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EXPLORING THE USE OF *MYRICA NAGI* EXTRACTS AS FOOD PRESERVATIVE

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ABSTRACT – This project involves the feasibility studies of the bark extract of *Myrica nagi* plant (Family : Myricaceae) as preservative for fruits and vegetables. Ethanolic extract of the bark of the above plant was tested against bacterial and yeast cultures isolated from selected raw fruits and vegetables. Testing was done at both room temperature and refrigeration temperature. Four mg/ml extract of the bark of *Myrica nagi* was found to be effective in preserving fruits and vegetables for one and a half week at room temperature and for four weeks at refrigeration temperature without altering the organoleptic properties. In conclusion, the alcoholic extract of the bark of *Myrica nagi* proved to be an ideal bio-preservative.

Key words : Preservative, *Myrica nagi,* organoleptic.

INTRODUCTION

Herbal products as preservatives are becoming more and more popular. These products are eco-friendly as well as harmless. There is awareness among public in using consumables preserved and prepared from the herbs. There are large number of herbs which are used to enhance the flavor and aroma in foods. One such natural product is spices. The ancient cultures have recognized the value of using spices and herbs in preserving foods. Spices and herbs contain a large number of compounds that are known to inhibit various metabolic activities of bacteria, yeasts and moulds. These compounds may either be lethal to microbial cells or simply inhibit the production of harmful metabolite, e.g. mycotoxins (Beuchat, 1994). Anti-microbial properties of spices (Zaika and Kissinger, 1981) have been documented.

Among the approaches employed in achieving food preservation by inhibiting growth of undesirable microorganisms, is the use of chemical agents exhibiting anti-microbial activity. These chemicals may be either synthetic compounds intentionally added to food or naturally occurring, biologically derived substances. (CAST news release, 1998) Chemical preservatives, such as sorbate, benzoate, etc., have been used to control microbial hazards for a long time. However, such compounds do not satisfy the concept of "natural" and "healthy" food that consumers prefer and the food industry, consequently, needs to manufacture. The use of industrially synthesized food antimicrobials may be associated with potential toxicological problems. This has warranted the use of natural compounds as preservatives in food industry.

Development of natural antimicrobial compounds from plants (collectively known as green chemicals) for food preservation, is focused on the potential use of phytoalexins, organic acids, and phenols. In addition, promising results have been obtained with essential oils from herbs and aromatic plants. Such essential oils consist of mixtures of esters, aldehydes, ketones, and terpenes with broad-spectrum antimicrobial activity. The toxicological studies of many herbs and spices as well as other active components have been carried out and this has revealed many of them to be food grade as well as GRAS (Generally Recognized As Safe). Flavor components of spices and herbs consist of compounds such as alcohols, aldehydes, esters, terpenes, phenols and organic acids. Essential oils extracted from spices and herbs generally contain the active antimicrobial principles.

MATERIALS AND METHODS

Procurement and Storage of the Spices

The spices were obtained from an authentic ayurvedic dealer of herbs and spices in Mumbai, India. The spice is obtained by collecting the bark of the plant *Myrica nagi*, drying and then crushing it. It was stored at room temperature in clean, dry plastic containers till further use.

Aqueous Extraction of the Powdered Spices

The aqueous extract was prepared by thoroughly mixing 20g of the fine powder of the spice in 100 ml of distilled water, with occasional shaking over a period of two days at room temperature. The liquid is strained off and clarified by filtration through Whatmann Filter paper

no. 1. The filtrates thus obtained were stored at refrigeration temperature till further use.

Extraction with Organic Solvents

Successive solvent extractions were carried out in a Soxhlet apparatus using four different solvents, viz, Petroleum ether, Chloroform, Ethanol, and Acetone. The order of addition of solvents is according to increasing polarity. 20g of the spice powder was taken in a Whatmann Cellulose Extraction thimble (Diameter x length; 30mm x 100mm) and placed into the wide tube of the apparatus. 300ml of solvent (AR grade) was added and the condensed vapor containing the material was collected. The solvent was distilled off from the extract so obtained, on a Flash Evaporator. The extract was then dried at room temperature overnight. It was then scraped off and stored in a desiccator at room temperature till further use.

