IARJSET

ISSN (Online) 2393-8021
ISSN (Print) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology
Vol. 3, Issue 3, March 2016

Foliar nutraceutical and antioxidant property of Diospyros lanceifolia Roxb. (Ebenaceae) – An important medicinal plant of Assam, India

Dipjyoti Kalita[1], N. Devi[2] and D. Baishya[3]

Department of Botany, Gauhati University, Guwahati[1,2]

Department of Bioengineering, GUIST[3]

Abstract: The genus Diospyros L. belongs to the family Ebenaceae is commonly known as "Persimmon". It is native to China and America but also found in almost every tropical parts of the world. The plant is attributed with medicinal properties like anti-fertility, anti-tumor, anti-malarial, anti-diarrheal, antimicrobial, anti-inflammatory activities etc.. The present study was designed to evaluate the nutraceutical and antioxidant activity of the leaves of Diospyros lanceifolia Roxb.. The antioxidant activity was evaluated by using 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radicals. The IC_{50} value of methanolic extract of D. lanceifolia was found to be 76.74 ± 1.9 µg/ml. The study has revealed that the leaves can be used as therapeutic agent as they exert several beneficial effects by virtue of their antioxidant activity.

Keywords: D. lanceifolia Roxb., Antioxidant activity, Total Phenolic compound, Ascorbic acid, Gallic acid, DPPH.

I. INTRODUCTION

Medicinal plants based traditional systems of medicines are playing important role in providing health care to large section of world's population, especially in developing countries. Interest in them and utilization of herbal products produced based on them is increasing in developed countries also [1]. The natural metabolic processes continuously goes on inside human body along with oxygen utilization. During the process of oxygen utilization in normal physiological and metabolic processes, a portion of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals etc. These free radicals and reactive oxygen metabolites can react with proteins, nucleic acids and lipids, causing changes in genetic material and inactivation of enzymes that have been implicated in the etiology of several degenerative disorders including cancer, diabetes, rheumatoid arthritis, atherosclerosis, liver cirrhosis, Alzheimer's disease and other neurodegenerative disorders associated with ageing [2]. Natural antioxidants such as vitamin C, E, carotenoids, phenolic compounds etc. are frequently present in herbs and spices which are responsible for inhibiting or preventing the deleterious consequences of oxidative stress exerted by the reactive oxygen species (ROS) and are much more safer and cheaper than the commercially available antioxidant products. The antioxidant property is strictly correlated with the activity of phenolic compounds. Since the naturally available antioxidants are more beneficial to mankind, so it may easily develop a great future prospect in the field of medicine and therapeutic drugs.

Diospyros L. of Ebenaceae family is characterized by tree or shrubby habit with glabrous branches. Leaves are usually alternate or sometimes sub-opposite or opposite, subsessile, simple, entire, exstipulate, narrow elliptic or lanceolate to oblong-acuminate or obtuse, glabrous or pubescent, often anisophyllous. Inflorescence axillary, cymose, fasciculate, pseudo-racemose, bracteates. Flowers usually unisexual rarely hermaphroditic, regular, 3-7 merous, dioecious very rarely polygamous. From old days, different *Diospyros* sp are known for their medicinal uses. In many traditional medicinal systems of the world, a number of *Diospyros* plants are used as medicinal agents against various diseases. All parts of these plants are used for medicinal purposes such as the leaves used for lumbago, fruits are carminative, astringent, and cure biliousness, the seed are sedative and the bark is bitter, astringent and febrifuge[3],[4].

