Journal of Experimental Sciences 2012, 3(3): 11-14
ISSN: 2218-1768
Available Online: http://jexpsciences.com/

journal of
experimental sciences

Evaluation of total phenolic contents and antioxidant activities in different solvent extracts of *Diospyros melanoxylon* Roxb. bark

B. V. Jaiwal[1*], F. K. Shaikh[1], H. B. Waghire[2] and B. P. Sarwade[3]

[1]Department of Biochemistry, Dr. Babasaheb Ambedkar Marathawada University, Aurangabad-431004, India.
[2]Department of Botany, Dr. Babasaheb Ambedkar Marathawada University, Aurangabad-431004, India.
[3]Swami Vivekanand Arts, Commerce and Science College, Mantha-431504, India.

Abstract
Antibacterial and anticardidal activities from the polar and non-polar solvent extracts of *Diospyros melanoxylon* Roxb. is established earlier. Here we have evaluated amount of total phenolics present in Aqueous, Methanol, Ethanol, Acetone, Ethyl acetate, and Chloroform solvents by using Folin-ciocaltuaeu reagent from *Diospyros melanoxylon* Roxb. bark. Anti-oxidative potential of phenolics present in different solvents were evaluated by DPPH free radical scavenging assay, O-phenanthroline assay and reducing power assay. It was observed that the Methanol is a better solvent for the total phenolics extraction. The amount of phenolics present in solvents were in the order of Methanol > Ethanol > Acetone > Aqueous > Ethyl acetate. The highest free radical scavenging potential 59.33 ± 1.52% was observed in Ethanol extract and the lowest scavenging potential 39.66 ± 1.52% in Aqueous extract. The highest scavenging potential 66.66 ± 2.4% was observed in Acetone extract in case of O-phenanthroline assay. The similar scavenging potential was observed in Aqueous, Methanol, Ethanol and Acetone extracts in case of reducing power assay. This study shows that the *Diospyros melanoxylon* Roxb. bark extract could be used as probable antioxidative agent.

Keywords: Antioxidant, *Diospyros melanoxylon* Roxb., DPPH, Folin-Ciocaltuaeu reagent.

INTRODUCTION

The medicinal plants are used in the cellular and metabolic diseases treatment such as diabetes, obesity and cancer. It is well known that the reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide are highly reactive and potentially damaging transient chemical species. The tissue damage results from an imbalance between ROS-generating and scavenging systems inside the body. The decrease or damage scavenging systems leads to variety of disorders including degenerative disorders of the CNS (central nervous system) such as Alzheimer's disease, cancer, atherosclerosis, diabetes mellitus, hypertension, AIDS and aging [1, 2].

In recent years, there has been a considerable interest in the finding of natural antioxidants from plant materials because synthetic antioxidants have been questioned due to their toxicity [3]. It is reported that the phytochemicals from plants, particularly flavonoids and other polyphenols, have been involved in antioxidants or scavenging of free radical reactions [4, 5]. Natural antioxidants could prevent the ROS related disorders in human beings without the use of synthetic compounds, which may be carcinogenic and harmful to the lungs and liver [6]. Also, antioxidants play an important role in nutrition by lengthening the shelf life of food and the reducing nutritional losses and formation of harmful substances [7, 8].

Received: Feb 04, 2012; Revised: March 05, 2012; Accepted: March 22, 2012.

*Corresponding Author

B. V. Jaiwal
Department of Biochemistry, Dr. Babasaheb Ambedkar Marathawada University, Aurangabad-431004, India.

Email: jaiwalbv@gmail.com

In the present study, an attempt has been made to evaluate the antioxidant activities and the total phenolics from different solvent extracts (Aqueous, Methanol, Ethanol, Acetone and Ethyl acetate) of *Diospyros melanoxylon* Roxb. bark Different polar and non-polar solvent extracts of bark of *Diospyros melanoxylon* Roxb bark exhibited promising antibacterial and anticardidal activities [9, 10].

