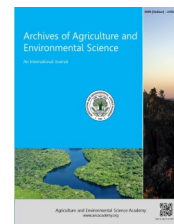




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ORIGINAL RESEARCH ARTICLE

Appraisal of phytochemical and antibacterial potential of *Myrica nagi* Willd. extracts against respiratory tract pathogens

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ABSTRACT

In the present study the antibacterial activity was studied against five bacterial pathogens i.e. *Haemophilus influenzae* MTCC 3826, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655 and *Streptococcus pyogenes* MTCC 442. Shade dried root were crushed and extracted in petroleum ether (PET), acetone (ACE), methanol (MeOH) and water (H₂O) by using Soxhlet apparatus. The agar well diffusion method was adopted to examine antibacterial activity of extracts against the susceptible organisms. Erythromycin was used as positive control to determine the sensitivity of the strains. Phytochemical analysis was done for plant extract. The results showed that MeOH extract was most active as comparison to other extract. The maximum inhibition was found against *H. influenzae* (18.4±0.07 mm) followed by *S. pyogenes* (17.3±0.13 mm), *S. pneumoniae* (16.2±0.07 mm) and *P. aeruginosa* (15.5±0.15 mm) respectively. The minimum inhibition was noted against *S. aureus* (14.4±0.13 mm). The phytochemical screening for MeOH extract has shown that plant contains flavonoids, glycosides, alkaloids, steroids, terpene, saponins and tannins. Therefore, *M. nagi* can be helpful as an alternative source of medicine and new drug discovery.

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INTRODUCTION

Extensive before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles was well accepted (Kumar *et al.*, 2015, 2016). Since ancient times, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. For example species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (R'ios and Recio, 2005). Natural products perform an assortment of functions, and any of them have interesting and useful biological activities. There are more than 35,000 plant species creature used in various human cultures around the world for medicinal purposes (Asolkar *et al.*, 1992; Kumar *et al.*, 2015). Researchers are increasingly turning their attention to natural products and looking for new leads to

develop better drugs against cancer, as well as viral and microbial infections (Chen *et al.*, 1987; Choe, 1986; Kumar *et al.*, 2014, 2015, 2016). Infectious diseases are subsequent leading cause of death worldwide. Treatment of infections continue to be challenging in modern time because of the severe side effects of antimicrobials and the growing resistance to these lifesaving drugs due to their intensive use (Kumar *et al.*, 2014, 2015; Kilani *et al.*, 2008). The problem of microbial resistance has become a global issue of concern, as about 70 % of the bacteria that cause infections in hospitals are resistant to at least one of the antibiotic most generally used for treatment (Kirtikar, 2001; Parekh, 2006; Kumar *et al.*, 2015, 2016). Because of this drug resistance, the search for new antibiotics continues unabated. The increasing malfunction of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Sanjay *et al.*, 2010; Kumar *et al.*, 2015). In this

relationship plants continue to be a rich source of therapeutic drugs. The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of plants to the drug industries was possible, because of the large number of the phytochemical and biological studies all above the world. The extensive use, mistreatment and overuse of antimicrobial in human have gradually more raised levels of antimicrobial resistance in wide range of microorganisms globally (WHO, 2013). It is quite difficult to combat for new drug resistance with such wide range of resistant organisms. Therefore, discovery of new drugs is somewhat essential to sort out major clinical and public health problems grown up by drug resistance.

The herb *Myrica nagi* belongs to the plant family Myricaceae and the order Fagales. It is also called the *Myrica esculenta*. This herb is commonly referred to by the name Kaiphula in Sanskrit and Box Myrtle and Bay Berry in English. In Hindi, it is known as the Kafal. The plant *Myrica nagi* is native to the Hilly regions of the countries Nepal and India. It is found extensively in the northern region of India, especially in the state of Punjab. It also grows wildly in the Garhwal and Kumaon region in Uttarakhand. The only part of the tree that has therapeutic value and is used for medicinal purposes is its bark. The bark of Kafal contains Myricitrin which kills bacteria and so the bark is used for arresting bleeding, removing wind from the stomach, arresting secretion, stimulating bile, and for the retention of potassium in the body. It is also used as an anti-septic, fever, cold, asthma, Intestinal Disorders, teeth disorders, wounds and ulcers (Parmar and Kaushal, 1982). The aim of this study was to find out antibacterial and phytochemical aspects of *Myrica nagi* root extracts against respiratory pathogens that usually cause infections in upper and lower respiratory tract region.

MATERIALS AND METHODS

Plant material: Plant was collected from Srinagar, Uttarakhand and authenticated at Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar. Collected roots were dried under shade at room temperature and crushed to small pieces by using pestle and mortar and powdered in an electric grinder.

