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# In Vitro Antioxidant Activity of Different Parts of the Plant *Diospyros discolor*

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**Abstract:** This study was conducted to evaluate the antioxidant activity of extracts from different parts of the plant *Diospyros discolor* including leaf, fruit and bark. Methanol was used as solvent and antioxidant effect was measured by DPPH method and total phenolic content. Extracts from all parts of the plant showed potential antioxidant activity. Among the three different parts bark showed highest antioxidant activity with $IC_{50}$ value of 45.78 µg/ml that is followed by the fruit ($IC_{50,}$ 69.13 µg/ml) and leaf ($IC_{50,}$ 72.50 µg/ml). On the other hand the antioxidant activity of standard (Ascorbic Acid) was with $IC_{50}$ value of 43.04µg/ml. Incase of phenolic content, bark contains (9.16 mg of GAE / gm of extractives) followed by fruit (5.95 mg of GAE / gm of extractives) and leaves (5.65 mg of GAE / gm of extractives)

**Key words:** *Diospyros discolor, Antioxidant activity, DPPH, Total phenolic content*

## INTRODUCTION

It is recognized that naturally occurring substances in higher plants have antioxidant activity. Recently, there is an increased interest in oxygen containing free-radicals in biological systems and their implied roles as causative agents in the aetiology of a variety of chronic disorders. That is why; attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them.[1]

The genus Diospyros (Ebenaceae) consists of woody shrubs and trees distributed in the tropical and sub-tropical regions of the world. Around 500 species [2] are known world wide of which 24 species[3] are native to India. This genus is a rich source of naphthols, naphthoquinones and naphthalene derivatives[4]. Previously, anthraquinone glycosides[5], ellagic acid glycosides[6] and the ubiquitous triterpenes lupeol, betulin and betulinic acid [6] have been reported from the stem bark.

*Diospyros discolor* belongs to the family Ebenaceae is a medium-sized tree growing to a height of 20 m. Leaves are leathery, oblong, up to 20 cm long, with a round base and acute tips. The blade is glossy green, smooth above and softly hairy below. Female flowers are axillary and solitary, larger than the male. Fruits are fleshy, globose, up to 8-10 cm diameter, densely covered with short brown hairs. The pulp is edible. The fruit hairs have to be rubbed off before eating as it can cause peri-oral itching and irritation[8].

Different parts of *Diospyros discolor* is used in different indications as traditional medicines which can be summarized as – bark is used for fevers; dysentery, diarrhea and itch skin ailments, decoction of bark for coughs[8].

In Southeast Asia, juice of unripe fruit is used for wounds. Oil from seeds is used for diarrhea and dysentery. Infusion of fruit is used as gargle in aphthous stomatitis. In Bangladesh, juice of bark and leave are used for snakebites. Bark and leaves are used as eyewash. In the Guianas, *D. discolor* is used for colds, diarrhea, heart problems, hypertension, spider bites, stomach aches, diabetes and eczema[8].

It is reported by Mei-Hsien Lee *et. al* that the plant extracts of *D. discolor*, abundant phenolic constituents (more than 30 mg of GAE per g) and superoxide anion radical scavenging activities and $IC_{50}$ values ranged from 12.9 to 28.5 µg/mL. But probably no work has been performed particularly to observe which parts of the plant *D. discolor* possess antioxidant activity and subsequent comparison among them. The aim of the present study is to explore and to compare the antioxidant potentiality of different parts of the plant *D. discolor*.

## MATERIAL AND METHODS

**Assessment of Free Radical Scavenging Activity by DPPH Method:** DPPH was obtained from Sigma

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Aldrich Co. (St. Louis, USA). All other chemicals used were of analytical grade.

**Preparation of Crude Plant Extract:** Different parts of the test plants were collected and identified from the Department Botany, University of Dhaka. About 200 g of dried, ground separate parts of the plant were soaked in 1.5 L of 98% methanol for 7 days, stirring every 18 h using a sterilized glass rod, separately. The final extracts were passed through No. 1 Whatman filter paper (Whatman Ltd., UK). The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4°C for further use.

**Antioxidant Activity (DPPH Free Radical Scavenging Activity) of Methanolic Extract:** The antioxidant activity of extract was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay[9-11]. The free radical scavenging capacity of the methnolic extract of different parts of the plant *Diospyros discolor* was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude methanolic extract of different parts of the plant *Diospyros discolor* was mixed with 95% methanol to prepare the stock solution (10mg/100mL). The concentration of extract of different parts of *Diospyros discolor* solution was 10 mg /100 ml or 100µg/ml. From stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes & by serial dilution with methanol and was made the final volume of each test tube up to 10 ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing extract of different parts of *Diospyros discolor* (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml) and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10mg/100mL or 100µg/ml) of extract of different parts of *Diospyros discolor*. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation-

**% DPPH radical-scavenging = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] x 100**

Then % inhibitions were plotted against respective concentrations used and from the graph (Fig 2) IC suffix was calculated.

