International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 10, Issue10-2023

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.10.011

# Phytochemical analysis, Anti-microbial and Anti-oxidant potential of *Myrica esculenta*

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## Abstract

Ayurveda is the traditional Indian system of medicine that focuses on the medicinal potential of plants. Plants are used as medicine to maintain health for ages and are also major natural sources of medicinal compounds in current pharmacopoeias. *Myrica esculenta* is a well-recognized species of plant in Ayurveda. In traditional systems of medicine different parts (flower, root, seeds, leaves, stem, bark and even a whole plant) of *Myrica esculenta* (known as "Kaphal" or ''Kathphala'' in the Indian subcontinent) are used to treat various ailments such as fever, diarrhea, asthma, inflammation, bronchitis, Anemia, bronchitis, ear, nose and throat disorders . It is an important indigenous plant native to India and usually distributed in the foothills track of mid Himachal Pradesh starting from Ravi eastwards to Assam as well as Arunachal Pradesh, Sikkim, Nagaland, Manipur, Mizoram in Khasi, Jaintia, Lushai hills or Meghalaya in between 900-2100 m above sea level. Some different varieties are also found in neighbouring countries like Nepal, China etc. and because of the richness of phytochemicals the plant is attributed to possess a number of therapeutic uses: antimicrobial, anti- cancerous anti-inflammatory, anti-diabetic, analgesic, anti-ulcer and anti-oxidant. Phytochemical studies of the different parts of plant have revealed the presence of various bioactive phytoconstituents such as phenolic compounds, alkaloids, glycosides, triterpenoids and volatile oils. Myricetin is the active constituent present in *Myrica esculenta* has found to be largely responsible for the therapeutic potentials.

**Keywords:** *Myrica esculenta*, Kaphal, Phytoconstituents, Therapeutic potentials, Myricetin, Anti-microbial and Anti-oxidant.

## 1. Introduction

India is a country rich in indigenous herbal resources. Usage of medicinal plant for the treatment has increased considering their minimal side effects and efficacy when compared to those of synthetic formulations. According to World Health Organization (WHO), around 2100, plant species have the potential of being used as medicine [Shukla, 2015]. Plant species of this genus is widely distributed in Japan, Taiwan, China, Western highlands of Cameroon, North America, Ethiopia, Nepal and India [Silva B.J.C., et.,al 2015]. The medicinal plants are rich sources

of secondary metabolities which are taxonomically and chemically diverse compounds. *Myrica esculenta* commonly known as "Kaphal" and "Boxberry" is a woody, evergreen dioecious tree belongs to family Myriacaceae [Haridson K, 1987].

*M. esculenta* is a moderate sized, evergreen, dioecious tree found mainly in subtropical temperate region [Paranjpe P *et.al* 2012]. It is considered as one of the most important source of medicine and drugs with many secondary metabolites recommended for the treatment of various ailments like anaemia, ulcer, diarrhoea, skin diseases, cough, pyrexia and ENT infections [Kabra *et.al*, 2019]. *Myrica* plants grow well in the soil depleted with nitrogen, mixed forests, agricultural and marginal lands [Bhatt *et al.* 2000]. *Myrica esculenta* also show several therapeutic effects including anti-diabetic, anti-hypertensive, anti-inflammatory, anti-cancerous and free radical scavenging properties [Wei *et al.*, 2011].

Myricanol-9-acetate is a novel compound identified in extract of plant that possesses anti-oxidant properties that plays major role in preventing the oxidative damage also they neutralize free radicals, thus controlling chronic diseases and are quite effective in the apoptosis of cancer cells [Ahmad, 2021]. This current study aimed at evaluating the phytochemical screening, in vitro anti- microbial activity and anti-oxidant potential of *Myrica esculenta.*

## 2. Materials and Methods

### Collection of Plant materials:

Fresh leaves of *Myrica esculenta* were collected from Solan and nearby area of Himachal Pradesh, India. Plant material was collected and washed to remove dust particles, soil and other unwanted substances**.** The leaves were shade dried at room temperature for 7-10 days until the water molecules evaporated and the plant was well dried for grinding. After drying, the plant leaves were grounded using a mechanical blender into fine powder. The powder was sieved to obtain uniformity and then packed in air tight containers for further studies. The extraction procedure depends upon the solubility of organic solvent. The solvent extract was prepared using fine powder of plant material. The extraction procedure was carried out with 10 grams of powder of leaves.