In order to avoid the use of heat, as in the soxhlet extraction, a modification of the cold percolation technique was used to obtain the cold extract of the spices with 80% and absolute ethanol in the following manner: 1 gram of spice powder was mixed with 50ml of 80% ethanol and absolute ethanol, respectively in 250 ml flasks. The flasks were incubated on shaker at room temperature for 30 minutes. The extract was filtered through Whatmann's filter paper No.1. The ethanol was allowed to evaporate off by pouring the extract in Petri dishes and keeping them under fan at room temperature. These concentrated, dried extracts were then collected in alcohol sterilized glass ampoules and stored at -20ºC.

Table 1 : Evaluation of the antimicrobial activity of cold ethanolic extracts.

Solvent		Diameter of Zone of Inhibition (mm)			
		80% ethanol control	80% ethanol extract of *Myrica nagi*	Ethanol control	Ethanol extract of *Myrica nagi*
Sr no.	Organism				
1	*Staphylococcus aureus*	-	15.0	12.0	15.0
2	*Streptococcus pnueumoniae*	-	-	-	-
3	*Sarcina lutea*	-	13.0	11.0	14.0
4	*Bacillus subtilis*	-	13.5	11.5	13.0
5	*Salmonella typhi*	-	-	-	-
6	*Salmonella paratyphi* A	-	-	-	-
7	*Salmonella paratyphi* B	-	-	-	-
8	*Pseudomonas aeruginosa*	12.0	18.0	11.0	18.0
9	*Pseudomonas marginalis*	-	-	-	-
10	*Klebsiella pneumoniae*	11.0	15.5	12.0	15.0
11	*Proteus vulgaris*	11.0	20.0	11.0	20.0
12	*Escherichia coli*	12.0	16.0	14.0	18.0
13	*Vibrio cholerae*	11.0	13.0	12.0	13.0
14	*Shigella sonnie*	-	-	-	-
15	*Corynebacterium diptheriae*	11.0	13.0	11.0	12.0
16	*Erwinia cartovora*	-	-	-	-
17	*Paeinbacillus polymyxa*	11.0	17.0	12.0	18.0
18	*Aeromonas hydrophila*	-	15.0	-	17.0
19	*Serratia marcesans*	-	-	-	-
20	*Moraxella osloensis*	11.0	13.0	11.0	14.0

Key: '-' : No inhibition

Note: Zone size also includes cork borer diameter (10 mm)

Evaluation of the Antimicrobial Activity

Preliminary antimicrobial evaluation of all the extracts was carried out using Agar cup diffusion method. Appropriate solvent controls were maintained. (Stokes, 1975).

The standard test cultures used were from the Departmental culture stock. In addition cultures were obtained from Microbial Type Culture Collection and Gene Bank (MTCC, Institute of Microbial Technology, Chandigarh). All these cultures were periodically checked for their morphological, cultural, and biochemical characteristics. These cultures were preserved on Nutrient agar slants at refrigeration temperature following subculture.

For the evaluation of antimicrobial activity of yeast isolates Sabouraud's agar, instead of Mueller Hinton agar, was used. The plates were incubated at room temperature for 48 hours and the zones of inhibition were recorded in millimeters.

Table 2 : Viable Count Study.