Diospyros species are a rich source of biologically active compounds and almost all parts of plants in this genus have been used as traditional medicine [3] [5]. Plants of this genus are well documented, and are reported to contain naphthoquinones, including 7-methyljuglone, diospyrin, isodisopyrin and triterpenes of the lupine series [6]. The latter have been found to exhibit ichthyotoxic, antimicrobial and antitumor activities [7] [8]. Naphthoquinones produced by this genus are usually in the form of dimmers [8]. Other biologically active compounds that have been reported from *Diospyros* species are coumarin, flavonoids and other phenolic compounds[3] [5]. Thus, *Diospyros* sp in traditional medicinal medicinal system of the world are used as (a) woman's medicine, for insomnia and hiccough, (b) for internal hemorrhage and bedwetting in children (c) anti-hypertensive (d) dysphonia (e) vermicide and vermifuge (f) sedative (g) antifebrile (h) promotes secretions (i) astringent and (j) antifungal and antibacterial [9] [10]. In Indian medicinal system also various *Diospyros* sp are used in folk medicines and constitute a part in formulation of drugs in Ayurvedic system [11] [12] [13] [14] [15].
D. lanceifolia occur throughout the tropical region of the world from India and Sri Lanka in the northwest through

IARJSET
ISSN (Online) 2393-8021
ISSN (Print) 2394-1588
International Advanced Research Journal in Science, Engineering and Technology
Vol. 3, Issue 3, March 2016

SE Asia to Sumatra, the Lesser Sunda Islands and Sulawesi [15] [16]. The bark, leaves and fruits of this plant is used as fish poison frequently by the tribal people whereas it has a high medicinal property to cure various teeth problem and skin disease also [17] [18] [19].

The present paper throws light on the evaluation of foliar nutraceutical and antioxidant activities of *D. lanceifolia* Roxb.

II. METHODOLOGY

1) Collection and preparation of plant materials:

Flowering twigs of *D. lanceifolia* were collected from various localities of Kamrup district of Assam. Voucher specimens were processed following standard herbarium techniques [20] and were identified with the help of relevant literatures [21] [22] and previously identified specimens at GUBH, ASSAM, CAL and also with images of herbarium specimens of online databases of various herbaria like K, JSTOR and EOL .

The collected samples were washed thoroughly, sliced and oven dried at 60^0C until they were completely dried and get constant weight. The dried slices were then powdered and kept at 4^0C for further analysis. The plant powder was used directly for the preparation of the crude extract in different solvents as per necessity of the experiment. The final filtrates were collected and dried under room temperature. Separate Soxhlet extraction was also done for 4-6 hours for the same experiment.

2) Total Phenol Content Estimation:

The total phenol content was determined by the Folin-Ciocalteau's method. For this purpose 1 gram each of the leaf samples were ground with a pestle and mortar in 10 ml of 80% ethanol. The homogenates were centrifuged at 10000 rpm for 20 minutes and then the supernatant was evaporated to dryness. The residue of each extracted sample was dissolved in distilled water so as to make final concentration of the extract 1mg/ml. 200 µl of each of these plant extract were taken and volume made up to 2 ml. 0.3 ml of Folin - Ciocalteau reagent was added. After 5 minutes, 0.8 ml of 20% Na_2CO_3 was added and the final volume was made 5 ml. Absorbance was taken by UV-Vis Spectrophotometer at 765 nm after 30 minutes incubation. The amount of phenol content was determined using Gallic acid as standard. Results were expressed as µg/mg (Gallic acid equivalent/dry weight).

3) Total Ascorbic Acid Content Estimation:

The amount of ascorbic acid present in the samples was calculated by extracting the sample in 4% oxalic acid and titrating the extract against the 2, 6-dichloro phenol indophenol dye until the end point where pink colour appears that persist for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid present in the samples. Standard ascorbic acid solution is used as the reference and the calculation is done by the following formula:

$$\textbf{Ascorbic acid (mg/100g)} = \frac{0.5 \times v1 \text{ ml} \times 100 \text{ml}}{v2 \text{ ml} \times 5\text{ml} \times S} \times 100$$

Where, v1 = volume of oxalic acid
v2 = volume of sample
S = sample

4) Antioxidant Activity Estimation:

The antioxidant activities of the plant extracts along with standard were assessed on the basis of the radical scavenging effect of stable DPPH. A solution of DPPH of concentration 0.2 mM was prepared in 70% methanol and kept overnight. Stock solution (1 mg/ml) of the extract was prepared in 70% methanol. Various concentrations of the extracts *viz.*10, 20, 50,100,150,200,300,400 and 500 µl were taken in different test tubes and the volume was made up to 1000 µl. 1 ml DPPH was added to each solution and kept at dark for 30 minutes. Ascorbic acid and Gallic acid were taken as standards. Optical density of these samples was measured at 517 nm along with blank where 1 ml methanol with 1 ml DPPH solution was taken. The activities of the samples are measured in terms of percent inhibition (IC50) and calculated by the following formulae:

$$\textbf{Percent inhibition, } \textbf{IC}_{50} \textbf{ (\%) of DPPH} = \frac{A-B}{A} \times 100$$