### *Ethanol, Methanol and plant extract*

About 10 grams of powder was taken and soaked in 100ml of solvent (Methanol, Ethanol, and Hexane) in a conical flask with occasional shaking for 36 hours. The liquid was strained and filtered through Whatmann filter paper and was stored to be analyzed for phytochemical, anti-microbial and anti- oxidant properties.

### Qualitative Phytochemical Analysis of (*Myrica esculenta* leaves extract)

The bioactive compounds present in (*Myrica esculenta*) leaves extract were analyzed qualitatively using ethanolic, methanolic and hexane, extracts of *Myrica esculenta* leaves were subjected to the qualitative phytochemical screening for the *esculenta* leaves. It was screened for the presence of alkaloids, saponins, phytosterols, flavonoids, proteins, phenol, cardiac glycosides and carbohydrates. The following phytochemical were tested as described:

### *Test for Alkaloids*

**Dragendroff's test:** 1 ml of plant extract was added in a test tube, adding 2 ml of Dragendroff's reagent. Appearance of orange red color indicates the presence of alkaloids.

### *Test for Flavonoids*

**Lead acetate test:** 1ml of plant extract was added in a test tube. Added few drops of lead acetate solution. Appearance of yellow precipitates indicates the presence of flavonoids.

### *Test for Saponins*

**Foam test:** 500 µl of plant extract was added in a test tube with 5 ml quantity of water. Appearance

of foam persisting for 10 minutes indicates the presence of Saponins.

### *Test for tannins*

**Gelatin test:** I ml of plant extract was treated with gelatin and sodium chloride solution. The presence of white precipitate showed that tannin was present.

### *Test for cardiac glycosides*

**Keller Killani test:** 1 ml of plant extract was added in a test tube with 1.5 ml of glacial acetic acid, 5 % of ferric chloride with few drops of concentrated sulphuric acid (along the side of test tube**).** Formation of brown ring between the layers indicates the presence of cardiac glycosides.

### *Test for phenols:*

**Ferric chloride test:** 1 ml of plant extract was treated with few drops of ferric chloride solution. Formation of bluish- black color indicates the presence of phenol.

### *Test for Phytosterols*

**Salkowski's test:** The 1 ml plant extract was treated with 2 ml of chloroform in a test tube followed by addition of 3 ml of conc. sulphuric acid from the side of test tubes. Appearance of reddish brown color in chloroform layer indicates the presence of Phytosterols.

### *Test for resins*

**Acetone water test:** 1 ml of plant extract was treated with 1 ml of acetone and distilled water. The mixture was shaken and turbidity indicates the presence of resins.

### *Test for Triterpenes*

**Copper acetate test:** 1ml of plant extract was treated with 3-4 drops of copper acetate. Appearance of emerald green color indicates the presence of Triterpenes.

### *Test for fixed oils and fats*

**Stain test:** 200 µl of plant extract was spread on a filter paper along with few drops of ether. Formation of oily stain indicates the presence of fats.

### *Test for proteins*

**Xanthoproteic test:** 1ml of plant extract was treated with few drops of nitric acid. Formation of yellow color indicates the presence of proteins

**Ninhydrin test:** 1 ml of plant extract was treated with Ninhydrin reagent. Appearance of blue color indicates the presence of proteins.

