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Original Article

Pharmacognostic Evaluation and Determination of Secondary Plant Metabolites by HPTLC and its Antioxidant Activity in *Myrica esculenta*

Prashant Kumar[1], Abhishek Gupta[2], Anita Singh[1]*

ABSTRACT

Introduction: *Myrica esculenta* Buch.-Ham. (Myricaceae) is commonly known as Box Berry, Kaiphal, and Katphala in Ayurveda. As per the Ayurvedic literature the palnt is used for variety of diseases and disorders. **Method:** The present study deals with the pharmacognostical standardization, HPTLC analysis and antioxidant activity of methanolict extracts of the leaves of *M. esculenta*. The plant showed high phenolic and flavonoid content. *In-vitro* antioxidant study of dried leaves of *Myrica esculanta* was performed using methanolic extract. **Results:** Antioxidant activity of *M. esculenta* methanolic extract showed the least IC_{50} value of 60 ± 1.15 µg/ml. Standard ascorbic acid showed an IC_{50} value of 2.03 ± 0.06 µg/ml. The calibration curve of Gallic acid showed r^2 of 0.949 and R_f of gallic acid was found to be 0.44 ± 0.006. Quantification of gallic acid in the samples of *leaves of M. esculenta* has been performed and the gallic acid was found to be 0.056%. **Conclusion:** The presence of gallic acid has not yet been reported and quantified in this species which may be utilized for the proper standardization of the drug. The present study showed new natural antioxidant that can replace the synthetic ones to be used in foods and cosmetics.

Key words: Antioxidant, DPPH, *Myrica Esculenta*, Gallic Acid, HPTLC.

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INTRODUCTION

Medicinal plants have been in use since ancient times by all cultures as medicines, as herbal remedies and are proved as economical with minor side effects. These attributes of a plant depend on the type of phyto-constituents present in it. Due to the high therapeutic values of the medicinal plants, during the last one decade, the efficacy and safety have been stressed out. *Myrica esculenta* Buch.-Ham. (Myricaceae) is commonly known as Box Berry, Kaiphal, and Katphala in Ayurveda. As per the Ayurvedic literature, stem bark of this plant is used in gulma (abdominal tumors), jvara (fever), arsa (piles), grahani (irregular bowel function), pandu roga (anemia), hrillasa (nausea), mukha roga (oral disorders), kasa (cough), svasa (dyspnea), agni-mandhya (indigestion), aruchi (anorexia),allergic disorders and kantharoga (ears, nose, and throat disorders).[1,2] It is a medium sized tree, 3-15 m high found in the subtropical or outer Himalaya. The medicinal properties of *M. Esculenta* are well known as per ethnobotanical surveys.

The stem bark mainly contains gallic acid, castalagin[3] myricanol, myricanone,[4] epigallocatechin 3-O-gallate, epigallocatechin-(4β→8)-epigallocatechin 3-O-gallate, 3-O-galloylepigallocatechin-(4β→8)-epigallocatechin-3-O-gallate, proanthocyanidin, catechine, delphinidine chloride[5] quercetin, β-sitosterol, taraxerol and triterpene diol.[6]

The bark Possess anti-inflammatory[7] anti-helmintic[8] anti-microbial,[9,10] anxiolytic,[11] while the fruits are known for their anti-oxidant properties and Anti-microbial nature[12,13] The whole plant is an effective chemo preventive agent in skin.[14] Conventionally, foremost tribal communities of Uttarakhand exploit the bark revealing its ethonobotanical importance. It is well known to be effective in chronic cough, asthma and ear ache. When the bark powder is applied externally, it heals chronic and malignant ulcers, and also to intoxicate the fishes.[15] In Ayurveda, the plant is known to have antispasmodic, anti-inflammatory, analgesic properties.[16] The antibacterial activity of fruit and bark of the plant has been performed earlier.[17] Till now there are so many Synthetic antioxidants are commercially available but quite unsafe, and their toxicity is a distressing trouble.[18] The natural antioxidants, mainly phenolics and flavonoids, are safe, having prominent biological activity, so the current focuses on natural antioxidants from natural plants. Some researchers have worked earlier on standardization of stem bark by using HPTLC and developed a method for the identification of the biomarkers gal-

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lic acid, lupeol, oleanolic acid, and stigmasterol in the stem bark extract of *M. esculenta,*[19,20] but no work has been explored on the phytochemical screening and physicochemical evaluation on leaves of this plant till date. Therefore the present study deals with the pharmacognostical standardization, HPTLC analysis and antioxidant activity of methanolic extract of the leaves of *M. esculenta*.

MATERIALS AND METHODS

Chemicals and Reagents

1,1Diphenyl -2-picrylhydrazyle (DPPH) and Gallic acid were obtained from Sigma Aldrich Co. All other chemicals used were of analytical grade.