MATERIALS AND METHODS

Materials

DPPH 2,2-diphenyl-1-picrylhydrazyl (sigma Aldrich), Ferric chloride, Potassium Ferricyanide, O-phenanthroline, Gallic acid and Folin-Ciocalteau reagent (Qualigens), Tricarboxylic acid (SRL), Methanol , Ethanol , Ethyl acetate and Acetone (Rankem).

Collection of samples

The barks of *Diospyros melanoxylon* Roxb. were collected from surrounding area of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.) India. The bark were thoroughly washed by distilled water and kept at room temperature until dried. The completely dried bark was crushed into fine powder by using mixer grinder. Dried fine powder was preserved at 15°C for further experiment.

Preparation of extracts

Total polyphenols from bark was extracted using the different solvent systems as mentioned earlier [11 and 12]. The fine powder was soaked in Aqueous, Methanol, Ethanol, Acetone, Ethyl acetate and Chloroform solvents (1:10 w/v) and mixed properly with continuous stirring for 30 min on stirrer. All samples were kept at

15°C for overnight in air tight bottles and were centrifuged at 6000 × g for 15 minutes. Resulting supernatants were stored at -20°C for further experiment.

Estimation of total phenolics

The total phenolics present in each solvent extracts were estimated by using Folin-Ciocalteau reagent with slight modifications [13]. Various concentrations of Gallic acid (Standard phenolic compound) and each solvent extracts were mixed with Folin-Ciocalteau reagent and incubated at room temperature for 3 minutes followed by 2% Na_2CO_3 was added and incubated for 1 minute in boiling water bath. Samples were cooled under tap water and optical density was recorded at 650 nm. The amount of phenolics was determined by using standard graph and the concentration of phenolics expressed in Gallic Acid Equivalents (GAE mg/ml).

DPPH radical scavenging assay

DPPH radical scavenging activity of the each solvent extracts were estimated using a slight modification of the protocol reported by Yamaguchi et. al. [14]. For a typical reaction, 2 ml of 100 µM DPPH in Ethanol /Acetone was mixed with 100 µg/ml phenolics concentration of each extract. The ascorbic acid was used as standard reference antioxidant. The reaction mixtures were incubated in the dark for 15 minutes and thereafter the optical density was recorded at 517 nm against the blank. For the control, DPPH in Ethanol /Acetone was taken without plant extracts and the optical density was recorded after 15 minutes. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity and percentage inhibition of DPPH radical scavenging calculated using the following equation:

Effect of scavenging (%) =
[1-A sample (517 nm) /A control (517 nm)] ×100

O-phenanthroline Assay

Ortho substituted phenolic compounds were found more active than unsubstituted phenol. Hence, these compounds may exert pro-oxidant effect by interacting with iron. In the presence of scavenger, reduction of ferric ions will occur which is measured at 510 nm. Typical reaction mixture contain 0.5ml O-phenanthroline (0.005g in 10 ml Methanol), 1 ml ferric chloride 200 M (0.0324 mg / ml in distilled water) was added in 1ml of each solvent extract containing 40 µg/ml phenolics. The mixtures were incubated at ambient temperature for 10 min and then the absorbance was recorded at 510 nm. For the control, O-phenanthroline and ferric chloride mixture was taken without plant extracts and the optical density was recorded at 510 nm. The assay was carried out in triplicate. The increase in optical density of reaction mixture on addition of plant extracts in relation to the control was used to calculate iron chelating activity [15, 16].

Measurement of Reducing Power

The reducing power of each solvent extract of *Diospyros melanoxylon* Roxb.bark was determined by using the method described previously with slight modifications [17]. A dilution of the each solvent extract was performed (100 µg/ml phenolics) in 2.5 ml of 0.2 M phosphate buffer pH, 6.6 containing 1% Potassium Ferrocyanide. The mixtures were incubated at 50ºC for 20 minutes. Ten percent Trichloroacetic acid (TCA, 2.5 ml) was added to a portion of these mixtures (5 ml) and centrifuged at 6,000 × g for 10 minutes. The supernatant of each mixture was separated and mixed with distilled water (2.5 ml) containing 1% ferric chloride (0.5 ml). For the control, reaction mixture was taken without plant extract. The absorbance of these mixtures was measured at 700 nm. The increase in optical density of reaction mixture on addition of plant extracts in relation to the control could be the measurement of antioxidant activity of the extract.