### *Test for Carbohydrates*

**Fehling test:** 1 ml of Fehling's sol A and 1ml of Fehling sol B was added to 1 ml of extract separately and heated on a boiling water bath for 5 min and observed the brick red color

**Seliwanoff's test**: 3 ml of Seliwanoff's 's reagent was added to 1 ml of plant extract and heated for 1 min in a water bath Presence of rose red color indicates the presence of carbohydrates.

## Microbial cultures and growth conditions

The microbial cultures included multi-drug resistant isolates of *E.coli, Staphylococcus aureus, Klebsilla pneumonia, Enterococcus faecalis*. The cultures of bacteria were maintained on Nutrient broth (1.3gm/100ml), and Nutrient agar (2.8/ 100ml) and antimicrobial activity was checked on Muller-Hinton Agar (3.9g/100ml).

## Anti-bacterial activity assay

To determine the anti-bacterial activities of *Myrica esculenta* in methanol and ethanol, Agar well diffusion method was employed. About 3.9 g of Muller Hinton Agar was dissolved in 100 ml of distilled water and was autoclaved at 121˚C. Inoculum containing 100 ml of each bacterial culture to be tested was spread on Muller Hinton agar plates with sterile L- shaped spreader.

Subsequently, wells of 8 mm diameter were punched aseptically with a tip into the agar medium, and a volume of 100 µl of antimicrobial agent or extract solution, antibiotic and solvent at desired concentrations was introduced into the well and allowed to diffuse at room temperature for 30 minutes. The plates were then incubated in the upright position at 37˚C for 24 hr. Wells containing solvents and distilled water served as a negative controls while standard antibiotic ciprofloxacin served as a positive control. After incubation at respective temperatures, the plates were observed for zones of growth inhibition.

### Antioxidant analysis:

The free radical scavenging ability of extracts against DPPH radical was evaluated. About (0.004%) of DPPH solution was prepared in methanol. Ascorbic acid was used as a reference standard and dissolved in distilled water.

Different dilutions of the sample (20µl, 40µl, 60µl, 80µl, 100 µl) were taken in five test tubes respectively. With the same solvent, the final volume of each test tube was made up to 3 ml. 1ml of freshly prepared DPPH solution was added to each test tube. The reaction was incubated for 30 minutes in dark and the absorbance was recorded at 517 nm. A blank sample containing only methanol was used to zero the spectrophotometer. The respective optical density of DPPH on the addition of test sample in relation to the control was used to calculate the antioxidant activity, as % inhibition of DPPH radical.

## 3. Results and Discussion

Phytochemical screening is used to detect the various bioactive compounds and thus may lead to its further isolation, purification, and characterization. The present study carried out revealed the presence of medicinal active constituents. The phytochemical active compounds of *Myrica esculenta* were qualitatively analyzed for leaf extract and the results are presented in Table1. The phytochemical analysis revealed the presence of most bioactive compound in alcoholic extract of leaves of *Myrica esculenta* plant. The hexane leaf extract of *Myrica esculenta* was analyzed for phytochemical screening and most of the vital secondary metabolites were absent except for the reducing sugars and fats due to its non polar nature.

Ethanolic leaf extract of *Myrica esculenta* showed the presence of phytosterols, cardiac glycosides, carbohydrates, flavonoids, alkaloids, steroids, tannin and phenol. Foam was not formed that indicate the absence of saponins. Turbidity is the significant property to identify the presence of resin but in our study no turbidity was found in acetone-water solution that indicate the absence of resin in ethanolic leaf extract. Formation of emerald green color indicate the presence of triterpenes that was found to be dark green that indicates the absence of triterpenes.

Further phytochemical investigation was done in the methanolic extract and the results in comparison to ethanolic leaf extract was found to be more accurate and clear. Methanolic leaf extract showed the presence of triterpenoids which were absent in the ethanolic leaf extract and foam formation was also observed other than that carbohydrates, tannins, alkaloids, cardiac glycosides, phenols, flavonoids are also present.