Preparation of extract: Plant extracts were prepared by immersing 200 g of powdered plant material in 600 ml of four different solvents i.e. petroleum ether (PET), acetone (ACE), methanol (MeOH) and water (H₂O), loaded in Soxhlet assembly and extracted for 72 h through successive method. Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30°C. Residues were stored at 4°C until further use. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/ml for agar well diffusion method.

Test microorganisms: Five bacterial strains causing respiratory infections used in this study were *Haemophilus influenzae* MTCC 3826, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus*

pneumoniae MTCC 655, *Streptococcus pyogenes* MTCC 442. Bacterial strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh.

Preparation of inoculums: Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 h at 37°C.

Antibacterial testing: The antibacterial activity of different extracts was determined by agar well-diffusion method (Ahmed *et al.*, 1998). 0.1 ml of 12-16 h incubated cultures of bacterial species were mixed in molten Mueller Hinton Agar medium no. 173 (Hi Media Pvt. Ltd., Mumbai, India) and poured in pre-sterilized petri plates. A cork borer (6 mm diameter) used to punch wells in solidified medium and filled with extracts of 45 µl of 200 mg/ml final concentration of extracts. DMSO was used as negative control. The efficacy of extracts against bacteria was compared with the broad spectrum antibiotic erythromycin (positive control). The plates were incubated at 37°C for 24 h in BOD incubator and the diameter of the zone of inhibition was measured in millimetre. Each sample was assayed in triplicate and the mean values were observed. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimetre (mm) as observed from the clear zones surrounding the wells.

Phytochemical screening: Major phyto-constituents present in *Myrica nagi* extracts were subjected to phytochemical analysis to determine the presence of bioactive components by using standard qualitative methods (Trease and Evans, 1996; Evans, 2009).

Test for alkaloids: Test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloids.

Test for flavonoids: On addition of conc. HCl in methanolic extract of material, a red colour appeared which indicated the presence of flavonoids.

Test for glycosides: Plant extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with Ba(OH)₂. The remaining extract contained the glycosides. The hydrolysis of solution was done with conc. H₂SO₄ and after hydrolysis the presence of sugars was determined with help of Fehling's solution.

Test for steroids: The extract mixed with 3 ml CHCl₃ and 2 ml conc. H₂SO₄ was poured from side of test tube and colour of the ring at junction of two layers was noted. A red colour showed the presence of steroids.

Test for saponins: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for tannins: Extract was added in 1% ferric chloride and observed the colour. Bluish black colour appeared which disappeared on addition of dilute H₂SO₄ follow a yellow brown precipitate indicates the presence of tannins.

RESULTS AND DISCUSSION

The results for antibacterial activity are depicted in Table 1 and Figure 1. MeOH extract was found most active against all test pathogens in comparison to other extracts. The maximum inhibition was found against *H. influenzae* (18.4±0.07 mm) followed by *S. pyogenes* (17.3±0.13 mm), *S. pneumoniae* (16.2±0.07 mm) and *P. aeruginosa* (15.5±0.15 mm) respectively. The minimum inhibition was noted against *S. aureus* (14.4±0.13 mm). *M. nagi* crude extracts was found less active in comparison to positive control (erythromycin). The inhibition zone diameters of various extracts of *M. nagi* root.

The decoction of ethanolic extract of the bark of *Myrica nagi* showed antibacterial activity was performed on five different species of bacteria that includes three gram positive bacteria (*Bacillus cereus*, *Listeria monocytogenes*, *S. aureus*) and two gram negative bacteria (*Escherichia coli*, and *Almonella anatum*) as also reported by Shan *et al.* (2007).

Chandra *et al.* (2012) has also been reported to assess the anti-bacterial and anti-fungal activity of *Myrica nagi* fruit pulp difference extracts (Pt. ether, chloroform, ethyl acetate, acetone, ethanol and water extract) active against Ten bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter gergoviae*, *Salmonella enter-*

icatyphim, *Shigella flexneri*, *Staphylococcus aureus*, *Staphylococcus pidermidis*, *Streptococcus pyogenes* and *Bacillus cereus*) and three fungal (*Candida albicans*, *Aspergillus flavus* and *Aspergillus parasiticus*) by using disc diffusion method. The *Myrica esculenta* bark of aqueous ethanolic extract active against adult Indian earthworm *Pheritima posthuma* and more potent than reference control piperazine citrate (Jan, 2010; Vaghasiya, 2009).

The phytochemical screening of *M. nagi* extract has shown that plant contains alkaloids, flavonoids, glycosides, Steroids, Saponins and Tannins which are very important constituents when looking for pharmacologically active phytochemical in the plant (Table 2). Chandra *et al.* (2012) had reported a variety of constituents i.e. alkaloids, flavonoids, glycosides, steroids, proteins, phenolics and Tannins present in *M. nagi*. Moreover, *M. nagi* is also used as traditional veterinary medicine in India; it is used as an ingredient of an ointment against sprains and sores (Mazars, 1994). By this study, it is concluded that *M. nagi* can be used as herbal medicine to treat respiratory infections caused by tested pathogens as compared to synthetic chemotherapeutic agents. It is urged that further research should be brought out to expose the bioactive constituents present in *M. nagi*.

