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Research Article

ASSESSING OF ANTILISTERIAL COMPOUNDS FROM *LACTOBACILLUS CASEI* AND *OCIMUM SANCTUM* AGAINST *LISTERIA MONOCYTOGENES*

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ABSTRACT

In this study extracts of *L*actobacillus casei and *Ocimum sanctum* were separated and analysed for their antibacterial activity against the food borne human pathogen *Listeria monocytogenes. Lactobacillus casei* and *Listeria monocytogenes* were isolated from milk sample.The bacterial culture of *Lactobacillus casei* was inoculated in nutrient broth and incubated for 24 hours and 7 days on rotary shaker at 28±2^{o}C to obtain their antilisterial compounds.The petroleum ether extracts of *L*actobacillus casei and *Ocimum sanctum* were analyzed for their antagonistic properties against *Listeria monocytogenes.*Their inhibitory patterns were measured in both individual and dual extracts.The petroleum ether extract of *Lactobacillus casei* culture broth showed good inhibitory result against *Listeria monocytogenes* when compared with *Ocimum sanctum* extract.*Lactobacillus casei* produced 54mm zone of inhibition on the growth of *Listeria monocytogenes* at a concentration of 150µl. *Ocimum sanctum* produced 46mm zone of inhibition on the *Listeria monocytogenes* at a concentration of 200 µl. Dual extract of petroleum ether of *Ocimum sanctum* extract and *Lactobacillus casei* extract (1:1 ratio) produced 40mm zone of inhibition on the *Listeria monocytogenes* at a concentration of 200 µl.GC-MS analysis revealed the various components present in the *Lactobacillus casei* and *Ocimum sanctum* extracts.

Keywords: *Listeria monocytogenes,Lactobacillus casei,Ocimum sanctum,*GC-MS analysis.

INTRODUCTION

The genus *Listeria* includes six different species (*Listeria monocytogenes*, *Listeria ivanovii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seemlier*, and *Listeria grays*). *Listeria ivanovii* and *Listeria monocytogenes* are pathogenic in mice, but only the *Listeria monocytogenes* is consistently associated with human illness. There are 13 serotypes of *Listeria monocytogenes* that can cause disease, but more than 90 percent of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b.

Listeria monocytogenes cause the infection listeriosis. Listeriosis is food poisoning caused by eating foods contaminated with the *Listeria monocytogenes.* It is one of the most virulent food-borne pathogens, with 20 to 30 percent of clinical infections resulting in death. Listeriosis is the leading cause of death among bacterial pathogens, with fatality rates exceeding even *Salmonella* and *Clostridium botulinum*. (Ramaswamy *et al.,* 2007).Nationwide, 1,651 cases of listeriosis during the period of 2009–2011 were reported. The case-fatality rate was 21%, most cases (58%) occurred among adults aging 65+, and 14% were pregnancy-associated.

Listeria monocytogenes is found in soil and water and vegetables can become contaminated from the soil or from manure used as fertilizer. Animals can carry the bacteria and can contaminate meats and dairy products, fishery products, processed foods.

The reduction or controlling of *Listeria monocytogenes* contamination in food materials may reduce the risk of outbreak of diseases. It is achieved by proper food processing or using antibiotic compounds as food or feed additives. Another choice to control the *Listeria monocytogenes* is incorporation of herbal extract

or probiotic microbal extract. These extract not only act as antilisterial agent, but also improve food quality and nutritive values and the health of consumers.

MATERIALS AND METHODS

Collection and enrichment of sample for isolation of *Listeria monocytogenes* (Jami *et al*., 2010)

The raw milk samples were collected from local farm house situated in and around Tiruchengode (Namakkal district). The samples were collected in sterilized polythene bag, brought to the laboratory and stored at 4°C in a refrigeratorA 10ml of raw milk sample was taken in a centrifuge tube and centrifuged at 6000 rpm for 15 min at 4°C. The supernatant was discarded and the pellet was inoculated in to 10ml of sterile *Listeria* Enrichment Broth (LEB) in a screw-cap tube, and incubated at 4°C for 10 days.