Plant material

Collection of Plant: The plant specimen, i.e. dried leaves of *Myrica esculanta (Myricaceae)* were collected from nothern himalayan region of Garhwal Uttarakhand, India in the month of March 2015 from the height of 1685.53 Meters N 29.8688° N, 78.8383° E.. The plants were identified and authenticated in BSI, Allahabad, India. A voucher specimen has been submitted in the Institute's herbarium.

Preparation of Plant Extracts

The fresh plant material was collected, thoroughly washed with water to remove all debris and then shade dried. The dried material was powdered by using an electric grinder at 100 mesh sizes. Extraction was performed by soxhlation process in two steps. Firstly the powdered material was defatted under soxhlet assembly using 250 mL of 98% ether for 6 hours. This is followed by 9 hours soxhlation of defatted powder by using 250 mL of methanol as solvent. The final extracts were passed through Whatman No. 1 filter paper. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use. The crude extracts were obtained by dissolving a known amount of dry extract in 98% methanol to obtain a stock solution of 1000 µg/ml. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions (25, 50, 100, 125,150, 200, 250, 300 and 500 µg/ml).

Physicochemical and Phytochemical Studies

Physicochemical and Phytochemical studies, like extrasfive values, total ash, acid insoluble ash, total sugar, starch, phenols and flavonoids were calculated from the shade-dried and powdered (60 mesh) plant material.[21-24]

Antioxidant Activity (DPPH Free Radical Scavenging Activity)

The antioxidant activity of the plant extracts and standard was assessed on the basis of the radical scavenging effect of the stable DPPH free radical by the method previously described.22 The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 50 µg/ml. 0.002% DPPH solution in methanol was prepared. Then 2 ml of this solution was mixed with 2 ml of sample solutions (ranging from 25 µg/ml to 500 µg/ml) and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and the optical density was measured at 517 nm using a Shimadzu spectrophotometer against methanol. The blank used was 2 ml of methanol with 2 ml of DPPH solution (0.002%). The optical density was recorded and percentage of inhibition was calculated using the equation: % of inhibition of DPPH activity = (A–B) / A × 100; where A is optical density of the blank and B is optical density of the sample.[25]

HPTLC Studies

Air dried (45-55°C) powdered *Myrica esculanta (Myricaceae)* (2.0 g) in triplicate were extracted separately with 3 X 20 ml methanol. Extracts were concentrated under vacuum and redissolved in methanol, filtered and finally made up to 100 ml with methanol prior to HPTLC analysis.[26] Reagents used were from Merk (Germany) and standard gallic acid was procured from Sigma-Aldrich (Steinheim).

Chromatographic Conditions

Chromatography was performed on Merk HPTLC precoated silica gel 60GF254 (20 X 20 cm) plates. Methanolic solutions of samples and standard compound gallic acid of known concentrations were applied to the layers as 6 mm-wide bands positioned 15 mm from the bottom and 15 mm from side of the plate, using Camag Linomat V automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nl/s from the application syringe. These conditions were kept constant throughout the analysis of samples.

Detection and Quantification of Gallic acid

Following sample application, layers were developed in a Camag twin trough glass chamber which was pre-satarrested with a mobile phase of toluene: ethyl acetate: formic acid (5:5:1) till the proper separation of bands up to 8 cm height. After development, layers were dried with an air dryer and gallic acid was simultaneously quantified using Camag TLC scanner model 3 equipped with Camag Wincats IV software. Following scan conditions were applied: slit width, 5 mm x 0.45 mm; wavelength, 320 nm; and absorption-reflection mode. In order to prepare calibration curves, a stock solution of Gallic acid (1 mg/ml) was prepared and various volumes of the solution were analyzed through HPTLC, calibration curves of peak area vs. concentration were also prepared.

RESULTS

Phytochemical screening

Phytochemical screening for the hydro-alcoholic extract showed positive test for flavonoids, proteins, carbohydrates, glycosides, phenolic compounds and saponins, Table1.

Physicochemical Studies

Parameters such as moisture content, extractive values (Water, alcohol and ether soluble), total ash and acid insoluble ash values, total sugar, total starch, and total phenolics were determined, Figure 1.

Antioxidant activity

In-vitro antioxidant study of dried leaves of *Myrica esculanta* was performed using methanolic extract. In this study *M. esculenta* methanolic extract showed the least IC_{50} value of 60 ± 1.15 µg/ml. Standard ascorbic acid showed an IC_{50} value of 2.03 ± 0.06 µg/ml. The methanolic plant extract showed a promising antioxidant activity, Table 2.

HPTLC Studies

The calibration curve of Gallic acid showed r^2 of 0.949 and R_f of gallic acid was found to be 0.44 ± 0.006. Quantification of gallic acid in the samples of *leaves of M. esculenta* has been performed and the gallic acid was found to be 0.056%. A Densitogram and banding pattern obtained from extract shows gallic acid, Figure 2.

