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"NATURE'S DEFENSE: HARNESSING THE POWER OF OCIMUM SANCTUM (TULSI) AND NISIN EXTRACTS FOR ENHANCED FOOD SAFETY AND EXTENDED SHELF LIFE"

Microbiology

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ABSTRACT

Background And Objective: Foodborne pathogen contamination is a severe public health hazard that can lead to foodborne illnesses. Foodborne infections continue to be a global public health issue, with an estimated 600 million people being ill each year.This study thus encompasses around studying the antimicrobial effects of Ocimum sanctum and extraction of Nisin from lactococcus sp. for formulation of a product which can enhance the food safety and preservation aspects in food industry. **Materials And Methods:** As a part of the experiment we prepared different concentrations of extract of Ocimum sanctum to check the antimicrobial activity against a set of 10 commonly occuring microorganims of food industry. For easy diffusion of the extract wells were punched in the media and the microorganisms were inoculated, followed by incubation at 37 0c for 24 hrs. Second part of the experiment was extraction of Nisin from lactococcus sp. which is known to extend shelf life of food products. The fermented broth was centrifuged at 10,000 rpm for 15 minutes after 24 hours of incubation, and the supernatant was precipitated with 80% ammonium sulphate overnight at 4°C. **Results And Discussion:** Zone of inhibition was observed in the media having the Ocimum sanctum extract which indicated its antimicrobial efficacy against all the 10 sets of microorganisms. Nisin was extracted. Further studies were conducted , calculations done regarding the concentrations of the two compounds that can be used to formulate the product. **Conclusion:** Prevention from contamination and an enhanced shelf life can be achieved using the product which can be a breakthrough in enhancing food safety in Food industries.

KEYWORDS

Ocimum sanctum, Nisin, Antimicrobial activity, Shelf life, Food safety.

INTRODUCTION:

Since the birth of civilization, medicinal plants have been used by humans to combat disease. *Ocimum sanctum* has been known for over 2000 years as one of the most versatile medicinal plants, with a wide range of biological activity. It is a well-known sacred plant in the Indian subcontinent. Tulsi is also known as holy basil. Basils are tropical Asian plants that are thought to have originated in India. It is an annual plant that is mainly grown by seeds. It is common in tropical places and can be found at a height of 1800 metres in the Himalayan region. Tulsi variants can be found growing wild in several parts of Asia and Africa (Fig. 1).The species differ from one another in terms of geographical location, chemical constituent type and percentage, and thus have varied pharmacological effects. Tulsi has an antimicrobial, immunomodulatory, antistress, anti-inflammatory, antiulcer, antidiabetic, hepatoprotective, chemoprotective, antihyperlipidemic, cardioprotective, antioxidant, antitussive, radioprotective, memory enhancing, antiarthritic, antifertility, antihypertensive, anticoagulant, anticataract, anthelmintic, and antinociceptive activity index. Potential antimicrobial activity of tulsi has also been shown in recent research which can lead to prevention of food contamination in industries to quite an extent [1].

Figure 1: a) ***Ocimum sanctum, b) Ocimum bascilicum,c) Ocimum gratissimum,d) Ocimum americanum, e) Ocimum canum*****[1].**

Nisin is an antimicrobial peptide that is produced by Gram-positive bacteria such as *Lactococcus* and *Streptococcus* (Lubelski et al., 2008; de Arauz et al., 2009). Nisin was discovered in fermented milk cultures in 1928 and was first commercially marketed in 1929. It was first used as an antibacterial agent in England in 1953 (Rogers and Whittier, 1928; Delves-Broughton et al., 1996). The Joint Food and Agriculture Organization/World Health Organisation (FAO/WHO) certified nisin as a safe food additive in 1969. Nisin is currently licenced in over 50 countries and has made a considerable impact in the food sector as a natural biopreservative for various types of foods (de Arauz et al., 2009). In the United States (US), the Food and Drug Administration approved nisin in 1988 and granted it a generally recognised as safe (GRAS) classification for use in processed cheeses (Cotter et al., 2005).The first nisin variation, known as nisin A, is made up of 34 amino acids and is synthesised by Lactococcus lactis (Gross and Morell, 1971). Nisin belongs to the Type A (I) lantibiotics class of cationic peptide antimicrobials (Smith and Hillman, 2008). Nisin and other lantibiotics have received a lot of attention because of their potent and broad-spectrum activity, low likelihood of promoting bacterial resistance, and low cellular cytotoxicity at antimicrobial concentrations (Asaduzzaman and Sonomoto, 2009; Van Heel et al., 2011; Cotter et al., 2013).Recent research is focussed on studying the efficacy of nisin extracts as a preservative to extend the shelf life of food products in food industries [2].