**Assays for Total Phenolics:** Total phenolic content of different parts of *Diospyros discolor* extractives was measured employing the method as described by Skerget *et al* 2005. involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard[12].

In brief, to 0.5 ml of extract solution (conc. 2 mg/ml), 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of $Na_2CO_3$ (7.5 % w/v) solution was added. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared (Fig 1) from gallic acid solution with different concentration, the total phenols content of the sample was measured. The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent) / gm of the extract.

## RESULTS AND DISCUSSIONS

Incase of DPPH method for studying the antioxidant potential, the bark of *Diospyros discolor* showed highest antioxidant activity having $IC_{50}$ value of 45.78 µg/ml that is followed by the fruit (69.13 µg/ml) and leaf (72.50 µg/ml). On the other hand the antioxidant activity of standard (Ascorbic Acid) was with $IC_{50}$ value of 43.04µg/ml.

It has been established that oxidative stress is one of the major causative factors in induction of many chronic and degenerative diseases which includes atherosclerosis, diabetes mellitus, cancer, Parkinson's disease and immune dysfunction and is involved in aging[13-15]. The antioxidative activity of natural sources is due to the active compounds present in the plants. According to Pratt and Hudson, most natural antioxidants can be found in wood, bark, stem, leaf, fruit, root, flower and seed. Most of these compounds are normally phenolic or polyphenolic compounds in nature, e.g. tocopherols, flavonoids and derivatives of cinnamic acid, phosphatidic and other organic acids.

It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH analysis is one of the tests used to prove the ability of the components of the different parts of *Diospyros discolor* extract to act as donors of hydrogen atoms[16].

**Total phenolic Content:** The amount of total phenolic content differs in different extractives and ranged from 5.65 mg of GAE / gm of extractives to 9.16 mg of GAE / gm of extractives (Fig 3). The highest phenolic content was found in bark (9.16 mg of GAE / gm of extractives) followed by fruit (5.95 mg of GAE / gm of extractives) and leaves (5.65 mg of GAE / gm of extractives).

