Research Article

Antioxidant Activity of Bark of *Myrica nagi*

Rinu K Rana*[1], Rakesh K Patel[2]
1. Laureate Institute of Pharmacy, Kathog, Himachal Pradesh, India.
2. S.K. Patel College of Pharmaceutical Education & Research, Kherva, Gujarat, India.
***Corresponding author's E-mail:** rinupharma@gmail.com

Accepted on: 26-06-2014; **Finalized on:** 31-08-2014.

ABSTRACT

Myrica nagi Thunb. (Syn. Box myrtle) (Myricaceae) is a subtropical tree widely distributed throughout the mid-Himalayas. It has been traditionally used for the treatment of various disorders such as liver diseases, fever, asthma, anemia, chronic dysentery, ulcer and inflammation but it is not scientifically proven. The present communication deals with the antioxidant activity of the polar, non polar and methanolic extracts of *Myrica nagi* bark powder. Phenolic and Flavonoid content was observed highest in the polar extract of *M. nagi* and least in the non polar extract of *M. nagi*. 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect of the extracts was determined spectrophotometrically. The highest radical scavenging was observed in the polar extract of *M. nagi* with IC_{50} = 296.53µg/ml. All these findings reveal the antioxidant activity of polar extract of *M. nagi* bark may be because of presence of high amount of flavonoids and phenolic constituents. The results indicate that *M. nagi* can be utilized as natural antioxidant.

Keywords: *Myrica nagi*, Phenolics, Flavonoids, Anti-oxidants, DPPH.

INTRODUCTION

Oxygen is an indispensable part of human life. But it can seriously affect human well being through the formation of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide which play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, carcinoma and Parkinson's disease[1,2]. Therefore, antioxidants which protect the cells against the damaging effects of reactive oxygen species may have great relevance in the prevention and therapeutics of free radical mediated diseases. The antioxidant compounds from natural sources are capable of providing protection against free radicals. It is necessary to screen out the medicinal plants for their antioxidant potential[3,4].

Myrica nagi Thunb. (Syn. Box myrtle) (Myricaceae) is a sub-temperate evergreen tree widely distributed throughout the mid-Himalayas, Khasia Hills, Sylhet and southwards up to Singapore and in Malayan islands and in China and Japan[5,6]. It is a medium to large woody, evergreen, dioecious tree of 12 to 15 meters height and 92.5 cm trunk girth. The bark is curved, channeled or sometimes recurved pieces up to 2cm thick. Outer surface is scabrous and warty carrying occasional transverse cracks or longitudinal fissures and is brownish, mottled with dirty white patches. Inner surface is dull red, leather in texture and fracture is tough and short[7,8,9]. Traditionally, this tree is utilized a great remedy in anemia, asthma, bronchitis, cough, chronic dysentery, fever, liver complaints, nasal catarrh, piles, sores, throat complaints, tumors, ulcers, urinary discharges[10].

It contains Gallic acid, myricanone, Myricanol, Myricinol, Myricitrin, Myristicin, epigallocatechin 3-O-gallate, two prodelphinidin dimers [epigallocatechin(48)-epigallocatechin 3-O-gallate and 3-Ogalloyl epigallocatechin -(48)- epigallocatechin 3-O-gallate], and the hydrolysable tannin castalagin[11]. In Ayurveda it is described as detoxifier, pain killer and healing herb. The objective of the present study is to evaluate the in vitro antioxidant activity using DPPH model.

MATERIALS AND METHODS

Myrica nagi bark was procured from Sanjivani Aushadhalya, Bhavnagar, Gujarat and authenticated in Mehsana Urban Bank Institute of Biosciences, Ganpat University, Kherva.

Preparation of Extracts: The air dried *Myrica nagi* bark powder was passed through 60 # sieve. The final uniform powder was used for the extraction of active constituents of the plant material. 100 gm of *Myrica nagi* bark powder was taken and extracted in soxhlet apparatus with methanol. The extract was collected and evaporated to dryness. This extract was mixed with silica for making free flowing powder. This powder was further extracted successively with petroleum ether, benzene, chloroform, ethyl acetate and methanol. All the extracts were concentrated by vacuum distillation. The qualitative tests and TLC fingerprinting for phytoconstituents were performed. From the observation of phytoconstituent screening and TLC fingerprinting, non polar fractions were collected and combined. Similarly polar fractions were collected and combined. Total phenolics and total flavonoids of these fractions were performed and used for in vitro antioxidant activity.

