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Antimicrobial and Anti Oxidant Activities of Bark Extract of *Myrica Esculenta*

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ABSTRACT

Medicinal plants constitute several bioactive compounds that show antimicrobial and antioxidant activities. Due to the extensive need of the new antimicrobial and antioxidant compounds of plant origin the study was conducted. The present paper deals with the qualitative antimicrobial and antioxidant assay of methanolic extracts of medicinal plant Myrica esculenta.

Keywords: *Myrica esculenta*, kaphal, bark, antimicrobial activity, antioxidant activity.

INTRODUCTION

Myrica esculenta commonly known as box berry (kaphal), is an important medicinal plant distributed all along outer Himalaya. The tree is moderate, and vertically wrinkled bark is brownish in color. The flowering start from last of the March and fruit setting begins in April and ripe fruits are made available from May to June. The bark of *Myrica esculenta* is said to posses many medicinal properties. It is stimulating, useful in catarrhal fever, cough, throat infection, asthma, urinary discharges, bronchitis, anemia, cholera, ulcers and is used in many other diseases. The fruits are used to heal ulcer [1]. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoids, bioflavonoids, bezophenones, xanthones as well as some metabolites such as tannins, saponins, cynates, oxalates and anthra-quinones [2]. Many kinds of plants are prevalent in this country and a large number of them have been used for antimicrobial assay [3]. However, Ayurvedic Sanhita mentions *Myrica esculenta* to be harmful to liver and spleen. In contrary to this, oil extracted from the flowers acts as a tonic, and has been used in earache, headache, diarrhea and paralysis [4], [5], [6]. Even the yellow color extracted from the bark is used as a medicinal colorant [7], [8]. Fruit constituents exhibit healing properties in case of different ulcers; it also finds application in retention of placenta and bone fracture [9]. The fruits of *Myrica nagi* are known for their ravishing taste and have been reported for reducing sugars, tannins and Vitamin C [10] [11]. Gallic acid, catechin, chlorogenic acid and *ρ*–coumaric acid in the ethanolic extract of the fruits [12]. The bark constitute gallic acid, myricanol, myricanone, epigallocatechin-3-*O*-gallate, two prodelphinidin dimmers [epigallocatechin-(4β→8)- epigallocatechin 3-*O*-gallate and 3- *O*-galloyl epigallocatechin-(4β→8)-epigallocatechin3-*O*-gallate], hydrolysable tannin castalagin. Prodelphinidin units with 2, 3-cis configuration having average of 5,000 mean molecular weight were found in the higher mean molecular weight fractions. A number of animal models have been

used for finding the pharmacological effects. These pharmacological activities prove the traditional utilization of the plant scientifically [13]. The ethanol extract of the stem bark possess potential anti-allergic activity when studied on mice. In the experiment allergic pleurisy and vascular permeability were induced by acetic acid in mice [14]. In rat paw edema ethyl acetate and aqueous extracts of bark showed anti-inflammatory activity. Challenged rats with histamine induced rat paw edema, showed 18-25 % inhibition with the ethyl acetate and aqueous extracts while the standard drug showed 27%. This study concluded that flavonoids and steroids might be responsible for the activity [15]. The fruits of the tree were studied [12], [16] for the antioxidant activities and it was found that they can be utilized as natural antioxidants. The study also revealed that phenolics and flavonoid contents were higher in *Myrica esculenta* fruits than *Myrica rubra*, another species of the same genus found in China. The same study proved that *Myrica esculenta* fruits possess strong antioxidant activity than *Myrica* rubra [12].

The aim of this study was to evaluate the antioxidant activity of the methanolic extract of the bark of *Myrica esculenta*. The antioxidant activity was assessed by studying radical scavenging ability of the methanol extract for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Methanol extract demonstrated significant inhibition of the DPPH free radical in the concentration range of 10-80 μg mL^{-1} Extract at the concentration of 80 μg mL^{-1} demonstrated 93.47% inhibition of the DPPH free radicals. These results indicate that the use of *Myrica esculenta* as folk medicine may be attributed to the presence of antioxidants. Isolation and characterization of active antioxidants compounds is in progress which can be used for treatment of oxidative stress related diseases.