RESULTS AND DISCUSSION

Phenolics are diverse groups of secondary metabolites and received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenol is due to their redox properties of hydrogen donors and singlet oxygen quenchers [18]. Also phytomedicines have been an integral part of traditional health care system and according to World Health Organization, greater than 80% of world population depends on traditional medicine [19].

The different solvents such as Aqueous, Methanol, Ethanol, Acetone and Ethyl acetate were used for extraction of phenolics from bark of *Diospyros melanoxylon* Roxb. The amount of extracted phenolics was estimated by using Folin-ciocalteau reagent. The highest concentration of phenolics 1.78± 0.06 mg/ml GAE was observed in Methanol extract and lowest concentration of phenolics 0.20 ± 0.03 mg/ml GAE was observed in Ethyl acetate extract. Almost similar concentrations of phenolics were found in Aqueous, Ethanol and Acetone extracts. There were no phenolics observed in Chloroform extract (Table 1). Our results show that Methanol was the best solvent for extraction of phenolics from bark of *Diospyros melanoxylon* Roxb. This finding correlates the study of Jerneja et. al. (12) who found that Methanol is a better solvent than Ethanol for extraction of phenolics from green walnut fruits.

Table 1. Total phenolic contents in different solvent extract of *Diospyros melanoxylon* Roxb. bark (Mean ± S. D.)

Name of Solvent Extract	Total phenolic contents in mg/ml GAE
Aqueous	1.10 ± 0.10
Methanol	1.78 ± 0.06
Ethanol	1.36 ± 0.08
Acetone	1.16 ± 0.04
Ethyl acetate	0.20 ± 0.03
Chloroform	-----

The free radical scavenging activity was determined by using DPPH reagent. The 100µg/ml phenolics from each solvent extract was used for DPPH radical scavenging assay. It was observed that the Ethanol extract showed highest free radical scavenging potential 59.33 ± 1.52% and the Aqueous extract showed lowest free radical scavenging potential 39.66 ± 1.52% (Fig. 1). The Acetone and Ethyl acetate extracts showed similar scavenging potential 42.33 ± 2.08% of ascorbic acid (Fig 1). The 40µg/ml phenolics from each solvent extract was used for O-phenanthrolin assay. The Acetone extract showed highest iron chelating potential 66.66 ± 2.4%, and remaining all solvent extracts showed approximately equivalent scavenging

potential 40.30 ± 4.08% of ascorbic acid (Fig. 2). Similarly, the 100 µg/ml phenolics from each solvent extract were used for reducing power assay. The Aqueous, Methanol, Ethanol and Acetone extracts showed almost similar scavenging potential and Ethyl acetate extract showed lowest scavenging potential 28 ± 3.5% (Fig. 3).

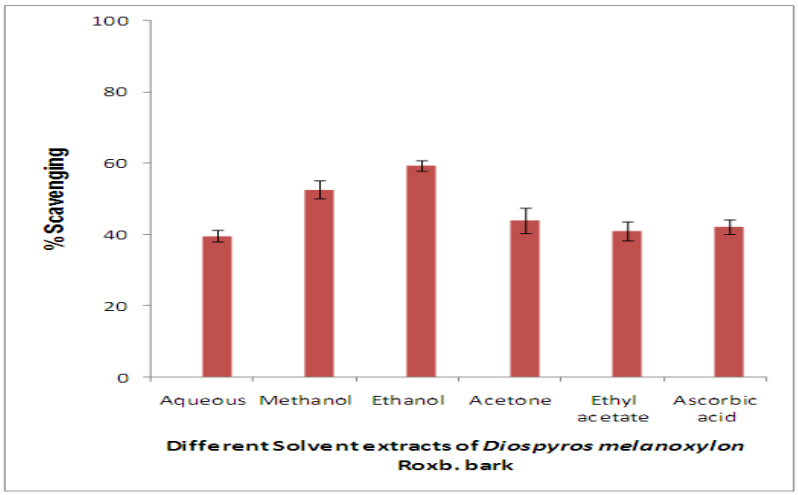

Fig1.DPPH radical scavenging activity by 100 µg/ml phenolics of *Diospyros melanoxylon* Roxb. bark.