Estimation of Total Phenolics

The amount of total phenolic content of plant extracts was determined according to Folin-Ciocalteu procedure with little modification. To 1ml of each extract

(100µg/ml), 1.5ml of Folin-Ciocalteau reagent (diluted two-fold) was added. The above mixtures were kept for 5min and then 4ml of 20% Na_2CO_3 was added and volume was made up to 25ml with methanol and mixtures were kept up to 30 min and absorbance of blue color was measured at 739nm using UV visible double beam Spectrophotometer (Shimadzu UV 1800)[12]**.** 1ml aliquots of 50, 75, 100, 125, 150, 175µg/ml methanolic gallic acid solutions were used as standard for calibration curve. The absorbances of sample mixtures were compared with Gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of total Flavonoids content

Total flavonoid content was measured by the aluminum chloride colorimetric assay[13]. An aliquot of 1 ml of each extract (1mg/ml) was mixed with 0.3 ml 5 % $NaNO_2$. After 5 min, 0.3 ml 10 % $AlCl_3$ was added. In this mixture, 2ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured spectrophotometrically at 510 nm using UV visible double beam Spectrophotometer (Shimadzu UV 1800). 1ml aliquots of 20, 40, 60, 80 and 100µg/ml methanolic Quercetin solutions were used as standard for calibration curve. The absorbances of sample mixtures were compared with Quercetin calibration curve. The total flavonoid content was expressed as Quercetin equivalents (QEg/100g dry weight of extract).

Antioxidant activity

The free radical scavenging activity of the polar extract (PE), non polar extract(NPE) and methanolic extract (ME) of *M. nagi* bark and Ascorbic acid as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl)[14]. Different solutions of 150,300.450,600 and 750 µg/ml concentration of each extract were prepared in DMSO. 2ml of DPPH solution was added to 0.6 ml solution of all extracts. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV visible double beam Spectrophotometer (Shimadzu UV 1800). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg /ml) that inhibits the formation of DPPH radicals by 50%.

RESULTS AND DISCUSSION

Total phenolics and total flavonoid content

The total phenolic content of the polar extract (PE), non polar extract (NPE) and methanolic extract (ME) of *M. nagi* bark were found to be 20.60 $g100g^{-1}$, 6.98 $g100g^{-1}$ and 14.28$g100g^{-1}$ respectively. The total flavonoid content of the polar extract (PE), non polar extract (NPE) and methanolic extract (ME) of *M. nagi* bark were found to be 5.18$g100g^{-1}$, 0.79$g100g^{-1}$ 3.41$g100g^{-1}$ respectively.

DPPH radical scavenging activity

DPPH radical scavenging activity of ascorbic acid is shown in Fig.no.1. DPPH radical scavenging activity of polar extract (PE), non polar extract (NPE) and methanolic extract (ME) of *M.nagi* bark is shown in Fig.no.2.

IC_{50} values

IC_{50} values of polar extract (PE), non polar extract (NPE) and methanolic extract (ME) of *M. nagi* bark and standard ascorbic acid for DPPH were found to be 296.53µg/ml, 1006.3µg/ml, 532.6µg/ml and 21.24µg/ml respectively. IC_{50} value of different extracts of *M. nagi* bark powder and standard ascorbic acid is shown in Fig.no.3. DPPH radical scavenging activity of different extracts *M. nagi* bark and standard is presented in the following order: ascorbic acid> polar extract >methanolic extract> non polar extract.

From the graph, it was observed that anti oxidant activity of polar extract of *M. nagi* possess higher degree of free radical scavenging property than all other fractions of *M.nagi*. It may be due to the presence of higher phenolic and flavonoids compounds like myricetin, myricanol, myricanone. But as compared to ascorbic acid, the IC_{50} value of all the fractions was very low.

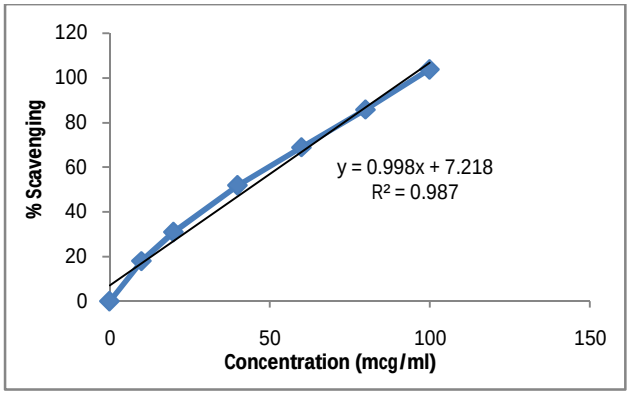

Figure 1: % scavenging effect of Ascorbic acid as a standard

The antioxidant activity of phenolics is mainly due to their redox properties which allow them to act as reducing agent, hydrogen donators and singlet oxygen quenchers[15]. Phenolics compounds are considered to be the major contributors to the antioxidant activity of vegetables, fruits or medicinal plants. Flavonoids are important secondary metabolites of plant modulating lipid peroxidation involved in atherogenesis, thrombosis

and carcinogenesis. It has been confirmed that pharmacological effects of flavonoids is correlating with their antioxidant activities[16].

