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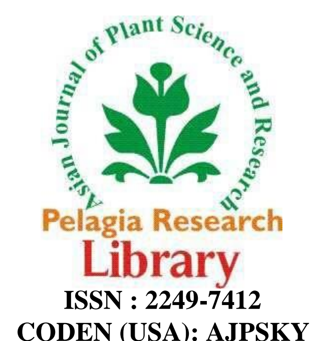

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Anti-microbial Effect of Selected Indian Spices on Lactobacillus acidophilus

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ABSTRACT

Indian spices which are regularly used in foods, on the other hand have great health benefits. Studies also proven that certain spices possess antimicrobial, microbicidal, fungicidal properties. The base of the project goes with this simple antimicrobial property of some selected spices that are: Carom seeds, Clove, Turmeric, Pepper, Nutmeg and Bayleaf

Initially, respective oils were produced by conventional methods using methanol as an extraction solvent for each of the selected spice

Microorganisms such as Lactobacillus acidophilus and Escherichia coli (E. coli) were isolated from mixed cultures and grown in Nutrient broth which is used as a stock solution.

Antimicrobial properties of the above oils were tested against Lactobacillus acidophilus and Escherichia coli (E. coli), using minimum inhibitory concentration technique on the nutrient agar media. The zones of inhibitions were observed for each oil and are measured

Key words: Turmeric, Bay leaf, Carom seeds, Clove, Turmeric, Pepper, Nutmeg

Introduction

An Indian meal is designed to have various kinds of spices included in it and these spices along with the addition of taste and flavour to the food they also claim to have antimicrobial properties **[1]**. A regular meal in India typically include a hand full of spices like Turmeric, Bay leaf, Carom seeds, Clove, Turmeric, Pepper and Nutmeg. Each of the spice has its own importance but most commonly these are believed to have antimicrobial properties. In order to evaluate the antimicrobial activity of these spices, these spices are selected and tested against our regular oral flora (*Lactobacillus acidophilus*) **[2,3]** which are regularly used as probiotics for their benefits **[4]** but on the other hand they were found to be responsible for dental caries **[5]**. *L. acidophillus* starts effecting tooth enamel which is a hard tissue of a tooth and later progresses towards dentin, or cementum. Early in its development, caries may affect only enamel. Once the extent of decay reaches the deeper layer of dentin, the term "dentinal caries" is used **(Figure 1)**. Since cementum is the hard tissue that covers the roots of teeth, it is not often affected by decay unless the roots of teeth are exposed to the mouth. Although the term "cementum caries" may be used to describe the decay on roots of teeth, very rarely does caries affect the cementum alone. Roots have a very thin layer of cementum over a large layer of dentin, and thus most caries affecting cementum also affects dentin **[6]**.

The respective organisms were isolated from mixed cultures using from oral swabs and grown in nutrient broth which were later plated to test their activity against the above mentioned spices. Then the activity of these spices against the organism was measured using the inhibition zones developed.

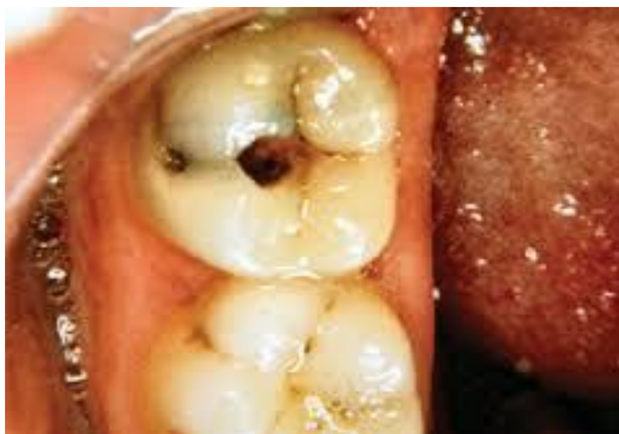

Figure 1: Tooth effected by *L.acidophillus*

Method

Preparation of culture

Mixed culture developed from soil **[7]** usually contains various types of microorganisms these cultures were grown in a nutrient broth and a ten-fold serial dilution was performed and grown in nutrient broth and checked for the development of pure cultures by plating them on the nutrient agar medium.

From these plates E.coli was isolated and pure cultures were obtained, this was inoculated into sterile nutrient broth and incubated at 37ºc for 12-24hrs. After incubation period, the turbid culture that is obtained is the subculture and it is further used for antibacterial testing.