Although protein and resins was found absent in either of the leaf extracts. Yellow precipitates indicates the presence of flavonoids. Orange red color indicates the presence of alkaloids. Bluish black color indicates the presence of positive test of phenol. Reducing sugars were also present in the extract and appearance of red precipitates in the solution proves its presence. White precipitates indicates the presence of tannins and no stain formation was observed in case of methanolic leaf extract which indicates the absence of fats and oils.

**Table 1: Phytochemical screening of *Myrica esculenta* leaves extract**

| Phytochemicals | Hexane extract | Ethanolic extract | Methanolic extract |
|---|---|---|---|
| Alkaloids (Dragendroff's test ) | - | + | + |
| Flavonoids (Lead acetate test) | - | + | + |
| Triterpenes (Copper-acetate test) | – | + | + |
| Tannins (Gelatin test | – | + | + |
| Phenols (Ferric chloride test) | – | + | + |
| Phytosterols (Salkowski's test) | – | + | + |
| Proteins (Ninhydrin test) (Xanthoproteic test) | – | – | – |
| Resins (Acetone water test) | - | – | – |
| Triterpenes (Copper acetate test) | - | - | + |
| Fixed oils and fats (Stain test) | + | – | - |
| Carbohydrates (Fehling test ) (Seliwanoff's 's test) | + | + | + |

(+) Represent the presence of phytochemicals and (-) represents the absence.

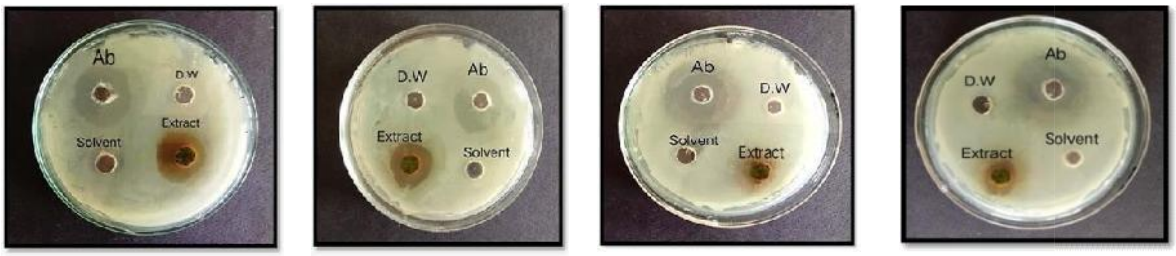
### *Screening of Anti-bacterial activity of Myrica esculenta leaf extract*

Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. The solvent extract of *Myrica esculenta* showed effective antimicrobial activity against the test pathogens. The antimicrobial activity of Ethanolic and methanolic solvent extracts of *Myrica esculenta* leaf against human pathogenic bacteria that including *E.coli, Klebsiella pneumonia, Enterococcus faecalis and S.aureus* was tested. The anti-bacterial effect was determined by agar well diffusion method against gram positive and gram negative bacteria. The inhibitory effect was observed in terms of diameter of zone formed around each well caused by diffusion of antibacterial substances. The diameter of zone of inhibition of each sample against every test microorganism was found to be less than standard antibiotic i.e. ciprofloxacin 0.5mg/ml used in assay.

In the present work leaves of methanolic and ethanolic extract exhibited antimicrobial activity, which is purely due to presence of phytochemical compounds present in leaf of *Myrica esculenta.*

*Table 2: Anti-bacterial activity of Ethanolic leaf extract*

| Test microorganism | Antibiotic | Ethanolic extract | Solvent | D.W |
|---|---|---|---|---|
| ***E.coli*** | 14mm | 10mm | - | - |
| ***K. pneumoniae*** | 12mm | 8mm | - | - |
| ***S.aureus*** | 13mm | - | - | - |
| ***E. faecalis*** | 9mm | 6mm | - | - |

