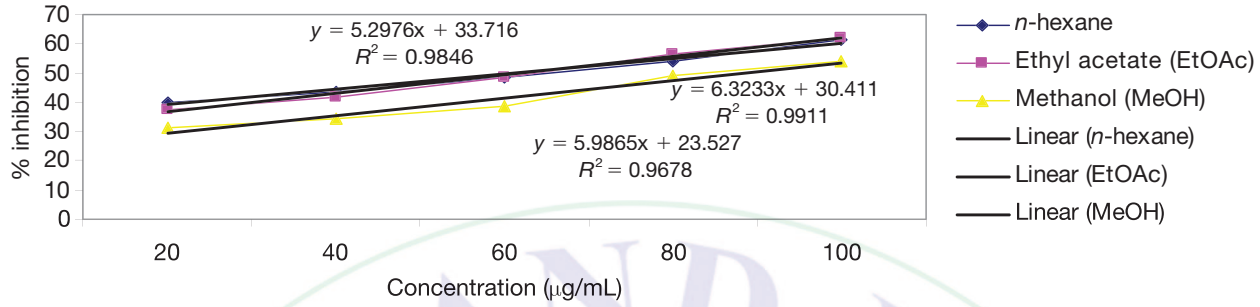
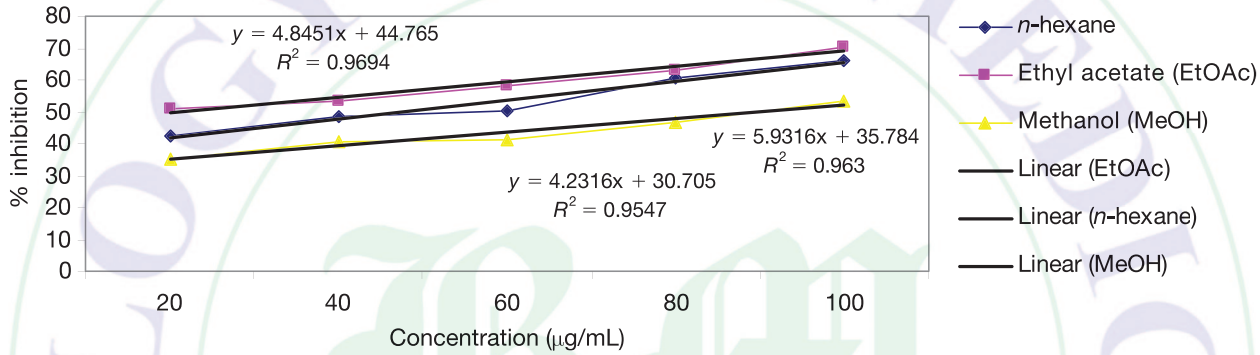


Figure 2: DPPH radical scavenging activity of the bark of *B. purpurea*.



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

Figure 3: NO scavenging activity of the leaves of *B. purpurea*.

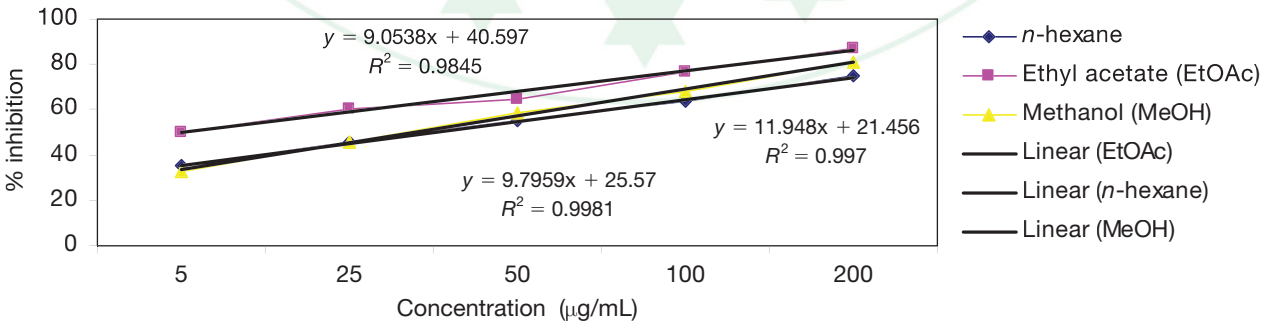
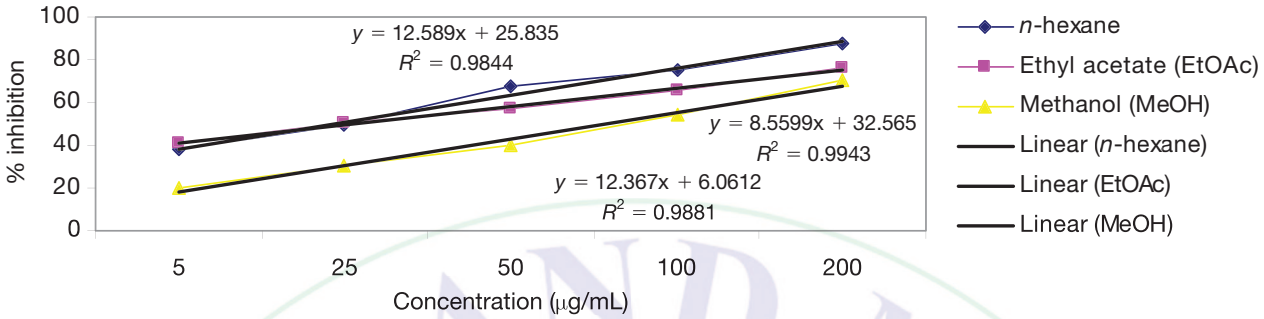


Figure 4: NO scavenging activity of the bark of *B. purpurea*.



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 spectrospic 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.

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