DISCUSSION AND CONCLUSION

The plant was initially evaluated for its phytochemical & physicochemical parameters. The plant showed high phenolic & flavonoid content, which was the basis for further evaluation of plant for its antioxidant activity

Table 1: Phytochemical Screening of *Myrica esculanta*

Metabolites	Hexane	Chloroform	Alcohol	Water
Alkaloids	-	+	+	-
Glycosides	-	-	+	+
Tannins	-	-	+	+
Saponins	-	-	+	+
Flavonoids	-	-	+	+
Protein	-	-	-	-
Carbohydrates	-	-	+	+

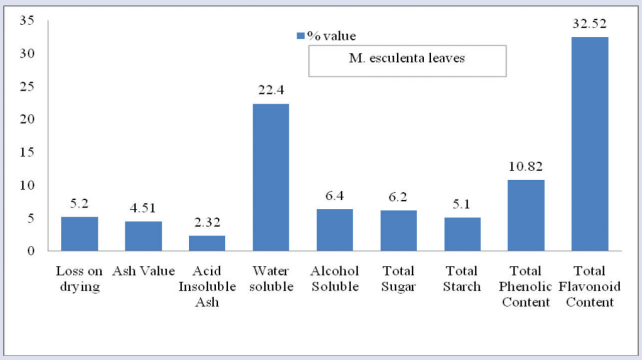

Figure 1: Percentage physicochemical values of *M. esculenta* leaves

Table 2: Antioxidant activity (DPPH Free Radical Scavenging Activity)

S.No.	Sample	DPPH assay IC_{50}(µg/mL)
1.	Ascorbic acid standard	2.03±0.06
2.	Methanolic plant extract	60±1.15

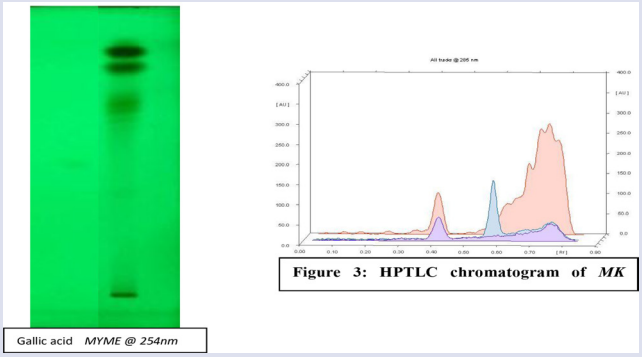

Figure 2: HPTLC Chromatogram of Plant Extracts showing different bands

and identification as well as quantification of its phenolic markers by HPTLC.

Ascorbic acid (standard compound) showed highest significant and potent antioxidant activity in DPPH free radical scavenging method. The methanolic extract showed a promising activity.

Presence of phenolic compounds in *M. esculenta* suggests that the antioxidant activity may be due to the polyphenolic content. Identification of all chemical constituents in extract those are responsible for antioxidant activity requires further investigation, the crude extracts merits further experiments *in-vivo*. Gallic acid, a potential antioxidant present in this species, has been studied through HPTLC. The presence of gallic acid has not yet been reported and quantified in this species which may be utilized for the proper standardization of the drug.

The present study showed new natural antioxidant that can replace the synthetic ones to be used in foods and cosmetics. Thus, the effective source of *M. esculenta* could be employed in all medicinal preparations to combat major problems associated with oxidative stress and related disorders.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATION USED

DPPH: 1,1Diphenyl -2-picrylhydrazyle; **HPTLC:** High Performance Thin Layer Chromatography; IC_{50}: percent inhibition at 50% concentration.

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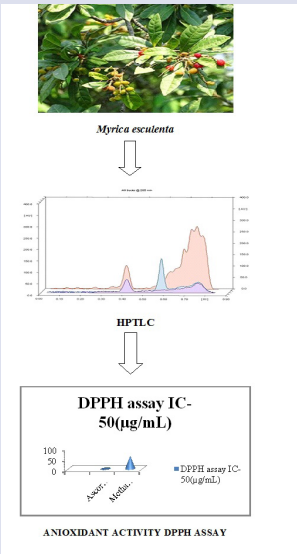
GRAPHICAL ABSTRACT



SUMMARY

- In present research work different phytochemical & physicochemical parameters were performed on *Myrica esculenta* leaves extract for its standardization.
- The quantification of gallic acid in the samples of *M. esculenta* leaves has been performed by HPTLC analysis and the gallic acid was found to be 0.056%
- Antioxidant activity of methanolic extract of *M. esculenta* was estimated by DPPH method and the IC_{50} value was found to be 60 ± 1.15 µg/ml.

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Prashant Kumar: Is working as research scholar in Department of Pharmaceutical Sciences, Kumaun University Bhimtal, Uttarkhand, India. Has sound experience in nanoparticulate drug delivery systems of medicinal plants.

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