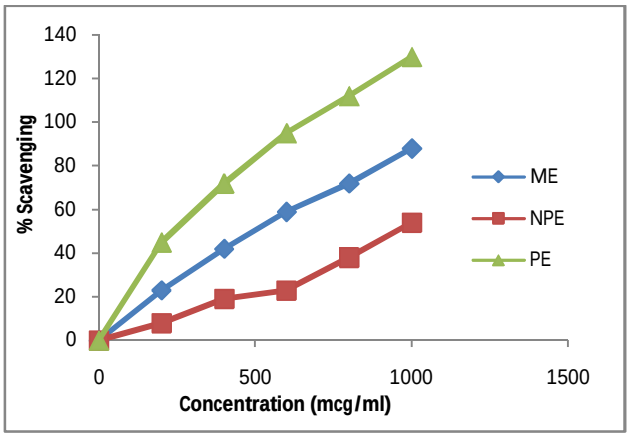

Figure 2: Comparison of % scavenging effect of Extracts

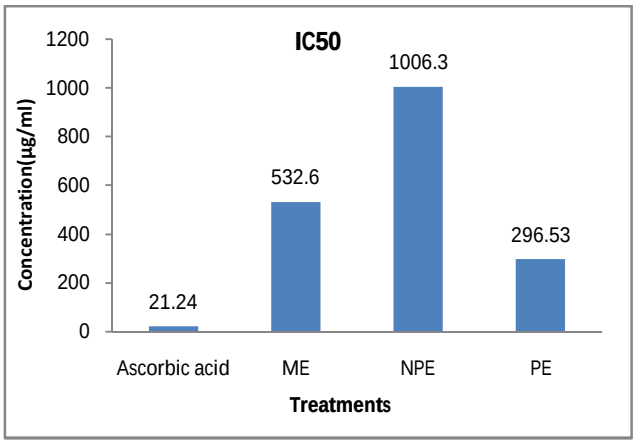

Figure 3: Comparison of IC_{50} value of different extracts of *M.nagi* bark powder with Ascorbic acid as a standard.

CONCLUSION

The present study indicates that this plant is a significant natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

REFERENCES

1. Yaushisakono, DPPH free radical scavenging activity of medicinal plant extract, Biochemistry and Biophysics, 136(1), 1978, 189-195.
2. Halliwell B, Gutteridge JMC, Role of free-radicals and catalitic metal ions in human disease: an overview, Methods Enzymol, 186, 1990, 1- 85.
3. Halliwell B, Antioxidant and human disease: a general introduction, Nat. Rev, 55, 1997, 44-52.
4. Patil AP, Patil VR, Evaluation of in vitro antioxidant activity of seeds of blue and while followed varieties of *Clitoria ternatea* Linn. Int. J. Pharm and Pharmaceut Sci., 3, 2011, 330-336.
5. Parmar C, Kaushal MK, *Myrica nagi*. In: Wild Fruits. Kalyani Publishers, New Delhi, India, 1982, 49–53.
6. Anonymous, Medicinal Plants of India, ICMR, New Delhi, vol. II, 1987, 304.
7. Kirtikar and Basu, Indian Medicinal Plants, New Delhi, Periodical Experts, Vol. II & III, 1975, 2350-2351.
8. Anonymous, The Wealth of India: Raw Materials, CSIR, New Delhi Vol. VI, 1962, 472.
9. Sarin YK, Principal crude herbal drugs of India, Bishen Singh Publication, Dehradun, India, 2008, 182-183.
10. Bhatt ID, Rawal RS, Dhar U, The availability, fruit yield and harvest of *Myrica esculenta* in Kumaun (West Himalaya), India, Mt Res Dev, 20, 2000, 146-153.
11. Sun D, Zhao Z, Wong H, Foo LY, Tannins and others Phenolics from *Myrica esculenta* bark, Phytochemistry, 27, 1988, 579-583.
12. Cetkovic G, Canadanovic J, Djilas S, Savatovic S, Mandic A, Tumbas V, Assessment of polyphenolic content and in vitro antiradical characteristics of apple pomace, Food Chemistry, 109, 2008, 340-347.
13. Marinova, D, Ribarova F, Atanassova M, Total phenolics and total flavonoids in Bulgarian fruits and vegetables, J. Univ. Chem. Technol. Metall., 40, 2005, 255–260.
14. Yaushisakono, DPPH free radical scavenging activity of medicinal plant extract, Biochemistry and Biophysics, 136(1), 1978, 189-195.
15. Chua MT, Tung TT, Chang ST, Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*, Bioresovr Technol., 99, 2008, 1918-1925.
16. Mbaebie BO, Edeoga HO, Afolayan AJ, Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq., Asian Pac J Trop Biomed., 2, 2012, 118-124.

Source of Support: Nil, **Conflict of Interest:** None.