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# Evaluation of the Antioxidant and Antimicrobial activity of the *Myrica nagi* Leaf extracts

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**A B S T R A C T**
Nutraceutical is a combination of two words nutrition and pharmaceutical which provides health and medical benefits, including the prevention and treatment of diseases. These are products derived from food sources that provide extra health benefits, in addition to the basic nutritional value found in foods. Products typically claim to prevent chronic diseases, improve health, delay the aging process, and increase life expectancy. All the samples tested using Nitric oxide radical inhibition method and ABTS method EEW extract exhibited potent activity with a IC50 value 140µg/m The main aim of the present study is to screen the antioxidant and anti-microbial activity of the leaf extract of *Myrica naga* with an objective of utilizing the extract as a neutraceuticals in future. The result of the study confirms the antioxidant property of the leaf extract of *Myrica naga* by Nitric oxide radical inhibition activity method. l and 175µg/ml. EEW extract exhibited moderate antioxidant activity under lipid peroxidation method. This result suggests that the selected herbal based anti-asthmatic constituents play a vital role in scavenging Nitric oxide which could prevent the bronchial inflammation in asthmatic patients. Because Nitric oxide is naturally formed in activated macrophages and endothelial cells and it is considered as an active agent in several pathologies based on inflammation. The extract also showed potent antioxidant activity under DPPH and Hydrogen peroxide method.

**Keywords:** Nutraceutical, Myrica nagi, Anti-microbial activity, Anti-oxidant activity

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## 1. Introduction

Nutraceutical is a combination of two words nutrition and pharmaceutical which provides health and medical benefits, including the prevention and treatment of diseases was defined first by Stephen DeFelice, MD, founder and

chairman of the Foundation for Innovation in Medicine (FIM), Cranford, NJ[2] in the year 1989.

Examples of nutraceuticals include fortified dairy products, fruits, vegetables and pulses.

A dietary supplement is

- ✓ A product (other than tobacco) that is intended to supplement the diet that bears or contains one or more of the following dietary ingredients: a vita-min, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract, or combinations of these ingredients.
- ✓ Intended for ingestion in pill, capsule, tablet, or liquid form.
- ✓ Not represented for use as a conventional food or as the sole item of a meal or diet.
- ✓ Tabled as a "dietary supplement."

**Sources of nutraceuticals:**

Nutraceuticals are products derived from food sources that provide extra health benefits, in addition to the basic nutritional value found in foods. Products typically claim to prevent chronic diseases, improve health, delay the aging process, and increase life expectancy. There are number of sources of nutraceuticals which include human and mammalian metabolites dietary components of plant and animal origin, synthetic constituents and from plant secondary metabolites they are increasingly produced by microbial fermentation6. Most of the nutraceuticals are of plant derived origin out of which they are used as single derivatives (eg: resveratrol), or as multi components products such as pycnogeol, or whole plant foods such as flax seed.

**Nutraceuticals as a novel source of antimicrobials:**

The traditional and herbal medicines are known as essential resources of nutraceuticals which could provide a variety of beneficial effects on human health. The secondary metabolites present in them give a specific medical benefit other than a purely nutritional.Nutraceuticals have thus dual role to play: as food and therapeutic agent i.e. aids in prevention and/or treatment of disease and/or disorder. The other benefit is, being natural, they have no side effects as other therapeutic agents. Nutraceuticals may range from single isolated nutrients, dietary supplements, or secondary metabolites to genetically engineered designer foods.

**Plant profile:**

**Plant Name:** *Myrica nagi* Linn

**Common Names:** Kayphal, Kandujai Kai, Marudam, Kaidaryamu

**Chemical constituent :** The bark contains tannins, proanthocyanidine, saccharine matter and salts. The ground bark yields a colouring principle 'Myricetin' and Myricetrin . Myriconol isolated from the stem bark and charecterized. B-sitosterol, taraxerol, myricadiol, anthocyanidines, constituent are Triterpenoids like myriconol, myricitrin, terexerone, terexerol, Diaryl heptanoids like myricanone and myricanol were also present.

