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Research Article

NUTRITIONAL EVALUATION, ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF WILD EDIBLE FRUIT OF *MYRICA NAGI* PULP

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ABSTRACT

The present study aimed at evaluating the in vitro antimicrobial activities, nutritional profile and phytochemical screening of wild edible fruit of *Myrica nagi* were investigated by disc diffusion method against ten bacterial strains and three fungal strains. The ethanolic fruits extracts of *Myrica nagi* showed significant activity 16±1mm, 15±1mm and 15±1mm against *Escherichia coli* (MTCC 729), *Streptococcus pyogenes* (MTCC 1925) and *Escherichia coli* (MTCC 443) against food poisoning bacteria, and the fruits have been found to rich in nutrients such as crude protein, carbohydrates, crude fiber, ash content (1.3, 16.13, 3.4, 1.25 %) and minerals as calcium, magnesium, potassium and phosphorus (1.0, 8.4, 1.98 and 0.24 mg/gm) respectively and phytochemical screening of plant for the presence of glycosides, flavonoids, phenols, resin and tannins. However, alkaloids were absent. This analysis revealed that, the fruits contained higher value of fat, protein, fiber and minerals as compared to the cultivated fruits with apple and 200 gm fruits contain sufficient amount of nutrients, required per day by a person. Consumption of fruits may promote general health and well-being as well as reduce the risk of chronic diseases.These findings confirms that the *Myrica nagi* may be potentional source for the formulation of nutraceuticals or natural foods.

Keywords: Nutritional value, Antifungal, Antibacterial and Phytochemical screening.

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. It plays an important role in the development of potent therapeutic agents. India has one of the richest plants medical traditions in the world. Uttarakhand is a vast repository of medicinal plants that are used in traditional medical treatments. *Myrica nagi* belongs to the family Myricaceae, is a popular, potentially income-generating wild edible fruit in the Indian Himalaya and commonly known as Kaphal in Uttarakhand state. The fruit of this plant are edible and prepare the refreshing drink. The bark is asstrigent, carminative, antiseptic and decoction used in asthma, fever, chronic bronchitis, lung infections, dysentery and in toothache. The leaf, fruit, root and bark are used for various body disorders such as liver diseases, worms, jaundice, fever, asthma, anemia, chronic dysentery, ulcer, and inflammation (Nadkarni, 1954 and Rastogi, et al 1995). Food poisoning is still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods. The increasing antibiotic resistance of some pathogens that are associated with food borne illness is another concern (Meng, 1998, Perreten, 1998 and Stermitz, et al 2000).

MATERIALS AND METHODS

Collection of plant material

The fresh part of fruit of *Myrica nagi* was collected from adjoining area of Khirshu village (Dist- Pauri Garhwal Uttarakhand) in the month of June – August 2010. The plant was properly identified from Taxonomy Laboratory, Department of Botany, H. N. B. Garhwal University (A Central University) Srinagar Garhwal Uttarakhand India.

Preparation of plant extract

The plant material was separated into its selected parts (fruit pulp, bark and root) air dried ground to moderately fine powder and soxhlet extracted with increasing polarity solvent (petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water) (Lin, et al 1999). Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of fruit bark and root was subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 100gm). The various concentrated extracts were stored in air tight container for further studies.

Media

Nutrient broth, nutrient agar, Muller Hinton agar, malt extract broth and Sabouraud dextrose agar, hydrochloric acid, alcohol, sulphuric acid and distilled water etc all product of Himedia Laboratories Mumbai (India) were used in this study.

Bacterial strains

Ten bacterial strains were used namely *Escherichia coli, Klebsiella pneumoniae, Enterobacter gergoviae, salmonella entericatyphim, shigella flexneri, Staphyloccus aureus, staphyloccus epidermidis, streptococcus pyogenes* and *Bacillus cereus.* The bacterial strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, Customer no, 3921.

Fungal strains

Three fungal strains were used namely *Candida albicans, Aspergillus flavus* and *Aspergillus parasiticus.* The fungal strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

Antibacterial assay

The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts (Iennette, 1985 and Rosoanaivo, et al 1993). Diluted bacterial culture (100µl) was spread over nutrient agar plates with a sterile glass L-rod. 10mg/ml and 50mg/ml of the each extracts were applied to each filter paper disc (Whatman No. 1, 5 mm diam.) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate (3 discs/ plate) and the plates were inoculated at 37°C for 24 h. After incubation, the diameter of inhibition zones was measured with a caliper.

Antifungal assay

The antifungal activity was tested by disc diffusion method (Saklani, Nov. 2011 and Espinel, et al 2002). The Sabouraud dextrose agar plates were each similarly seeded with each fungal strain. The 24 hrs both culture of each bacterium and 7 days inoculated fungus culture were used to seed sterile Sabouraud dextrose agar at 45°C respectively, and fungal plates were incubated at 25-28°C for 7 days after which diameter of zones of inhibition were measured. Each disc filled with extract.