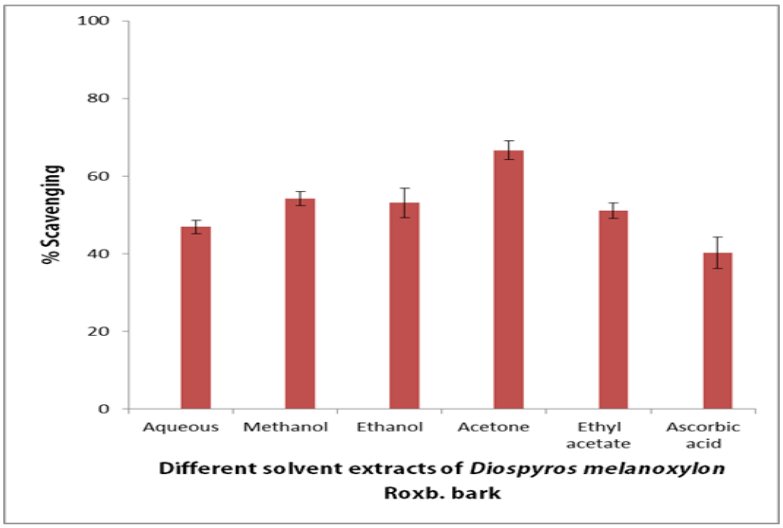

Fig 2. O- phenanthroline assay by 40 µg/ml phenolics of *Diospyros melanoxylon* Roxb. bark.

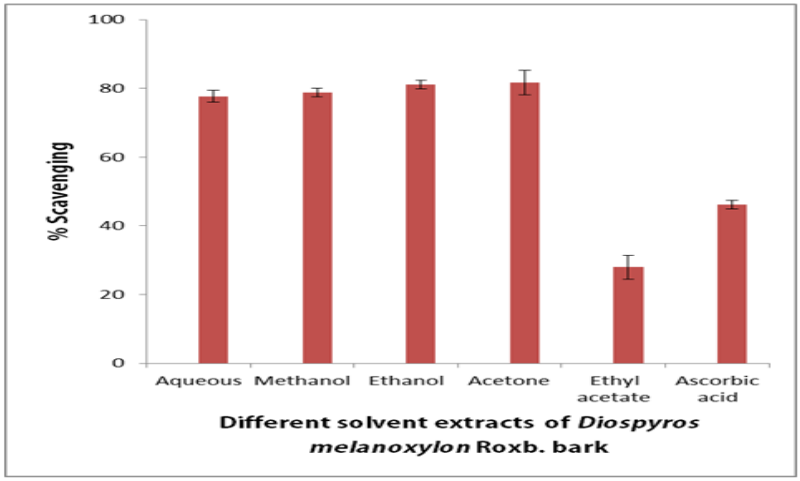

Fig 3. Reducing power assay by 100 µg/ml phenolics of *Diospyros melanoxylon* Roxb. bark.

Our results indicate that Ethanol is a better solvent for extraction of free radical scavenging agents from bark of *Diospyros melanoxylon* Roxb. Also Acetone is a better solvent for extraction of iron scavenging agents from bark.

CONCLUSION

In the conclusion, 1) The Methanol is better solvent for total phenolics extraction. 2) Different solvent extracts of *Diospyros melanoxylon* Roxb. bark exhibits anti-oxidative potential. 3) The anti-oxidative potential might be due to chelating property of phenols.

The present study suggests that the *Diospyros melanoxylon* Roxb.bark.is a natural source of antioxidants and could be used as therapeutic agent for aging associated disorders.