Table 1. The percentage of potency of *M. nagi* extracts against respiratory tract pathogens.

S.N.	Pathogens	Diameters of inhibition zone (mm)			
		PET	CHCl ₃	MeOH	H ₂ O
1.	<i>H. influenza</i>	12.3±0.12	14.3±0.1	17.3±0.1	9.2±0.05
2.	<i>H. influenzae</i> (MTCC 3826)	11.2±0.1	13.7±0.07	18.4±0.07	10.4±0.16
3.	<i>P. aeruginosa</i>	9.5±0.07	10.2±0.1	15.5±0.15	11.2±0.07
4.	<i>P. aeruginosa</i> (MTCC 2474)	10.4±0.07	11.4±0.05	15.1±0.07	10.3±0.07
5.	<i>S. aureus</i>	9.4±0.12	11.3±0.07	15.3±0.05	9.2±0.1
6.	<i>S. aureus</i> (MTCC 1144)	10±0.15	10.4±0.07	14.4±0.13	11.1±0.05
7.	<i>S. pneumonia</i>	10.1±0.13	9.5±0.1	16.2±0.07	12.5±0.1
8.	<i>S. pneumoniae</i> (MTCC 655)	9.4±0.07	10.7±0.07	15.1±0.08	11.3±0.07
9.	<i>S. pyogenes</i>	10.3±0.07	11.7±0.05	17.3±0.13	12.3±0.05
10.	<i>S. pyogenes</i> (MTCC 442)	10.4±0.11	10.7±0.05	16.1±0.03	13.1±0.02

*Values are means of three replicates, Cork borer diameter: 6 mm.

Table 2. The phytochemical screening of crude extracts of *M. nagi*.

S.N.	Phytoconstituents	Solvents			
		PET	ACE	MeOH	H ₂ O
1.	Alkaloids	-	+	+	+
2.	Flavonoids	-	+	+	+
3.	Glycosides	-	+	-	+
4.	Steroids	+	+	+	+
5.	Saponins	-	-	+	+
6.	Tannins	+	+	+	+

+ = Present, - = Absent.

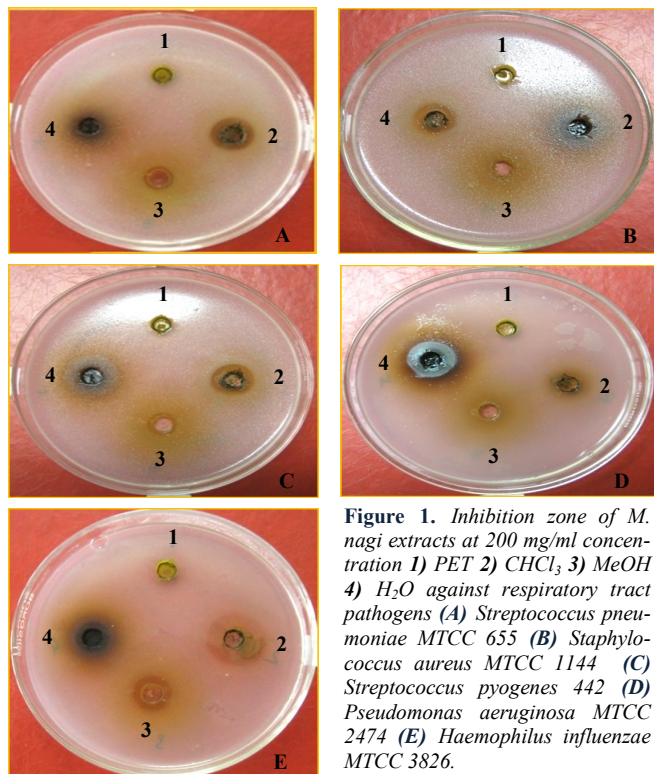


Figure 1. Inhibition zone of *M. nagi* extracts at 200 mg/ml concentration 1) PET 2) CHCl_3 , 3) MeOH 4) H_2O against respiratory tract pathogens (A) *Streptococcus pneumoniae* MTCC 655 (B) *Staphylococcus aureus* MTCC 1144 (C) *Streptococcus pyogenes* 442 (D) *Pseudomonas aeruginosa* MTCC 2474 (E) *Haemophilus influenzae* MTCC 3826.

Conclusions

The investigation for antibacterial activity of *M. nagi* root extracts revealed that plant has broad spectrum activity against selected bacteria which explain the basis for its use in traditional medicines. The significant activity was exhibited by MeOH extract against test respiratory pathogenic microorganisms. The study supported the usefulness in term of availability of phytoconstituents. By results, it can be concluded that *M. nagi* can be helpful as an alternative source of medicine and new drug discovery.

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