**Pharmacology** : The bark is useful in fever, asthma, urinary discharge, piles, bronchitis, throat complaints, tumours, anemia, chronic dysentry, ulcer, a good snuff in headache, useful collyrium for opthalmia. Tonic, carminative, useful in inflammation and uterine stimulent.


**Fig 1:** *Myrica nagi* Linn plant profile and picture

## 2. Material and Methods

**Equipment's:**

- ✓ Autoclave - Equitron.
- ✓ Autoanalyser - Micro lab 200.
- ✓ Balance - Denver instruments Apx 203.
- ✓ Centrifuge - R24, Remi instruments.
- ✓ Elisa –micro plate reader - Bio rad 550.
- ✓ Filtration Unit - Millipore.
- ✓ Glassware's - Borosil.
- ✓ Hot air oven - American universal.
- ✓ Laminar air flow - Klenzaids.
- ✓ Magnetic stirrer - Remi instruments.
- ✓ Microtitre plates - Tarsons.
- ✓ Milli-Q - Millipore.
- ✓ Spectophotometer - Shimadzu.
- ✓ Water bath maintained at $37^0$C - NSW India.

**Chemicals:**

**1**,1-Dipheny1-2picryl hydrazyl (DPPH), 2,2' –Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), were obtained from Sigma Aldrich Co., St. Louis, USA. Phosphate Buffer Saline from Gibco-BRL. Rutin from Acros Organics., New Jersey, USA. Naphthyl Ethylene Diamine Dihydrochloride (NEDD) from Roch – Light Ltd., Suffolk, UK. Ascorbic acid from SD Fine Chemicals Ltd., Mumbai, India. Sodium nitroprusside, Dimethyl sulphoxide, Potassium chloride and Sodium chloride from Ranbaxy Laboratories Ltd., Mohali, India. Sulphanilic acids, Sodium bicarbonate, Disodium hydrogen phosphate from E-Merck (India) Ltd., Mumbai, India. All chemicals used were of analytical grade.

**Pharmacognostic and Phytochemical evaluation**

**Collection and authentication of plant:**

The leafs of plant *hydroalcoholic leaf extract of Myrica nagi* were collected from the pharmacognosy lab in Himalaya drug company Banglore and was authenticated.

**Ash values determination:**

Ash values are helpful in determining the quality and purity of crude drugs in powder form. Ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash values were determined.

**Extractive values determination:**

These help in evaluating the constituents of crude drug and also indicate the nature of the constituents present in the

drug. There are different types of extractive values, according to the Indian Pharmacopoeia, Indian Herbal Pharmacopoeia, and Ayurvedic Pharmacopoeia of India. The powder of hydroalcoholic leaf extract of *Myrica nagi* was subjected for ethanol and water.

**Preliminary Phyto chemical tests:**

Phytochemical tests were performed for the determination of various chemical constituents such as alkaloids, tannins, proteins and carbohydrates etc.

**Extraction of *Myrica nagi* plant using various solvents:**

The powdered plant material (leaf) was subjected to maceration. For maceration three solvents were used i.e. methanol : water(1:1).The choice of these three solvents and solvent mixture was based on the literature survey. In maceration process the powdered material was kept for maceration for 12 hrs for methanol and ethyl acetate solvents. The filtrate was subjected to rota vaccum evaporator for recovering the solvent, and the concentrated extract was kept on water bath for drying. For the solvent ratio of ethanol: ethyl acetate: water(4.5:4.5:1) was taken and the powdered material was kept for maceration for 120 hrs, and filtered using muslin cloth and the filtrate is concentrated on rota vaccum evaporator the precipitate so formed is dried on water bath and the yield is noted .

***In vitro* antioxidant evaluation by standard methods**

**Diphenyl Picryl Hydrazyl (DPPH) radical Scavenging Method:**

**Preparation of test solutions:** 21 mg of each of the extracts of methanol, ethyl acetate, EEW (ethanol: ethyl acetate: water (4.5:4.5:1)) were weighed; and were dissolved in distilled DMSO separately to obtain a solution of 21 mg/ml concentration. Each of these solutions was serially diluted separately to obtain the final concentration ranging from 1,000 µg/ml to 0.9765 µg/ml.