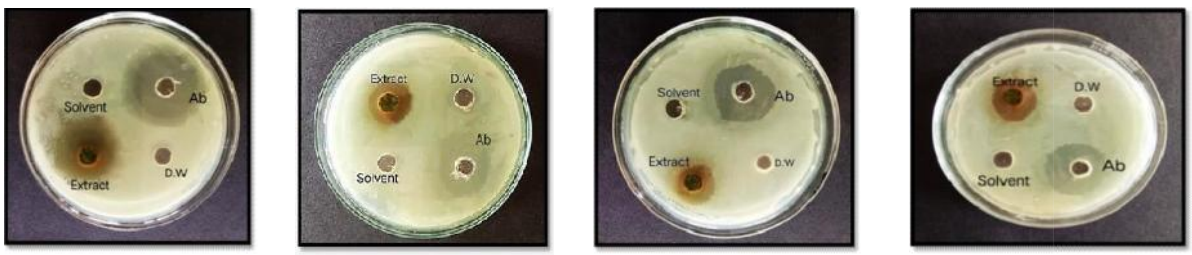



(a) *E.coli* *(b)K.pneumoniae* *(c) S.aureus* *(d) E. faecalis*

**Figure1: Anti-bacterial activity of ethanolic extract against test microorganism**

**Table 3: Anti-bacterial activity of Methanolic leaf extract**

| Test microorganism | Antibiotic | Methanolic extract | Solvent | D.W |
|---|---|---|---|---|
| ***E.coli*** | 16mm | 13mm | - | - |
| ***K. pneumoniae*** | 11mm | 9mm | - | - |
| ***S.aureus*** | 13mm | 6mm | - | - |
| ***E. faecalis*** | 13mm | 8mm | - | - |




(a) *E.coli* *(b)K.pneumoniae* *(c) S.aureus* *(d) E. faecalis*

**Figure 2: Anti-bacterial activity of methanolic extract against test microorganisms**

Methanol has higher polarity in comparison to other solvents and thus tends to dissolve different compounds from the plant materials soaked in them. This property of Methanol thus explains the fact that all the four bacterial species tested were inhibited by the methanolic leaf extract. The maximum zone of inhibition was observed in case of *E.coli* bacteria (13mm) gram negative bacteria and the minimum was observed in case of *S. aureus* (6mm) gram-positive bacteria. Similarly, ethanolic leaf extract exhibited a similar property at which it could inhibit the growth of 3 out of 4 species tested. The negative one is the *S. aureus* and the maximum zone was observed in case of *E.coli* (10mm). The non - polar hexane extract doesn't show any antibacterial activity against all the four species as many reports suggested that secondary metabolites are mainly responsible for the anti-microbial activity.

## Analysis of Anti-oxidant potential:

### *DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging assay*

The antioxidant activity of *Myrica esculenta* was done by using DPPH free radical scavenging assay. DPPH (1, 1-diphenyl- 2picrylhydrazyl) is a Stable free radical, due to the delocalization of the spare electron on the whole molecule. In the present study the antioxidant activity of different extracts of Myrica esculenta leaf was done. The absorbance was recorded at 517 nm and the percentage inhibition was analysed. The capability of scavenging DPPH radical was calculated by the following equation. DPPH Scavenging (%) = DPPH radical scavenging activity % = Ac- As / Ac × 100. Where Ac stands for Absorbance of control, As for Absorbance of test Sample.