Where, A= optical density of the blank
B= optical density of the sample

The quantitative phytochemical analysis of the plant extracts along with standard were assessed with the help of UV-VIS spectrophotometer (540nm) and the comparison between the various concentrations were calculated by the following formula –

$$\textbf{\% transmittance} = \frac{\text{amount of light transmitted through the solution}}{\text{amount of light transmitted through the solvent}} \times 100$$

$$\textbf{Absorbance (A)} = -\log\left(\frac{\% \text{ transmittence}}{100}\right)$$

5)Statistical Analysis:

The data were subjected to statistical analysis. All the asseys were recorded in triplicates and the values were expressed as mean ± S.D. IC_{50} value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis.

III. FINDINGS

Experiments on *D. lanceifolia* showed that the ascorbic acid concentration was highest i.e. 76.85 ± 0.6 mg/100gm among all other triplicates while the inhibition concentration (IC_{50}) recorded was 76.74± 1.9 µg /ml and Gallic acid concentration recorded was 31.13± 0.9.

Table 1: Antioxidant property analysis of the leaves of *Diospyros* L.

Leaf extract	Ascorbic acid (mg/100gm)	Gallic acid (µg GAE/ mg)	Inhibition concentration (IC_{50})(µg /ml)
D. lanceifolia	76.85 ± 0.6	31.13 ± 0.9	76.74 ± 1.9

Thus, the antioxidant property analysis showed that the leaf of *D. lanceifolia* is very promising as a medicne. Also, the total phenolic content and ascorbic acid content of the leaf sample of *D. lanceifolia* were found to be of significantly high concentration which will really be

helpful in drug discovery and isolation. The graphical representation of the IC_{50} value of *D. lanceifolia* is plotted in fig 1 & 2.

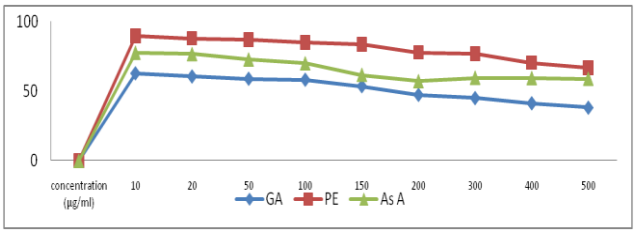

Figure 1: DPPH activity of *D. lanceifolia* Roxb.

IV. CONCLUSION

The most common antioxidants present in herbs and fruits are Ascorbic Acid (vitamin C) and E, carotenoids, flavanoids etc. The selected plants contain a diverse number of phytochemicals which are experimentally proven [3], thus the present study unveiled the role of phenolic components as major source of antioxidant property in *D. lanceifolia*. The protection provided by medicinal plants against oxidative damage to body tissues has been attributed to the fact that these foods may provide an optimal mix of phytochemicals that could be isolated and used to develop new drug to control the human and animal infectious disease. Therefore more studies are recommended for the assessment of health benefits of *D. lanceifolia* leaf in molecular level.

ACKNOWLEDGEMENT

Authors are grateful to the Head, Department of Botany, Gauhati University and Department of Bioengineering, GUIST, Assam for providing facilities to carry out the work and to the Director, BSI, Shillong for allowing them to consult herbarium specimens and literature.