**Preparation of standard solution:** 21mg of each of ascorbic acid and rutin were weighed separately and dissolved in 2ml of DMSO to get 21 mg/ml concentration. This solution was serially diluted with dimethyl sulfoxide to get lower concentrations.

**Procedure:**

The assay was carried out in a 96 well microtitre plate. To 200µl of DPPH solution, 10µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used are 1000 to 1.95µg/ml. The plates were incubated at $37^0$C for 20 minutes and the absorbance of each well was measured at 490 nm, using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated. $IC_{50}$ (Inhibitory Concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals.

**Scavenging of Hydrogen peroxide radicals:**

**Preparation of test and standard solutions:** 30 mg of each extracts and the standards (rutin and ascorbic acid) were accurately weighed and separately dissolved in 10 ml of methanol. These solutions were serially diluted with methanol to obtain the lower dilutions.

**Procedure:** A solution of $H_2O_2$ (20 mM) was prepared in PBS, (pH 7.4). Various concentrations of 1 ml of the extracts or standards in methanol were added to 2 ml of $H_2O_2$ solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without $H_2O_2$.

**Nitric oxide radical inhibition activity:**

**Preparation of standard solution:** 42 mg of each of ascorbic acid and rutin were weighed separately and dissolved in 2ml of DMSO to get 21mg/ml concentration. This solution was serially diluted with dimethyl sulfoxide to get lower concentrations.

**Procedure:** The reaction mixture (6 ml) containing SNP (10 mm, 4ml), PBS (1 ml) and ml of extract in DMSO were incubated at 25°C for 150 minutes. After incubation 0.5 ml of the reaction mixture containing nitrate was removed and 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 ml of NEDD was added, mixed and allowed to stand for 30 minutes in diffused light at room temperature. The absorbance of these solutions was measured at 540 nm using ELISA reader against corresponding blank solution. $IC_{50}$ value obtained is the concentration of the sample required to inhibit 50 % nitric oxide radical.

**Antimicrobial Activity**

**Disc diffusion method:**

- Sabourd Dextrose Agar plates were prepared, by pouring the sterile agar into sterile Petri dishes under aseptic conditions.
- 0.1 ml of the test organisms spread on agar plates.
- Sterilized Whatman No.1 filter paper discs (5mm diameter) that were impregnated with the pure Phytoconstituents and extracts (500µg/ml) were applied to the inoculated media.
- The plates were then incubated at 37°C overnight and were examined for a zone of clearance around the discs.
- The plates were maintained at +4°C for 1 hour to allow the diffusion of solution into the agar medium.
- The plate containing fungi were incubated at 28°C for 48 hours.
- Amphotericin – B (40µg/ml) was used as standard drug.

## 3. Results and Discussion

**Collection and authentication of plant:**

Leaf the of plant *Myrica nagi* (Betulaceae) were collected from the pharmacognosy lab, Banglore and was authenticated.

**Ash values determination:**

Ash value such as total ash, acid insoluble ash, water soluble ash and sulphated ash were determined. The results were given in table 1.

**Antioxidant Studies**

**Diphenyl picryl hydrazyl (DPPH) mehtod:**

The EEW extract exhibited potent antioxidant activity with a $IC_{50}$ value of 68 µg/ml among all the test samples under DPPH method. The standard antioxidants ascorbic acid and rutin (controls) exhibited $IC_{50}$ values of 56 and 53 µg/ml respectively.

**Hydrogen peroxide method:**

All the samples tested using hydrogen peroxide method, EEW extract exhibited moderate activity with $IC_{50}$ value 270.7 µg/ml. When compared to all the test samples under hydrogen peroxide method. The standard antioxidants ascorbic acid and rutin (controls) exhibited $IC_{50}$ values of 172.36 and 46.15 µg/ml respectively.

**Nitric oxide radical inhibition activity:**

All the samples tested using Nitric oxide radical inhibition method EEW extract exhibited potent activity with a $IC_{50}$ value 140 µg/ml. The known standard antioxidants ascorbic acid and rutin (controls) exhibited $IC_{50}$ values of 30 and 185.5 µg/ml respectively.