Nutritional & Mineral assay

The edible portion of fruits was analyzed for moisture, ash, fat (Saklani, et al, Sept. 2011). Fiber as per method reported in AOAC. Total nitrogen was analyzed by microkjeldhal method (Ward, et al 1962) and for crude protein the value was multiplied by 6.25. Total carbohydrates were obtained by subtracting the value moisture, crude protein, crude fat crude fiber and ash from 100% (Negi, et al 1992). The total energy value equal to addition of fat, protein and sugars calorie, each gram of fat give 9 kcal, protein and sugar give 4 kcal energy. The minerals analyzed were Potassium using atomic absorption spectrophotometer, Calcium and Phosphorus by flame photometer. Ascorbic acid in fruits was estimated (Jayaraman, et al 1956).

Phytochemical analysis

The qualitative phytochemical properties of the dried powdered sample were determined using standard methods (Kokate, et al 2005).

RESULTS AND DISCUSSION

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity assay. The results of antibacterial, antifungal, nutritional value and phytochemical screening activity, table 1, 2, 3 and 4 & 5 reveals that antibacterial, antifungal, nutritional, and phytochemical screening activity of fruit of *Myrica nagi* was evaluated against ten bacterial and three fungal pathogenic strains.

Antibacterial and antifungal activity

Myrica nagi ethanolic fruits pulp extract significant activity 16±1mm, 15±1mm and 15±1mm against *Escherichia coli* (MTCC 729), *Streptococcus pyogenes* (MTCC 1925) and *Escherichia coli* (MTCC 443) against food poisoning bacteria, the order of the species based on total antibacterial activity is as follows: *Escherichia coli* (MTCC 729) > *Streptococcus pyogenes* (MTCC 1925) > and *Escherichia coli* (MTCC 443).

Nutritional value

The level of nutrients such as crude protein, carbohydrates, crude fiber, and ash content 1.3, 16.13, 3.4, and 1.25 % and minerals as calcium, magnesium, potassium and phosphorus 1.0, 8.4, 1.98 and 0.24 mg/gm respectively.

Phytochemical screening

The phytochemical screening for the presence of glycosides, flavonoids, phenols, resin and tannins. However, alkaloids were absent. This analysis revealed that the fruits contained higher value of fat, protein, fiber and minerals as compared to the cultivated fruits with apple and 200 gm fruits contain sufficient amount of nutrients, required per day by a person.

Table 1: Antibacterial activity of ten bacterial strains against *Myrica nagi* plant fruits pulp extract.

Bacterial Name		Petroleum ether Extract		Chloroform Extract		Ethyl acetate Extract		Acetone Extract		Ethanol Extract		Water Extract	
Genus /Species/Subspe.	MTCC (Code)	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/Ml	50 Mg/ml	10 Mg/ml	50 Mg/ml
Bacillus cereus	1272	-	-	-	-	8	9	10	12	9	13	9	11
Escherichia coli	729	-	7	-	8	8	9	-	9	11	16	-	9
Enterobacter gergoviae	621	-	-	-	-	7	9	9	9	9	13	-	7
Klebsiella pneumonia	432	-	-	-	9	-	9	-	12	9	12	7	10
Salmonella entericatyphim	98	-	-	-	-	-	8	-	9	7	10	-	8
Shigella flexneri	1457	-	7	-	-	8	10	-	10	8	11	-	7
Staphyloccus aureus	902	-	8	-	8	7	11	11	13	9	14	8	11
Staphyloccus epidermidis	435	-	-	-	-	7	9	-	10	8	12	-	8
Streptococcus pyogenes	1925	-	-	-	7	-	8	-	11	10	15	9	10
Escherichia coli	443	-	8	-	9	8	9	-	10	9	15	-	9

Disc size, 5 Mm, Inhibitory zone size ±1 Mm, Mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Table 2: Fungal activity of three fungal strains against *Myrica nagi* plant fruits pulp extract.

Fungal Name		Petroleum ether Extract		Chloroform Extract		Ethyl acetate Extract		Acetone Extract		Ethanol Extract		Water Extract	
Genus /Species/Subspe.	MTCC (Code)	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml
Candida albicans	3017	-	-	-	-	-	6	-	8	-	8	-	6
Aspergillus flavus	2798	-	6	-	-	-	-	-	-	-	8	-	7
Aspergillus parasiticus	2796	-	8	-	-	-	-	-	6	-	7	-	6

Disc size, 5 Mm, Inhibitory zone size ±1 Mm, Mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Table 3: Nutritional value of *Myrica nagi* fruit pulp.