REFERENCES

[1] Ravishankar B. and Shukla V.J.2007. Indian Systems of Medicine: A Brief Profile. Afr J Tradit Complement Altern Med 4: 319–337.
[2] Divya N, Nagamani JE and Suma P.2012. Antioxidant and antihemolytic activities of Bombax ceiba pentandra spike and fruit extracts. Int J Pharm Pharm Sci 4: 311-315.
[3] Kalita D., Devi N. & Baishya D. 2016. Comparative Preliminary Foliar Phytochemical Screening of Diospyros malabarica (Desr.) Kostel and Diospyros lanceifolia Roxb.
[4] Watt, J.M. and M.G. Breyer-Brandwijk, 1932. The Medicinal and Poisonous Plants of South Africa E. and S. Livingstone: Edinburgh, pp: 92-95.
[5] Akak, C.M., Djama, C.M., Nkengfack, A.E., Tu, P-P., & Lei L-D. (2010). New coumarin glycosides from the leaves of Diospyros crassiflora (Hiern). Fitoterapia, 81, 873-877.
[6] Li, X.C., van der Bijl, P., & Wu, C.D. (1998). Binaphthalenone Glycosides from African Chewing Sticks, Diospyros lycioides. Journal of Natural Products. 16(6), 817-820.
[7] Chen, C.R., Cheng, C.W., Pan, M.H., Liao, Y.W., Tzeng, C.Y., & Chang, C.I. (2007). Lanostane-type triterpenoids from Diospyros discolor. Chemical and Pharmaceutical Bulletin, 55(6), 908-911
[8] Zakaria, M.N., Jeffreys, J.A.D., Waterman, P.G., & Zhong, S-M. (1984).
[9] Tezuka, M., C. Takahashi, M. Kuroyanagi, M. Satake, K. Yoshihira and S. Natori, 1973. Phytochemistry, (12): 175-183.
[10] Thomas, P.S., G. Karagianis and R. Brun, 2006. Phytochemistry, 67: 1950.
[11] Chopra, R.N.; Nayar, S.L. & Chopra, I.C. 1956. Glossary of Indian Medicinal Plants. CSIR, New Delhi, India. Pp. 98 – 99.
[12] Mallavadhani, U.V., Panda, A.K. & Rao, Y.R. 1998. Pharmacology and chemotaxonomy of Diospyros. Phytochemistry 49: 901 – 51.
[13] Bhushan P.; Dnyaneshwar W.; Pushpangadan P. & Narendra B. 2005. Ayurveda and traditional Chinese medicine: a comparative overview. Evid Based Complement Alt. Med. 2: 465 – 473.
[14] Laloo, R.C.; Kharlukhi, L.; Jeeva, S.; Mishra, B.P. 2006. Status of medicinal plants in the disturbed and the undisturbed sacred forests of Meghalaya, northeast India: population structure and regeneration efficacy of some important species. Curr. Sci. 90: 225 – 232.
[15] Kalita D & Devi N. 2015. Observations on the foliar architecture and micro-morphology of Diospyros lanceifolia Roxburgh (Ebenaceae). Pleione 9(2): 449-455.
[16] Gratiana, E.; Tenggara, N. & Jøker, D. 2004. Diospyros malabarica (Desr.) Kostel. Harum, F. & Schmidt, L. H. (eds.), Seed Leaflet, Forest & Landscape, Denmark. P. 105.
[17] Meyanungsang, 2010, Ph.D thesis "Documentation And Biological And Phytochemical Analysis Of Chungtia Medicinal Plants Of Nagaland, India", pp 68
[18] Jamir, T.T., Sharma, H.K. and Dolui, A.K., 1999. Folklore medicinal plants of Nagaland, India. Fitoterapia 70, 395-401.
[19] Wiart, C., 2006. Medicinal Plants of Asia and the Pacific. CRC Press, 2006.
[20] Jain, S.K. & Rao, R.R. 1977. A Handbook of Field and Herbarium Technique. Today & Tomorrow's Publication, New Delhi.
[21] Hiern, W.P. 1872. A Monograph of Ebenaceae. Transactions of the Cambridge Philosophical Society 12(1): 213 – 214.
[22] Francis, S.P. Ng. 2002. Ebenaceae. In Soepadmo, E.; Saw, L.G. & Chung, R.C.K. (eds.), Tree Flora of Sabah and Sarawak. Forest Research Institute Malayasia (FRIM), Kuala Lumpur, Malayasia. 4: 29 – 100.