REFERENCES

[1] Halliwell, B. and J. M. C Gutteridge. 1998. Free radicals in biology and medicine, London: Oxford University Press.

[2] Mantle, D., Eddeb, F. and A. T. Pickering. 2000. Comparison of relative antioxidant activities of British medicinal plant species in vitro, *J. Ethno. pharmacol.* 72: 47–51.

[3] Valentao, P., Fernandes, E., Carvalho, F., Andrade, P., B., Seabra, R., M., and M. Bastos. 2002. Antioxidative properties of cardoon (*Cynara cardunculus L.*) infusion against superoxide radical, hydroxyl radical and hypochlorous acid, *J. agric. Food Chem.* 50: 4989–4993.

[4] Kinsella, J. E., Frankel, E., German,B., and J. Kanner. 1993. Possible mechanisms for the protective role of antioxidants in wine and plant foods, *Food Technol.* 47: 85–89.

[5] Terao, J., and M., K. Piskula. 1997. Flavonoids as inhibitors of lipid peroxidation in membranes, In C. A. Rice-Evans & L. Packer (Eds.). *Flavonoids in health and disease* 277–295.

[6] Branen, A. L. 1986**.** Toxicology and Biochemistry of Butylated Hydroxyanisole and ButylatedHydroxytoluene*, J. Am. Oil Chem. Soc. 52*: 59-63.

[7] Grice, H. C. 1986**.** Safety Evaluation of Bbutylated Hydroxytoluene (BHT) in the Liver, Lung and Gastrointestinal Tract, *Food Chem. Toxicol.* 24: 1127–1130.

[8] Wichi, H. P. 1988. Enhanced Tumor Development by Butylated Hydroxyanisole (BHA) from thePerspective of Effect on Forestomach and Oesophageal Squamous Epithelium, *Food Chem. Toxicol.* 26: 717–723.

[9] S. K. Rath, N. Mohapatra, D. Dubey, S. K. Panda, H. N. Thatoi and S. K. Dutta. 2009. Antimicrobial activity of *Diospyros melanoxylon* bark from Similipal Biosphere Reserve, Orissa, India. *African J. Biotechnol.* 8 (9):1924-1928.

[10] S. K. Panda, D. Dubey and S. K. Dutta. 2010. Anticandidal activity of *Diospyros melanoxylon* Robx.Bark from Similipal Biosphere Reserve,Orissa, India*. Int. J. Green Pharm*. (4):102-107.

[11] Chavan, U. D., Shahidi, F. and Naczk. 2001. Extraction of condensed tannins from beach pea (Lathyrus maritimus L.) as affected by different solvents. *Food Chem.* 75:509–512.

[12] Jerneja J., V. Robert, Š. Franci. 2009. Extraction of phenolic compounds from green walnut fruits in different solvents. *Acta agric. Slov. 93* – 1.

[13] J. A. Vinson, X. Su, L. Zubik, and P. Bose. 2001. Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chem.* 49 (11) : 5315–5321.

[14] Yamaguchi, T., Takamura, H., Matoba, T., and Terao J. 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl , *Biosci. Biotechnol Biochem.* 62: 1201-1204.

[15] Sreejayan, N., and M. N. A. Rao. 1996. Oxygen free radical scavenging by curcuminoids, *Arzneim-Froschun/Drug Res*. 2:169-171.

[16] John, D. 1984. One husndred useful new drug of the kani Tribes of Trivandrum Forest Division, Kerala, India. *Int. J. Crude Res.* 22(1): 17-39.

[17] Yen, G.C., Duh, P.D. 1994. Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free Radicaland Active Oxygen Species, *J. Agric. Food Chem.* 42: 629-632.

[18] Rice-Evans, C. A., Miller, N. J., Bollwell, P. G., Bramley, P. M., and J, B. Pridham.1995. *Free Radical Res*. 22**:** 375.

[19] Duraipandiyan, V., Ayyanar, M., S. Ignacimuthu. 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India, *BMC Complementary Altern. Med.* 6: 35-51.