L. acidophilus bacterial strains were obtained from a Probiotic source (spores sachet), and then these organisms were made into pure cultures by serial dilution and streak plate methods. The obtained organism is confirmed to be *L .acidophilus* by observing colony morphology and conducting IMVIC and catalase tests. The obtained cultures are preserved and sub-cultured on to sterile nutrient broth after every 24hrs. These cultures were used as inoculums for further testing.

Preparation of culture media

Culture media provides all essential nutrients for the growth of microorganisms. Nutrient agar and nutrient broth were used to inoculate the bacterial strains **(Figure 2)**.

Nutrient media thus prepared was sterilized by autoclaving at 121ºc for 20mins at 15 lbs pressure.

Figure 2: Spread plate of *Lactobacillus acidophilus*

Preparation of solvent extracts:

- Completely dried spices- Carom seeds, Nutmeg, Clove, Pepper, Turmeric and Bay leaf (5gm each) are cut and

ground to fine powder using mortar and pestle, so that no heat is generated.

- Then each of these powders were soaked separately in 50ml of Methanol in the conical flasks and are covered with Aluminum foil. These powders were allowed to soak for 48hrs (Figure 3).
- After the incubation period, the extract is filtered with the help of Whattman filter paper and funnel.
- The filtrates are then boiled at 40oc in a heating mantle for an hour by continues stirring. (40°C is preferred since the selected solvent is Methanol which is inflammable if the temperature increases)
- Then, the obtained oils are collected and stored in closed vials at lower temperatures (4°C) in order to prevent evaporation.

Figure 3: Solvents extracted using methanol

Agar disc diffusion technique

Agar disk-diffusion testing **(Figure 4)** developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. In this well-known procedure, agar plates are inoculated with standardized inoculums of the test microorganism **[8]**. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured. Shows the growth media, temperature, period of incubation and inoculums size required by CLSI standards.

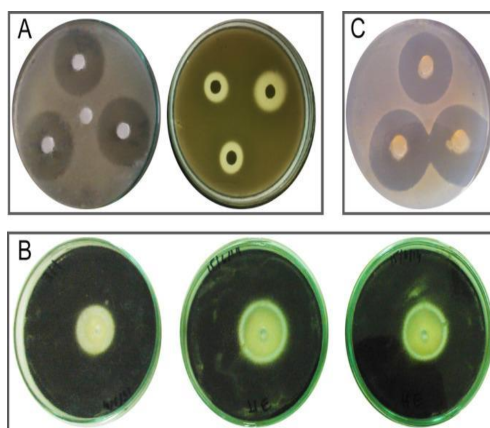

Figure 4: Demonstration of agar disc diffusion

Plating

Under Laminar Air Flow, Sterilized nutrient medium is poured in to Petri dishes, to a depth of 3-4 mm. 500µl of well grown E. coli culture broth is plated on to solidified Nutrient agar plates. Each plate was divided into six equal portions along the diameter. Then six bores are punched on media with the help of borers each for respective oil extract five for the extracts and one is used as control (Methanol). Then in the first plate, 20µl of each oil extract is pipetted in to each well. So that each well accommodates one of the oil extracts. In the same way 20µl, 30µl, 40µl, 50µl and 60µl **(Figure 5,6)** are pipetted in to the respective four plates. Such that each plate accommodates all the oil extractions of same concentration. Then these plates are incubated for 24hrs. After incubation period, zone of growth inhibition is developed. Then anti-microbial activity was measured based on the diameter of zone of inhibition observed.

Figure 5: Plates showing the inhibition zones against the organisms at concentrations 20µl and 30µl