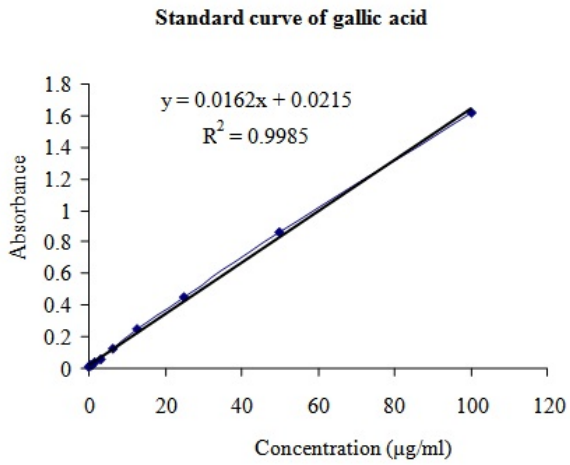


**Fig. 1:** Standard curve of Gallic acid

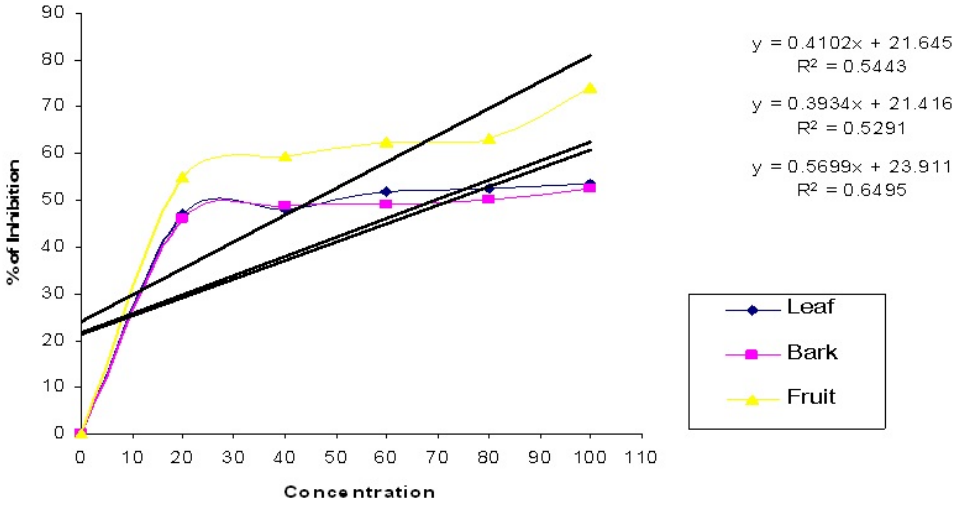


**Fig. 2:** IC $_{50}$ values of different parts of the plant *Diospyros discolor*

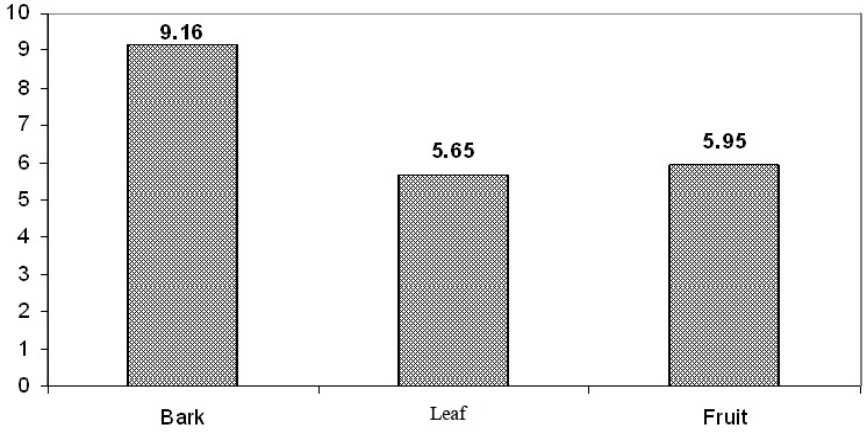


**Fig. 3:** Total phenolic content of different parts of the plant *Diospyros discolor*

In this study we have observed a relationship between total phenolic content and with $IC_{50}$ values that is the higher the phenolic content, the lower the $IC_{50}$ values. Incase of the $IC_{50}$ value is 45.78 µg/ml and phenolic content is (9.16 mg of GAE / gm of extractives) which followed by fruit ($IC_{50,}$ 69.13 µg/ml and phenolic content 5.95 mg of GAE / gm of extractives) and leaves ($IC_{50}$ , 72.50 µg/ml and phenolic content 5.65 mg of GAE / gm of extractives).

In conclusion, the findings of this study support this view that some medicinal plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. The data of this study will enrich the existing comprehensive data of antioxidant activity of plant materials.

## REFERENCES


1. Larson, R.A., 1988. The antioxidants of higher plants, *Phytochemistry*, 27: 969-978.
2. Willis, J.C., (revised by K.A. Airy Shaw) 1966. A Dictionary of Flowering Plants and Ferns, seventh ed. Cambridge University Press, London, pp: 360.
3. Gamble, J.S., 1997. Flora of the Presidency of Madras, Bishen Singh, Mahendra Pal Singh,Dehradun, India, pp: 772-778.
4. Hegnauer, R., 1989. Chemotaxonomie der Pflanzen, vol. 8. Birkhauser Verlag, Basle, pp: 402-411.
5. Srivastava, S.K., S. Pitre, 1985a. Planta med., 51: 537.
6. Srivastava, S.K., S. Pitre, 1986. J. Indian Chem. Soc., 63: 1004.
7. Zakaria, M., J.A.D. Jeffreys, P.G. Waterman, S.M. Zhong, 1984. Phytochemistry, 23: 1481.
8. http:// WWW. Philipinemedicinal plants.com.
9. Hasan, M.S., M.I. Ahmed, S. Mondal, S.J. Uddin, M.M. Masud, S.K. Sadhu, M. Ishibashi, 2006. Antioxidant, antino-ciceptive activity and general toxicity study of *Den-drophthoe falcata* and isolation of quercitrin as themajor component, *Oriental Pharmacy and* Experimental Medicine, 6: 355-60.
10. Koleva, I.I., T.A. Van Beek, J.P.H. Linssen, A.De Groot and L.N. Evstatieva, 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods" Phytochemical Analysis, 13: 8-17.
11. Lee, S.E., H.J. Hwang and J.S. Ha, 2003. Screening of medicinal plant extracts for antioxidant activity, Life Science, 73: 167-179.
12. Lucija Majhenic, Mojca S kerget, Zeljko Knez, 2007. Antioxidant and antimicrobial activity of guarana seed extracts, Food Chemistry, 104: 1258-1268.
13. Halliwell, B., 2000. The antioxidant paradox. Lancet, 355: 1179-1180.
14. Metodiewa, D., C. Koska, 2000. Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders. An overview. Neurotox Res., 1(3): 197-233.
15. Young I.S., J.V. Woodside, 2001. Antioxidants in health and disease. J Clin Pathol, 54: 176-186.
16. Stoilova, I., A. Krastanov, A. Stoyanova , P. Denev, S. Gargova, 2007. Antioxidant activity of a ginger extract (Zingiber officinale), Food Chemistry, 102: 764-770.