Isolation and identification of *listeria monocytogenes* (Molla *et al.*, 2004)

A loopful of the enriched broth was streaked on PALCAM agar plates supplemented with Natamycin (25mg/ml) and Colistin sulphate (20gm/ml). (*Listeria* selectivial- SV33 Series-MAST International). The plates were incubated at 30°C for 48 hours. The plates were examined for the presence of characteristic colonies presumed to be *Listeria*. Identification of the isolates were performed according to their morphological, cultural and biochemical characteristics by following Bergey's Manual of systematic Bacteriol (Kandler and Weiss, 1986). All the isolates were subjected to Gram staining and specific biochemical tests.

CAMP TEST (Christie, Atkins, and Munch-Peterson Test) (Nsagha *et al.*, 2000)

For the CAMP test, fresh isolates of bete-haemolytic *Staphylococcus aureus* and *Rhodococcus equi* were streaked vertically on a sheep blood agar plate. The vertical streaks are separate so that rest strains may be streaked horizontally between them without touching the vertical streaks. After 24-48 hours incubation at 35°C, the plates for haemolysis in the zone of the vertical streaks were examined. The bacterial cultures were maintained on nutrient agar slants stored at 4°C for further use.

Collection of antagonistic bacteria and Herbal plant

Isolation of *Lactobacillus casei* from Milk sample collected from Tiruchengode. The Herbal plant is *Ocimum sanctum* from Poosaripatti region Virudhunagar district.

Isolation of *Lactobacillus (Kasra and Peymanfar, 2012)*

10ml of milk sample was homogenized in 90 ml of sterile saline solution (0.85%, pH 7). Five 5-fold dilutions of the homogenates were prepared and inoculated on plates of MRS agar (Man, Rogosa and Sharpes) and the plates were incubated at 32°C for 48 hours. Colonies with typical characteristics of *Lactobacillus* were randomly selected from the plates and subjected to Gram's staining and biochemical test.

Production of antilisterial compounds

The bacterial culture was inoculated in nutrient broth and incubated for 24 hours and 7 days on rotary shaker at 28±2°C respectively. The culture was then centrifuged at 10,000 rpm for 5 min. The supernatant fluid was collected for testing of antilisterial compound.

Preparation of *ocimum sanctum* leaf extracts (Sundaramurthy *et al.*, 2012)

The O*cimum sanctum* leaves were collected from plant and washed with tap water followed by sterile distilled water. The preweighed leaf material was crushed by mortar and pestle. The content was transferred to soxhlet apparatus extracted with 100ml of petroleum ether.

Antagonistic activity by well diffusion assay

The molten Muller Hinton agar medium was poured into petriplates and allowed to solidify. About 0.1ml of *Listeria monocytogenes* broth culture was spread on the agar surface using sterile cotton swab. Then a well of 10mm was made in the agar medium by using a sterile cork borer. Different concentration (50µl, 100µl, 150µl, and 200µl) of extract was transferred into separate wells and the plates were incubated at 37°C for 24 hours. The development of inhibition zone around the sample loaded well was recorded. Petroleum ether was used as the control.

RESULT AND DISCUSSION

Listeria monocytogenes was isolated from milk sample. 2.1% of samples possessed *Listeria monocytogenes*. It grown on PALCAM agar media formed olive green colonies with a halo black center and surrounded by black zones. The colony shape was concave with 1.7-1mm diameter. In Brain heart infusion agar medium *Listeria monocytogenes* formed cream color colonies. It was gram positive, motile with rod shaped organisms. The results were show in Table -1. When the colonies reach confluence, the medium becomes brown-black. PALCAM agar is a selective medium used for the differentiation and isolation of *L.monocytogenes* from milk and cheese, as well as in other food products

(McLauchlin, 1987).

L.monocytogenes in bulk tank milk samples have been reported from different countries such as Austria 1.5% (Deutz *et al.,* 1999), Spain 3.6% (Gaya *et al*., 1998). India 1.7% (Bula *et al*., 1995), USA 4.1% (Roberts, 1994), Canada 1.9% (Fedio and Jackson, 1990) and Iran 1.6% (Moshtaghi and Mohammadpour, 2007).