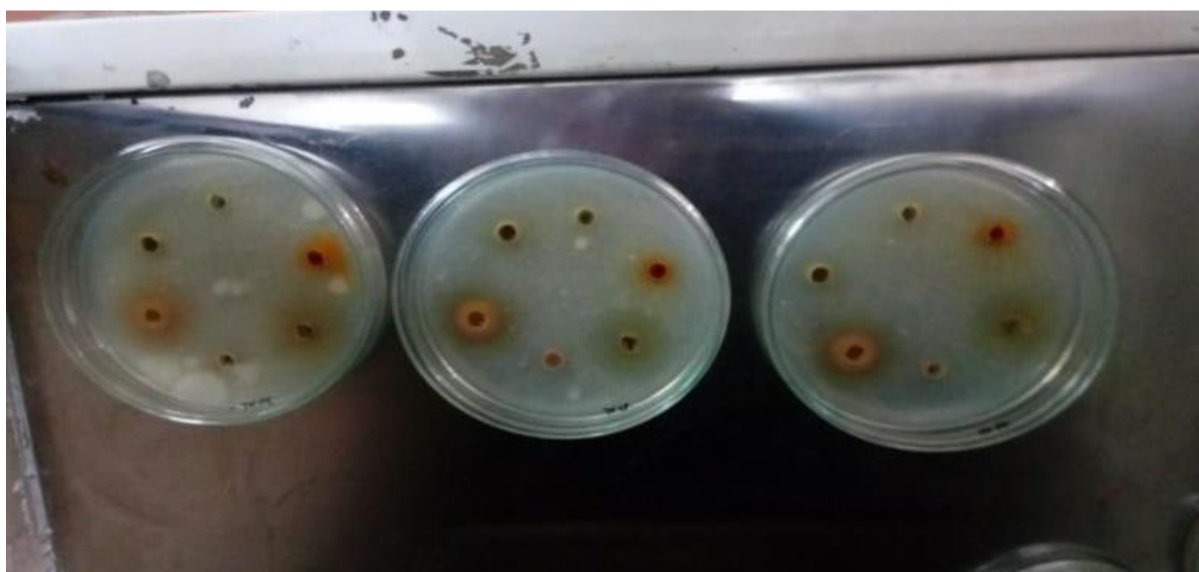

Figure 6: Plates showing the inhibition zones against the organisms at concentrations 40µl, 50µl and 60µl

Results

All the solvents containing the spices were tested against the microbial plates containing the well grown cultures of *L. acidophilus* at different concentrations (20, 30, 40, 50 & 60µl) using *E. coli* as a standard. These plates were incubated for 48 hrs and their zone of inhibitions were observed.

L. acidophilus culture after incubation time for 24hours, with 20µl, 30µl **(Figure 5)** concentrations. Spices- carom seeds, clove and nutmeg were showing some antibacterial property.

L. acidophilus culture after incubation time for 24hours, with 40µl, 50µl and 60µl **(Figure 6)** concentrations. At high concentration of spices- carom seeds, clove, nutmeg, pepper were showing good antibacterial property.

In order to obtain the median of all the above observations we have considered the plate treated with 40µl **(Figure 7)** and the results were as follows **(Table 1)**

Table 1: Results developed at concentration of 40µl in the spread plate cultures of *L.acidophillus*

Spice	Ring diameter
Carom seeds	2.6cm
Clove	2.2cm
Nutmeg	1.7cm
Turmeric	1.3cm
Pepper	Not clear but present
Bay leaf	Negative or no zone

For *E. coli* which was used as a control is treated with 40 µl **(Figure 8)** and incubated for 24hrs and the results were recorded (**Table 2**).

Zones of growth inhibition where observed for carom seeds, clove and turmeric, pepper. No clear zone observed for nutmeg. While, no zone of growth inhibition observed for bay leaf

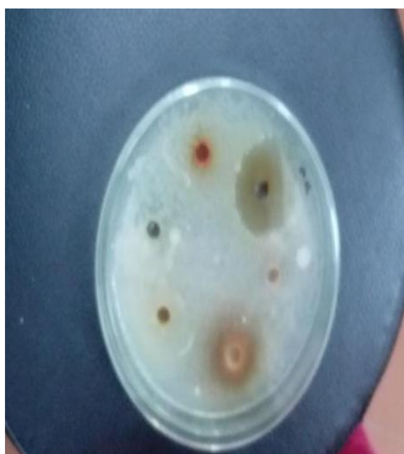

Figure 8: E.coli culture treated with 40 µl

Table 2: Results developed at concentration of 40µl in the spread plate cultures of *L.acidophillus*

Spice	Ring diameter
Carom seeds	1.9cm
Clove	1.3cm
Turmeric	1.1cm
Nutmeg	1cm
Pepper	Visible but not clear
Bay leaf	Negative or no zone

Conclusion

Usage of Indian spices in our regular diet helps us maintain our oral health and oral hygiene.

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