Nutrients	Value
Moisture (%)	76.60 ± 0.25
Ash (%)	1.25 ± 0.05
Total nitrogen (%)	0.20 ± 0.07
Total protein (%)	1.30 ± 0.04
Crude fat (%)	0.02 ± 0.25
Crude fibre (%)	3.40 ± 0.01
Carbohydrate	16.13± 0.64
Organic matter	98.10± 0.11
Ascorbic acid mg/100gm	4.10± 0.11
Ca mg/100gm	1.0 ± 0.15
Mg mg/100gm	8.4 ± 0.20
K mg/100gm	1.98± 0.30
P mg/100gm	0.24 ± 0.25
Energy value %	123.79 ± 0.05

Table 4: Qualitative estimation of *Myrica nagi* fruit and bark phytochemical screening

Test	*Myrica nagi* fruit pulp	*Myrica nagi* bark
Carbohydrates/ glycosides		
(1)Molish test	(+)	(-)
(2)Fehling test	(+)	(-)
(3)Benedict test	(+)	(-)
Alkaloid		
(1)Mayer's test	(-)	(+)
(2) Dragondroff test	(-)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(-)
Tannins		
(1)Pyrogoll & catechol	(+)	(+)
(2)Gallic acid	(+)	(+)
Unsaturated sterol/triterpenes		
(1)Liebermann Burchard test	(+)	(+)
(2)Salkowiskis test	(+)	(+)
Resin	(-)	(+)

Table 5: Qualitative estimation of *Myrica nagi* fruit pulp amino acid screening.

Amino acid test	*Myrica nagi* fruit pulp
L- Hydroxy proline	(+)
DL Serine	(-)
DL Iso-leucine	(+)
DL Valine	(+)
DL-2-Aminobutyric acid	(+)
L-Ornithin	(-)
L-Cystein hydroxyl	(+)
DL-Nor-leucine	(-)
DL-Tryptopham	(+)
DL-Alanine	(+)
L-Glutamic acid	(+)
Glycine	(-)
L –Proline	(-)
L- Arginine	(-)
DL – Aspartic acid	(+)
L –Cystein hydroxychloride	(+)
L- Histidine	(-)
L – Leucine	(+)
L –Lysine monochloride	(+)
DL – Methionine	(-)
DL – β-Phenyl alanine	(-)
DL – Threonine	(+)
L – Tyrosine	(+)
3-C-3-4Dihydroxy phenyl	(-)