**Anti-microbial study**

**Disc diffusion Method:** All the three extract namely Methanol MEE, Ethylacetate EE, Ethanol: Ethylacetate: Water (4.5:4.5:1)EEW were investigated for their anti-microbial activity against five fungal strains by disc diffusion method and MIC was determined by two fold serial dilution method.The EEW extract showed good antifungal activity with zone of inhibition of 22 and 20mm against *Aspergillus niger* and *Crytococcus neoformans* respectively which was comparable with standard antibiotic used. Whereas the EE extract showed moderate activity against the *Aspergillus niger* and *Aspergillus flavus* against the selected strains which was not comparable with the standard. Two fold serial dilution method, the Minimum Inhibitory Concentration of EEW extract is above 200µg/ml against the selected fungal strains.

**Discussion:**

All the samples tested using Nitric oxide radical inhibition method and ABTS method EEW extract exhibited potent activity with a $IC_{50}$ value 140 µg/ml and 175 µg/ml. EEW extract exhibited moderate antioxidant activity under lipid peroxidation method. The EEW extract exhibited potent antioxidant activity with a $IC_{50}$ value of 68 µg/ml and 270.7 µg/ml among all the test samples under DPPH method and hydrogen peroxide method.

All the three extract namely Methanol MEE, Ethylacetate EE, Ethanol: Ethylacetate: Water (4.5:4.5:1) EEW were investigated for their anti-fungal activity against five fungal strains by disc diffusion method and MIC was determined by two fold serial dilution method. The EEW extract showed good antifungal activity with zone of inhibition of 22 and 20mm against *Aspergillus niger* and *Crytococcus neoformans* respectively which was comparable with standard antibiotic used. Whereas the EE extract showed moderate activity against the *Aspergillus niger* and *Aspergillus flavus* against the selected strains which was not comparable with the standard. Two fold serial dilution method, the Minimum Inhibitory Concentration of EEW extract is above 200µg/ml against the selected fungal strains.

This result suggests that the selected herbal based anti-asthmatic constituents play a vital role in scavenging Nitric oxide which could prevent the bronchial inflammation in asthmatic patients. Because Nitric oxide is naturally formed in activated macrophages and endothelial cells and it is considered as an active agent in several pathologies based on inflammation. The extract also showed potent antioxidant activity under DPPH and Hydrogen peroxide method. Which confirms that scavenging ability of the extract towards the stable radical DPPH and Hydrogen peroxide radicals. Although these results were obtained at a comparatively higher dose, they are encouraging for further *in vivo* studies with an aim of obtaining clinically useful results.

**Table 1** Ash Value of Powdered leaf

| **Ash values % w/w*** | | | | |
|---|---|---|---|---|
| **Part of plant used** | **Total ash** | **Acid insoluble Ash** | **Water soluble ash** | **Sulphated ash** |
| Leaf powder | 2.84% | 2. 33% | 0.61 % w/w | 0. % w/w |
| *Average of three determinations | | | | |

**Table 2** Extractive Value of leaf powder

| **Extractive values % w/w*** | | |
|---|---|---|
| Part of plant used | Alcohol soluble | Water soluble |
| Leaf powder | 16.79% | 1.12% |
| *Average of three determinations | | |

**Table 3** Preliminary Phytochemical tests

| **S. No** | **Phytochemical tests** | **Leaf extract** | | |
|---|---|---|---|---|
| | | **Methanol fraction** | **Ethyl acetate fraction** | **Ethanol: Ethyl-acetate: Water (4.5:4.5:1) fraction** |
| 1 | Test for alkaloids | + | - | - |
| 2 | Test for carbohydrates | + | - | - |
| 3 | Test for steroids and terpenoids | - | - | + |
| 4 | Test for glycosides | - | - | + |
| 5 | Test for saponins | - | - | - |

| | | | | |
|---|---|---|---|---|
| 6 | Test for protein and amino acids | - | - | - |
| 7 | Test for flavonoids | - | + | + |
| 8 | Test for phenols | - | + | - |
| 9 | Test for acidic compounds | - | - | - |
| 10 | Test for mucilage | - | - | - |
| 11 | Test of fixed oils | - | - | - |