MATERIALS AND METHODS:

Collection of plant leaves:

Indian Academy Degree College Autonomous , bangalore was where the leaves of *Ocimum sanctum* (tulsi) were gathered from . For 4-5 days, leaves were carefully separated, washed, and dried. The leaves were then ground using a mortar and pestle into a coarse powder. Weighed and kept in an airtight container was around 50 g of powder from each leaves.

Preparation of Plant leaf Extracts:

A stock solution of 0.2 g/ml of each leaf powder was created by adding 200 ml of methanol to a separate conical flask along with 50 g of each leaf powder using a cold maceration extraction technique for concentration. The working solution of each extract was then created using the formula "C1V1=C2V2" at concentrations of 0.2 g/ml, 0.4 g/ml, 0.6 g/ml, and 0.8 g/ml, where C1 is the stock solution's concentration, C2 is the new solution's final concentration, V1 is the stock solution's volume, and V2 is the new solution's final volume. Then, by combining each extract concentration in a 1:1 ratio, mix solutions of neem and tulsi were also created at concentrations of 0.2 g/ml, 0.4 g/ml, 0.6 g/ml, and 0.8 g/ml[3].

Test Organisms:

The ten bacterial cultures used to check the antimicrobial activity of *Ocimum sanctum were : E. coli, S. aureus , Pseudomonas sp, Bacillus cereus, Salmonella sp, Proteus sp, Shigella sp, Klebsiella sp, Bacillus pumilus , Lactococcus sp.*

Antimicrobial Activity Test :

Ocimum sanctum leaves extract's antimicrobial activity was determined. Antibacterial activity were determined using the agar-well

diffusion technique. *Ocimum sanctum's* dried leaves were utilised to make a leaf extract. Methanol was used as the solvent to extract the leaf extract from the dried leaves. To make the extracts solvent-free, they were dried. Before usage for antibacterial activity, extracts were kept at 4°C (NCCLS, 2002). All bacteria were diluted to 106 CFU/ml and suspended in sterile water. The suspension was applied to the NA medium's surface in a quantity of 200 l. Using a sterile borer, wells (4.6 mm in diameter) were drilled into the agar. The extract of O. sanctun leaves was used at concentrations of 0.2 g/ml, 0.4 g/ml, 0.6 g/ml and 0.8 g/ml. The water was used to prepare the negative controls. To ascertain the sensitivity of each examined microbial species and to assess the relative percent of antibacterial activity, artificial streptomycin at a concentration of (0.8 g/ml) was employed as a positive reference standard. The inoculation plates underwent a 24-hour incubation period at 37°C. By measuring the diameter of the inhibitory zone (DIZ) of the investigated bacteria, antibacterial activity was assessed[4].

Extraction of Nisin:

Bacterial Culture and conditions of growth:

Lactococcus sp. was used and grown at 35°C with shaking at 200 rpm to a final concentration of 106 CFU/ml in tryptic soy broth plus 0.5% yeast extract .The organism was serially diluted in sterile 0.1% peptone water and surface plated onto tryptic soy agar prior to inoculation into test media. Plates were incubated 35°C for up to 48 hours, and colonies were counted[5].

Creating nisin standards:

Nisin (2.5% nisin, 106 IU/g powder) was solubilized in 20 mM HCl to a concentration of 10,000 IU/ml (0.1 g/10 ml). Nisin stock was then diluted in 20 parts water. Stock solutions were stable at 5°C for up to 7 days, and working solutions were made fresh daily[5].