***Table 3: Antioxidant activity of standard (Ascorbic acid) at 517nm.***

| Concentration of Ascorbic acid (µl) | O.D at different conc. | DPPH scavenging% |
|---|---|---|
| **20** | 0.132 | 86.6% |
| **40** | 0.112 | 88.0% |
| **60** | 0.095 | 89.9% |
| **80** | 0.087 | 90.7% |
| **100** | 0.076 | 91.9% |

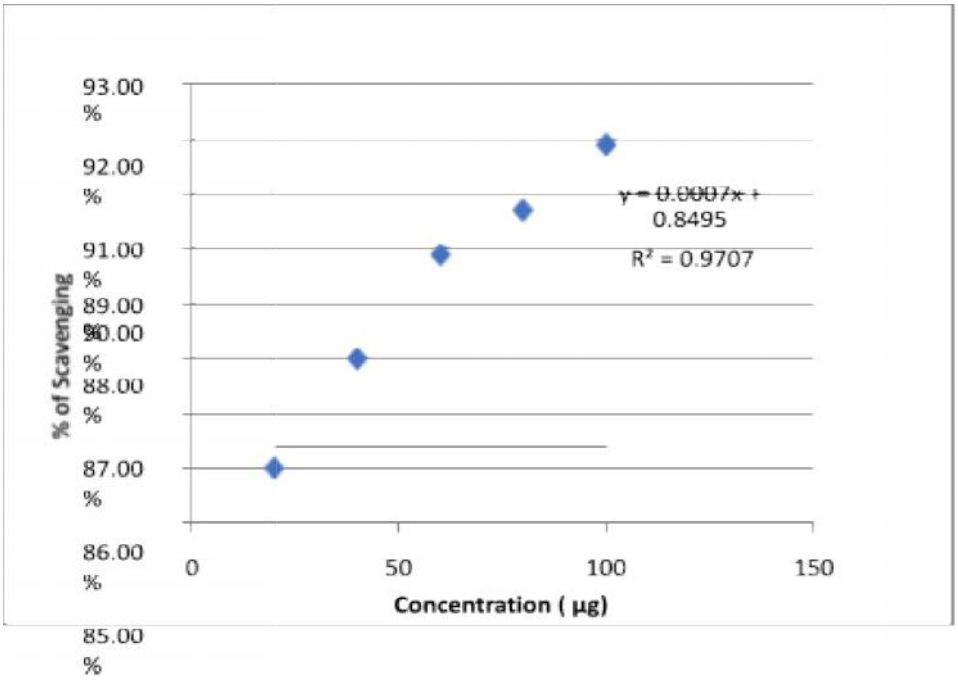



**Figure 3: Ascorbic acid standard for analysis of antioxidant activity of *Myrica esculenta***

**Table 4: DPPH scavenging % activity of methanolic and ethanolic leaf extract**

| Extract | Absorbance | DPPH scavenging% | Concentration (µg) |
|---|---|---|---|
| **Methanolic leaf extract** | 0.121 | 87.60% | 44.420 |
| **Ethanolic leaf extract** | 0.081 | 89.20% | 61.611 |

The methanolic leaf extract showed 87.6% of DPPH scavenging activity which is equivalent to the 44.4 µg of ascorbic acid. Ethanolic leaf extract showed 89.2% of DPPH scavenging activity which is equivalent to the 61.6 µg of ascorbic acid.

## 4. Conclusion

We conclude from the results of the present study, that *Myrica esculenta* has phytochemical compounds, anti-microbial and anti-oxidant activity. From the result of anti- microbial activity of *Myrica esculenta* methanolic and ethanolic leaf extract, it is clear to know that the extract also has ability to inhibit the growth of various microorganism like *(E.coli), (K. pneumoniae*), *(E. faecalis)* and *(S.aureus). Myrica* esculenta leaves extract also act as therapeutic agent which is of great interest in pharmaceutical and Ayurvedic industry. It may be concluded that in the near future, *Myrica esculenta* extract can be implemented as an anti- microbial agent.

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| DOI:10.22192/ijarbs.2023.10.10.011 | |

How to cite this article:
Shalini Sharma and Mehak Mehra. (2023). Phytochemical analysis, Anti-microbial and Anti-oxidant potential of *Myrica esculenta.* Int. J. Adv. Res. Biol. Sci. 10(10): 113-121.
DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.10.011