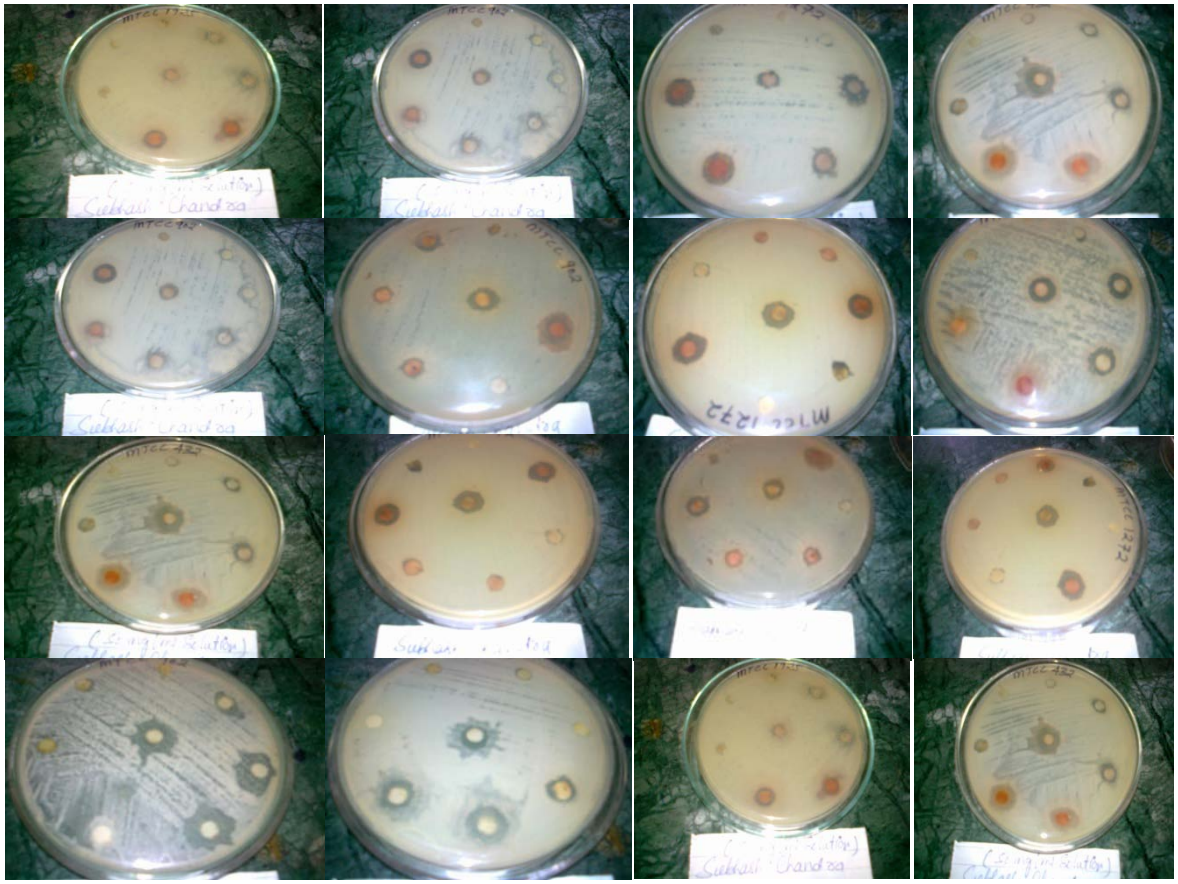

Fig. 1.1 & 2.1: Antibacterial and antifungal activity of ten bacterial strains & three fungal strains, minimum zone of inhibition against *Myrica nagi* plant fruit pulp extract.

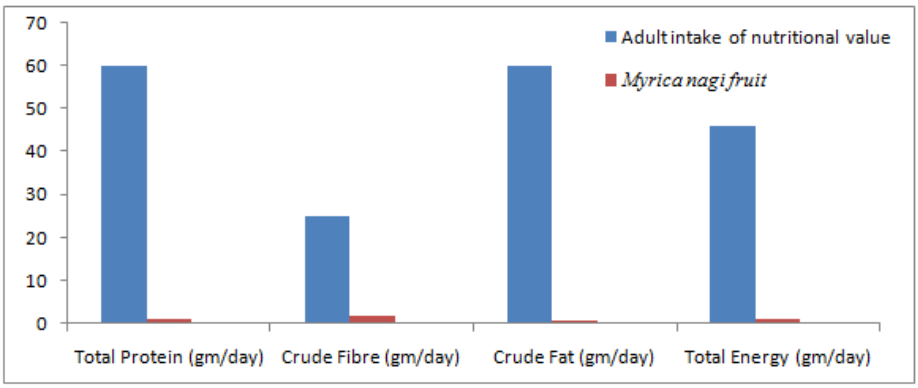

Fig. 3.1: Comparison of per day intake of nutrients by Adults with the nutrients present in the fruit pulp of *Myrica nagi*.

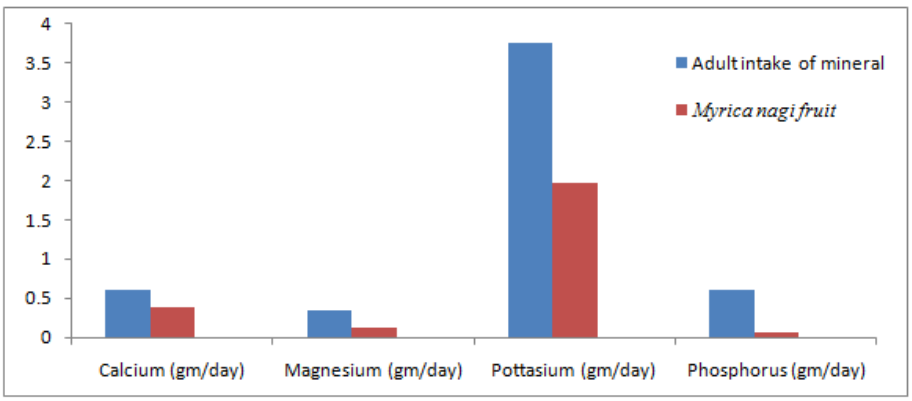

Fig. 3.2: Comparison of per day intake of minerals by Adults with the mineral present in the fruit pulp of *Myrica nagi*.

DISCUSSION

The results of this investigation revealed that antimicrobial and antifungal activity against selected bacterial and fungal strains. The differentiating activities against variety of microorganisms of these five fraction encourage developing a novel broad spectrum antimicrobial formulation in future. Now our research will be directed to develop a broad spectrum antimicrobial herbal formulation with this plant. Even at low concentrations, these species showed high antimicrobial and antifungal activity nearly equal to that of the commercial fungicide used as a positive control. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antimicrobial and antifungal activity. The fruit and bark of the plant is a good source of essential nutrients and medicinal property including minerals, carbohydrates, proteins and lipids.

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