Food Sample	Initial count cfu/gm	At room temperature (viable counts at RT)			At refrigeration temperature (viable counts at RT)			At refrigeration temperature (viable counts at refrigeration temperature)		
		Sprayed with								
		Un-sprayed	Ethanol	Ethanolic extract of Myrica nagi	Un-sprayed	Ethanol	Ethanolic extract of Myrica nagi	Un-sprayed	Ethanol	Ethanolic extract of Myrica nagi
Oranges	20	$3x10^5$	$4x10^4$	3	$3x10^2$	$5x10^2$	2	22	20	-
Beans	43	$2.5x10^6$	$3x10^6$	20	$9x10^3$	$7x10^3$	15	10	8	5
Capsicum	45	$9x10^8$	$8x10^7$	17	$8x10^2$	$7x10^2$	10	10	9	-
Carrot	22	30	43	$5x10^2$	20	18	22	-	-	-
Arbi	10	50	40	5	8	6	6	-	-	-

Isolation and Identification of Food Spoilage causing Organisms from Spoilt Raw Vegetables and Fruits

Spoilt food samples were purchased from the common market and processed within an hour of purchase. 1g of each spoilt food material was suspended in 10 ml of sterile Nutrient broth and sterile Saboraud's broth, respectively for isolation of bacteria and yeasts. The tubes were incubated at room temperature for 24hrs. Following incubation a loopful was isolated on sterile Nutrient agar plates from sterile nutrient broth and on sterile Saboraud's agar plates from sterile Saboraud's broth, respectively. The Nutrient agar plates were incubated at room temperature for 24 hours. The Saboraud's agar plates were incubated at room temperature for 48 hours.

Bacteria were identified as per the scheme given by Bergey's Manual of Determinative Bacteriology (1994). Yeasts were identified as per the scheme proposed by Barnett, Payne and Yarrow (1990).

Preservation of raw vegetables and fruits

Raw vegetables and fruits were preserved at room temperature and refrigeration temperature using *Myrica nagi* extracts.

At each temperature four sets of vegetables and fruits were taken. One set was sprayed with ethanol, the second and third was sprayed with 2 and 4 mg/ml of extract in ethanol, respectively and the fourth set served as the untreated control.

Following spraying, the food samples were enclosed in transparent, alcohol sterilized, plastic bags and preserved for a period of 3-4 weeks.

The food samples were then macroscopically (physical appearance) observed and photographed. The organoleptic characteristics such as colour, odour and taste of all the preserved food samples were studied and recorded. The surface viable counts of the food samples before and after treatment with extract were also determined as follows: 1 gram of the fruit or vegetable was cut aseptically with alcohol sterilized sharp scalpel and added to 10 ml sterile Phosphate buffer (pH 7.0-7.2). Ten–fold dilutions were prepared and 0.1 ml of it was spread on sterile nutrient agar plates with a sterile glass spreader. For the food samples preserved at room temperature the plates were incubated at room temperature for 24 hours. For the food samples preserved at refrigeration temperature 2 sets of viable count plates were spread. One set was incubated at room temperature and the other set was incubated at refrigeration temperature to count psychrophiles. The counts were recorded as colony forming units (cfu) / gram (g).

Determination of the probable composition of the extracted active principle by GC – MS Analysis

GC – MS analysis was carried out for the original spice powders of Myrica nagi and ethanolic extract of Myrica nagi using 5973 N Agilent Technologies Mass Coupled with 6890 GC. The column used was DB – 5, 30 m length, 250 microns internal diameter and 0.25film thickness. The method employed was Head Space – Solid Phase Micro-extraction method (HS – SPME). Sample was heated at 1000 C for 30 minutes followed by 5 minute absorption and deabsorption time using a 100 PDMS (Poly Dimethyl Siloxane) SPME fiber. The molecular weights of the compounds present in the alcoholic extracts were determined by comparison with standards listed in Wiley Library.

RESULTS AND DISCUSSION

Evaluation of the antimicrobial activity

The aqueous and soxhlet extracts were not found to exhibit significant antimicrobial activity. The cold 99.98% ethanolic extract of *Myrica nagi* was found to inhibit the growth of *Escherichia coli, Proteus vulgaris, Aeromanas hydrophila, Staphylococcus aureus* and others. Thus the ethanolic extract of the spice exhibited significant and a broad spectrum of antimicrobial activity.