MATERIALS AND METHODS

Materials and reagents used were of Analytical grade and were obtained from Ranchem and CDH, India. The media used for the growth of bacterial and fungal cultures and the reagent 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were procured from Hi-Media Pvt. Ltd., Bombay, India. Microbial Cultures (pure bacterial and fungal) were obtained from NCL, Pune, India and were revived for further use.

Collection of Plant Material and Extraction: Plant materials were collected from forest of Pauri-Garhwal (Uttarakhand) in the month of October and were dried under shade for 15 days. The plant was identified with the help of available literature and authenticated by Botanical Survey of India. The dried bark was crushed and extracted with methanol using soxhlet apparatus and concentrated in vacuum rotary evaporator.

Determination of antibacterial and antifungal activity (Agar Cup Diffusion Method): The agar cup diffusion method (Perez *et al.*, 1990) was modified. Soyabean casein digest agar (SCDA) was used for bacterial cultures. The culture medium was inoculated with the microorganism separately suspended in soyabean casein digest broth. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (ethanol). Standard antibiotic (Chloramphenicol, concentration 1mg mL^{-1}) was simultaneously used as positive control. The bacterial plates were then incubated at 37^{o}C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed.

Antioxidant assay: The radical-scavenging capacity of Methanolic extract of *Myrica esculenta* was determined using the DPPH radical method. 2 ml aliquot of test solutions was added to 2 ml of 2 x 10^{-4} mol L^{-1} ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was measured at 517 nm immediately. The decrease in absorbance was determined at 15, 30 and 60 min until the absorbance reached a steady state (after 60 min). All the tests were performed in triplicate and mean values calculated. The antioxidant activity was expressed according to the ability of an extract to scavenge DPPH free radicals and was determined using the following equation:

% Inhibition = $[1- (A_1-A_2)/A_0] \times 100$

Where A_0 is the absorbance of negative control (original DPPH solution without sample), A_1 is the absorbance of test sample (DPPH solution in presence of sample) and A_2 is the absorbance of sample without DPPH.

The IC_{50} value is the concentration (µg mL^{-1}) of extract/standard necessary to reduce the absorbance of DPPH by 50% compared to the negative control. The IC_{50} was determined by interpolation from linear regression analysis of the antioxidant activity (% Inhibition) against sample concentration (µg mL^{-1}) and the IC_{50} value decreases as a function of increasing antioxidant activity of samples. Results were also expressed as AEAC (ascorbic acid equivalent antioxidant capacity) in grams and calculated as follows:

AEAC (g) = IC_{50} (ascorbic acid) / IC_{50} (sample)

RESULTS AND DISCUSSION

The methanolic extract of plant, *Myrica esculenta* was tested for antimicrobial activity using agar well diffusion method at sample concentration 200µg 100 mL^{-1}. The methanol extract of plant showed strong and broad spectrum antibacterial activity against *Salmonella. aureus, S. typhimurium, S. epidermidis, E. salazakii, E. gergovios, B. cereus, K. pneumoniae, E. coli* but antibacterial activity against *S. entrica and S. flexineri* is negative. Antifungal activity against *C. albicans* was observed negative at 200 µg 100 mL^{-1}.

Table-1 Antibacterial activities of kaphal bark extract in the concentration of 200 µg 100 mL^{-1}

	Strain	Z I (mm) of PE	Z I (mm) of AB CH (standard)
1.	*Salmonella typhimurium*	7	33
2.	*E.coli*	19	26
3.	*Staphyloccocus epidermidis*	9	28
4.	*Enterobacter salazakii*	7	26
5.	*Enterobacter gergoviae*	8	28
6.	*Bacillus cereus*	8	27
7.	*Klebsiella pneumonia*	8	26
8.	*Salmonella entrica*	-ve	20
9.	*Shigella flexineri*	-ve	24
			Fucanozole (Standard)
10.	*Candida albicans*	-ve	28