**Table 4** Extraction and percentage yield of powdered leaf

| Part of plant used for extraction | Solvent used for extraction | Weight of crude drug | Quantity of solvent used | Weight of crude extract obtained | Percentage of yields |
|---|---|---|---|---|---|
| Leaf powder | Methanol | 100 g | 2500 ml | 13.5 g | 13.5% |
| | Ethyl acetate | 100 g | 2800 ml | 12.32 g | 12.32% |
| | EtOH: EtOAc: Water; (4.5:4.5:1) | 1 kg | 12 litres | 200g | 20% |

**Table 5** Anti-oxidant activity of leaf extracts using Diphenyl Picryl Hydrazyl

| S.No | Test extracts | $IC_{50}$ VALUE (µg / ml) |
|---|---|---|
| 1. | Methanol MEE | > 500 |
| 2. | Ethylacetate EE | >500 |
| 3. | Ethanol: Ethylacetate: Water (4.5:4.5:1)EEW | 68 |
| | **Standards** | |
| 1. | Ascorbic acid | 56 |
| 2. | Rutin | 53 |

**Table 6** Anti-oxidant activity of leaf extracts using hydrogen peroxide

| S.No | Test extracts | $IC_{50}$ VALUE (µg / ml) |
|---|---|---|
| 1. | Methanol MEE | > 500 |
| 2. | Ethylacetate EE | >500 |
| 3. | Ethanol: Ethylacetate: Water (4.5:4.5:1)EEW | 270.7 |
| | **Standards** | |
| 1. | Ascorbic acid | 172.36 |
| 2. | Rutin | 46.15 |

**Table 7** Anti-oxidant activity of leaf extracts using Nitric oxide

| S.No | Test extracts | $IC_{50}$ VALUE (µg / ml) |
|---|---|---|
| 1. | Methanol MEE | 128 |
| 2. | Ethylacetate EE | 246 |
| 3. | Ethanol: Ethylacetate: Water (4.5:4.5:1)EEW | 140 |
| | **Standards** | |
| 1. | Ascorbic acid | 30 |
| 2. | Rutin | 185.5 |

**Table 8** In-vitro antifungal activity of leaf extracts of *Myrica nagi* of against different fungai (Disc Diffusion Method)

| Zone of Inhibition in mm (concentration 500 µg/ml) | | | | |
|---|---|---|---|---|
| **Microorganism Fungus** | **Methanol MEE** | **Ethylacetate EE** | **Ethanol: Ethylacetate: Water (4.5:4.5:1)EEW** | **Standard (40 µg/ml)** |
| *Candida albicans* | 13 ± 0.1 | 11 ± 0.1 | 15 ± 0.1 | 20 ± 0.1 |
| *Aspergillus niger* | 15 ± 0.2 | 09 ± 0.2 | 22 ± 0.2 | 25 ± 0.05 |
| *Aspergillus flavus* | 14 ± 0.2 | 12 ± 0.2 | 18 ± 0.2 | 21 ± 0.1 |
| *Cryptococcus neoformans* | 17 ± 0.1 | 07 ± 0.1 | 20 ± 0.1 | 23 ± 0.1 |
| *Trichophyton rubrum* | 16 ± 0.1 | 10 ± 0.1 | 19 ± 0.1 | 23 ± 0.1 |

Means of 3 values ± S.E.M (Std. Amphotericin – B)

## 4. Conclusion

The present study is to screen the antioxidant and anti-microbial activity of the leaf extract of *Myrica naga* with an objective of utilizing the extract as a neutraceuticals in future. The result of the study confirms the antioxidant property of the leaf extract of *Myrica naga* by Nitric oxide radical inhibition activity method. The result suggests that the selected herbal based anti-asthmatic constituents play a vital role in scavenging Nitric oxide which could prevent the bronchial inflammation in asthmatic patients. Because Nitric oxide is naturally formed in activated macrophages and endothelial cells and it is considered as an active agent in several pathologies based on inflammation. The extract alsoshowed potent antioxidant activity under DPPH and Hydrogen peroxide method. Which confirms that scavenging ability of the extract towards the stable radical DPPH, Hydrogen peroxide radicals. Although these results were obtained at a comparatively higher dose, they are encouraging for further *in vivo* studies with an aim of obtaining clinically useful results.

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