There are several reports in the literature about the antibacterial activity of spices. Aqueous extracts of fresh garlic bulbs inhibited the growth of *Bacillus cereus* in concentrations of 3, 5, and 10% (w/v). There was gradual increase in the percentage of organisms inhibited as the concentration of the extract incorporated in the agar increased (3 -10%) (Saleem and Al-Delaimy, 1982). The extracts of both garlic and onion bulbs were found to inhibit the growth of *Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Pseudomonas aeruginosa* (Abdou *et al*, 1972).

Isolation and Identification of Food Spoilage causing Isolates

Food spoilage bacteria, yeasts and fungi were isolated from 7 vegetable samples and 2 fruit samples, on sterile Nutrient agar plates and sterile Saboraud's agar plates. In all 51 bacterial isolates and 27 yeast isolates were obtained. The ethanolic extract of *Myrica nagi* showed significant antibacterial activity against 34 bacterial and 5 yeast food spoilage isolates. These bacterial and yeast food spoilage isolates were then identified.

Preservation of raw vegetables and fruits

Selected raw fruits like Oranges and vegetables like Arbi, Beans, Capsicum and Carrots were employed for the study. Appropriate control comparison revealed the efficacy of 4mg/ml *Myrica nagi* extract to preserve the fruits and vegetables for 1.5 weeks at room temperature and for a period of four weeks at refrigeration temperature. The taste of the fruits and vegetables was also unaltered at a concentration of 4 mg/ml of *Myrica nagi* extract.

The 4 mg/ml *Myrica nagi* ethanol extract was found effective in preserving Oranges, Beans, Capsicum and Arbi at room temperature. At refrigeration temperature the extract was found to be effective in preserving Oranges, Beans and Capsicum. Smid *et al* (1996) investigated the reduction of spoilage- associated fungi and bacteria on whole tomatoes which were treated for 30 mins. with a solution containing 13 mM cinnamaldehyde, the major compound in cassia oil and stored at 18°C in sealed plastic bags. The calyx of cinnamaldehyde-treated tomatoes remained free from visible fungal growth for at least 9 days whereas on day 4, visible fungal growth was observed on calyces of untreated fruits.

After 11 days, a significant spoilage was observed on control samples preserved at room temperature. In contrast hardly any development of the bacterial population was observed on the samples treated with the extracts of *Myrica nagi*. For the fruits and vegetables preserved at refrigeration temperature, after a period of 4 weeks, the counts on control samples were less as compared to the controls kept at room temperature. However, the samples treated with the extracts of *Myrica nagi* and preserved at refrigeration temperature showed hardly any growth. The psychrophile count was also negligible.

Mantis *et al* (1978) studied the effect of garlic extract on *Staphylococcus aureus* in culture media and reported that a 5% garlic extract concentration had a germicidal effect, whereas concentrations of garlic extract equal to or greater than 2% had a clear inhibitory effect. Concentrations less than1% were not considered to be inhibitory. In a related study it was reported that garlic extract of concentration higher than 1% showed bacteriostatic activity against *Lactobacillus plantarum* in culture media, whereas concentrations between 2% and 5% were clearly germicidal (Karaioannoglou *et al*, 1977).

Determination of the probable structure of the extracted active principle

The GC-MS analysis of the *Myrica nagi* extract showed the presence of 2 major components, namely Phenol,2,4-bis(1,1-dimethylethyl)- and Anisole, 2,3,4,5,6-pentachloro- benzene indicating these compounds to be responsible for the preservative effect .

Wilkins and Board (1989) reported that more than about 1,340 plants are known to be potential sources of antimicrobial compounds. These compounds include many low molecular-weight substances, phytoalexins, among which phenolic compounds predominate (e.g., caffeic, Cinnamic, ferulic, and gallic acids; oleuropein, thymol, and eugenol). About 60 are mentioned by Nychas (1995). Beuchat (1994) listed about 60 plants that are commonly used as herbs or spices and also are known to contain low-molecular-weight antimicrobial substances with activity against a wide range of bacteria, yeasts, and molds.

Thus, the studies undertaken with *Myrica nagi* for preservation of food showed encouraging results.

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