Z I - Zone of Inhibition, AB - Antibiotic, CH - Chloramphenicol

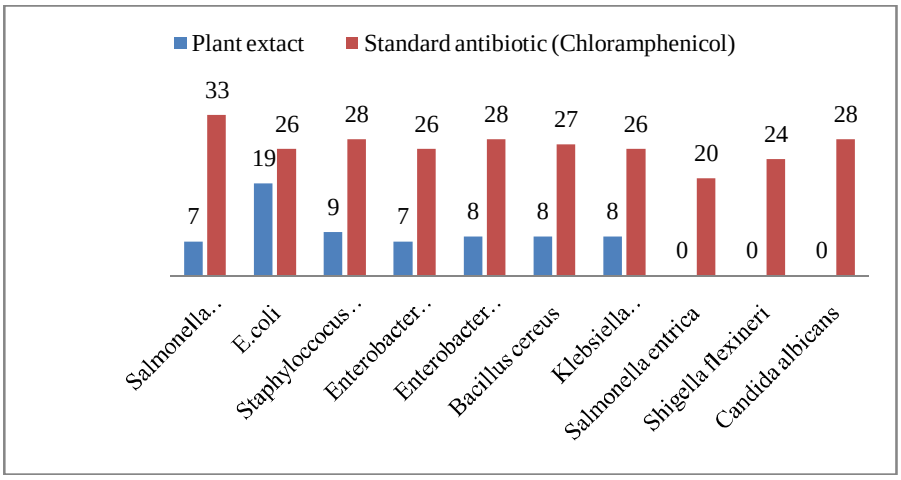
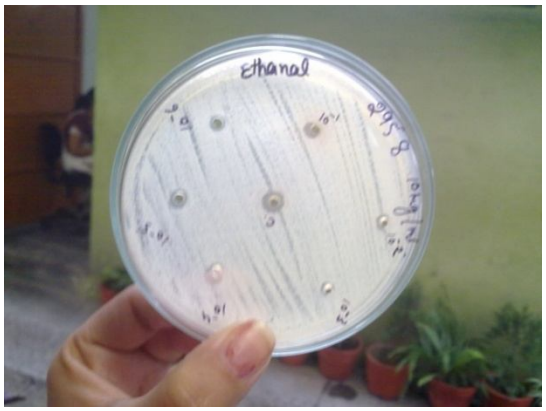


Fig.1. Graphical representation of zone inhibition on different strains

The above table depicts that in food samples *E coli* shows higher diameter of zone of inhibition.



Photographs of Zone of inhibition on various strains

It is well known that naturally occurring substances in plants have antioxidant activity. Among those substances, the flavonoids that are widely distributed in plants have the ability to scavenge free radicals, superoxide and hydroxyl radicals by single-electron transfer [35]. An antioxidant can exert its antioxidant activity through various mechanisms, including chelating ferrous iron, degrading peroxide, and scavenging free radicals. In this research work the antioxidant activity was assessed by the ability of the extract to scavenge the DPPH free radicals. The DPPH free radical is extensively used to evaluate the free-radical scavenging capacity of antioxidants. Scavenging of the DPPH free radical is related to the inhibition of lipid per oxidation and involves a hydrogen atom transfer process.

It was found that the Methanolic extract of bark of *Myrica esculenta* is effective at reducing the stable radical DPPH □ to the yellow-colored diphenylpicrylhydrazine, indicating that the extract is active in DPPH □ radical scavenging. The methanolic extract was found to be significant scavenging effects with increasing concentrations in the range of 10-80µg mL^{-1}. Extract at the concentration of 80 µg mL^{-1} demonstrated 93.47% inhibition of the DPPH free radicals (fig. 2). However, the scavenging effect of the extract was significantly higher than that of standard ascorbic acid. The IC_{50} value of the methanolic extract was found to be 3.14818 (fig. 3). A lower IC_{50} value indicating the higher DPPH □ radical

scavenging activity. In this assay, the antioxidant activity of methanolic extract on the DPPH □ radical may be attributed to a direct role in trapping free radicals by donating a hydrogen atom.