Solvent Extraction:

The powdered nisin preparation was suspended at a concentration of 10 mg/ml in sterile distilled water diluted with 10, 50, 90, 98, or 100% (vol/vol) ethanol or methanol. 2 ml of each sample was withdrawn after 0.5, 2, 4, 6, and 8 hours of vigorous stirring at room temperature to assess nisin activity. The samples were immediately centrifuged at 1,520 × g for 5 minutes. The agar diffusion assay was used to determine the quantity of nisin in supernatants. Glass vials were used whenever possible to reduce errors caused by the hydrophobic surfaces of disposables. The following formula was used to calculate the yield of an extraction process:

Yield (%) = measured nisin concentration (IU/ml) / 1000 IU/ml × 100

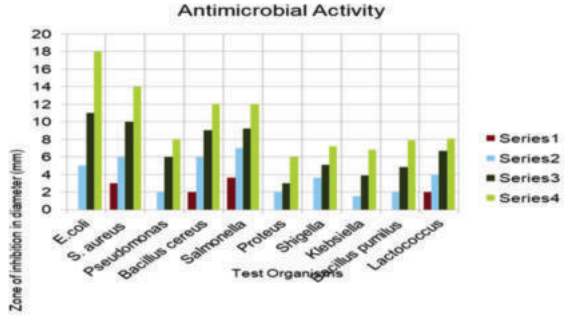
where 1,000 IU/ml in the denominator represents a full extraction, and 1,000 IU/ml corresponds to a dry preparation concentration of 1 mg/ml[5].

Formulation of the product:

Suitable concentrations of tulsi extracts and nisin extracts were then used to formulate the product .

Table 1: Tulsi leaf extracts in methanol at varied concentrations and their unique ZOI(Zone of Inhibition) in diameter(mm) for the ten sets of microorganisms mentioned below:

Concentration (Tulsi) g/ml	Zone of Inhibition in diameter (mm)									
	E.coli	S. aureus	Pseudomonas	Bacillus cereus	Salmonella sp.	Proteus sp.	Shigella sp.	Klebsiella sp.	Bacillus pumilus	Lactococcus sp.
0.2	0	3	0	2	3.6	0	0	0	0	2
0.4	5	6	2	6	7.0	2	3.6	1.5	2	4
0.6	11	10	6	9.1	9.2	3	5.1	3.9	4.8	6.7
0.8	18	14	8	2	12	6	7.2	6.8	7.9	8.1



Graph 1: Based on the values of table 1 a graph is plotted to indicate Tulsi leaf extracts in methanol at varied concentrations and their unique ZOI(Zone of Inhibition) in diameter(mm) for the ten sets of microorganisms mentioned below :

RESULTS AND DISCUSSION :

The antimicrobial activity of tulsi was assesed by measuring the Zone of inhibition in the petridishes .It was seen that there was a significantly greater Zone of inhibition observed when the extracts of Tulsi were used in concentrations of 0.6 g/ml and 0.8 g/ml in case of all the ten microorganisms. Zone of Inhibition was nil in case of few microrganisms like *Proteus, Shigella, Klebsiella , Bacillus pumilus* when 0.2 g/ml of tulsi extracts were used, but significant Zone of Inhibition was observed when greater concentrations of 0.6 g/ml and 0.8 g/ml of tulsi extracts were used . Nisin was extracted from *lactococcus* sp. and was used to preserve certain common food products for an extended period . Results noticed were positive , there was significant increase in shelf life of the products preserved with nisin.Then extracts of Nisin at suitable concentrations can be used to formulate with the tulsi extracts to use it as a food safety product.

CONCLUSION:

Thus the study concludes that *Ocimum sanctum* has potential in preventing the growth of the 10 sets of microorganisms which were tested for the antimicrobial activity . It has been observed that there has been significant Zone of Inhibition formation in all the sets of microorganisms tested at the concentrations of 0.6g/ml and 0.8 g/ml of Tulsi extracts used .Thus it can be concluded that *Ocimum sanctum* can be used as a potent antimicrobial agent in food industries to prevent food contamination by the abovementioned group of microorganisms .Nisin extracts were used to increase the shelf life of the food products to quite an extent , thus it can be concluded that nisin can be used as a food preservative in the food industry .Thus the formulation of a product with suitable concentrations of *Ocimum sanctum* extracts and nisin extract can lead to a breakthrough in the food contamination and preservation aspects in food industries .

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