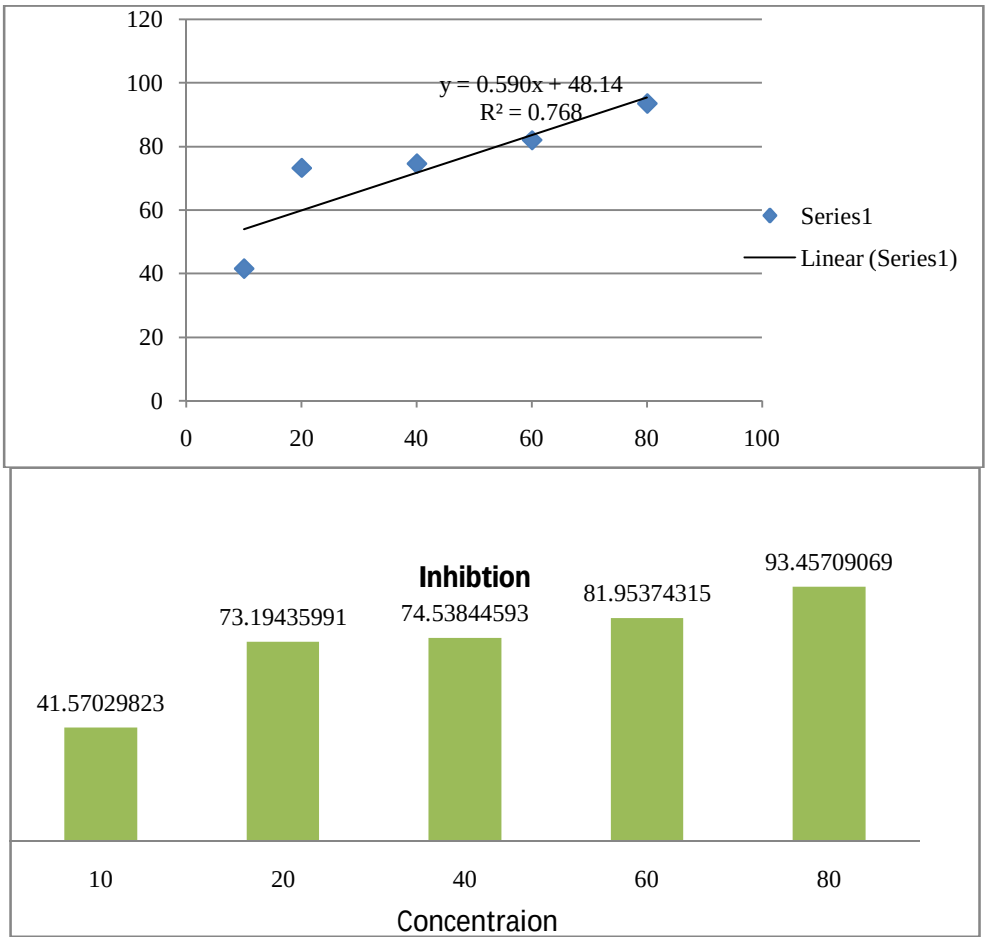


Fig 2. Graphical representation of inhibition of free radical in different concentration of solution.

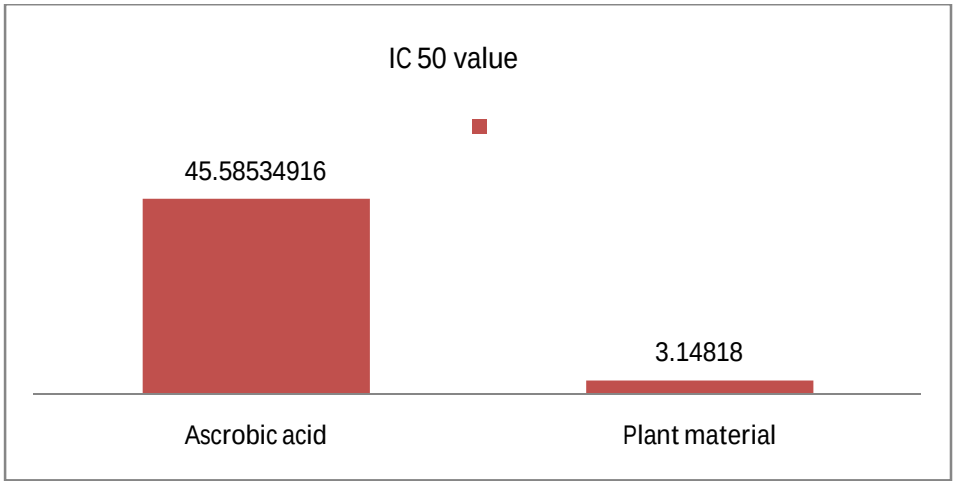


Fig.3. Graphical representation of IC_{50} of Standard (Ascorbic Acid) and Plant material

APPLICATIONS

This study was useful to evaluate the antioxidant activity of the methanolic extract of the bark of *Myrica esculenta*. The antioxidant activity was assessed by studying radical scavenging ability of the methanol extract for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical.

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