Biochemical tests were performed for the isolates according to an established protocol for the identification of *Listeria monocytogenes* in Bergy's Mannual of Systemic Bacteriology Table-2.This isolate shows positive reaction for catalase, methyl Red, voges proskauer, citrate utilization test where as oxidase and indole tests are negative. CAMP test reaction was positive, glucose, lactose and sucrose were utilized by fermentation mechanism. TSI shows acid slant and acid butt, trehalose, mannose, dextrose and xylose were utilized by *Listeria* species. The similar results were reported by Priyanka and Alka (2008) isolate the *Listeria* monocytogenes from milk product.

Isolation and characterization of *Lactobacillus casei*

Table 3 showed the morphological characterization *Lactobacillus casei* on MRS Agar media. *Lactobacillus casei* produced straw coloured colonies of smaller size. It was found to be Gram positive spiral shaped rod, non motile, catalase negative, and endospore absence.

Lactobacillus casei is a bacterium probiotic selected *Lactobacillus casei* strain was examined for their potential use in new probiotic fermented milks by evaluation of their technological performances, *in vitro* adhesion capacity and intestinal transit tolerance after administration to rats (Elisa *et al.,* 2004).

MRS agar is a selective medium used for the differentiation and isolation of *Lactobacillus casei* from milk products. This isolate shows gram straw colored colonies gram positive rod, non motile, and endospore negative. Galactose, glucose, lactose were utilized by *Lactobacillus casei.* The similar results were agreed with report of Bayane *et el.,* (2006).

Antilisterial activity of *Lactobacillus casei* and *Ocimum sanctum* extracts

The petroleum ether extract of *L*actobacillus casei, culture broth showed good inhibitory result against *Listeria monocytogenes* when compared with *Ocimum sanctum* extract. *Lactobacillus casei* produced 54mm zone of inhibition on the growth of *Listeria monocytogenes* at a concentration of 150µl. *Ocimum sanctum* produced 46mm zone of inhibition on the *Listeria monocytogenes* at a concentration of 200 µl. Dual extract of petroleum ether of *Ocimum sanctum* extract and *Lactobacillus casei* extract (1:1 ratio) produced 40mm zone of inhibition on the *Listeria monocytogenes* at a concentration of 200 µl. The results were show in table - 4

Antagonistic potential of dual extract, petroleum ether of *Ocimum sanctum* extract and *Lactobacillus casei* extract (1:1 ratio) against *Listeria monocytogenes* were shown in figure-1.

Lactobacillus casei antibacterial activity of probiotics may also partially explain their protective in vivo effect. It has been previously shown that production of biosurfactants by some strains of *Lactobacillus* can prevent adhesion of pathogens to intestinal cells (Reid, 1999). In this study, *Lactobacillus casei* culture extract demonstrate a growth inhibitory effect of *Listeria monocytogenes.* Zone of inhibition on different concentration *of Lactobacillus casei* culture filtrate were 50µl in 50mm, 100µl in 50mm, 150µl in 54µl and 200µl in 54mm.

Ocimum sanctum belongs to the family *Limiaceae* and is used as an important component for the ayurvedic treatment of various diseases and also possesses several pharmacological properties such as antifertility anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions. Various types of chemical compound such as phospholipids, glycolipids, steroids, caretenoids, phenolic compounds have been isolated from this plant. This similar report agreed with (Huma and Savita 2012). In this study, *Ocimum sanctum* extract was demonstrated to possess a growth inhibitory effect of *Listeria monocytogenes.* Zone of inhibition on different concentration *Ocimum sanctum extract* were 50µl in 40mm, 100µl in 44mm, 150µl in 43µl and 200µl in 46mm.

GC-MS analysis

Lactobacillus casei culture filtrate GC-MS analyzed compounds were Pentadecane, Sulfuric acid, 2ethyl hexyl, 3 isobutyl hexahydro, Butanic acid, Tetracontane, Dotricontane, Hexatricontane, 1,2 benzedicarboxylic acid, Tetra penta contane, Squalene. *Ocimum sanctum* extract GC-MS analyzed compounds were 1,6 octadien-3-ol, Heptan-2-ol, Pentadecane, Phenol,2methoxy-3-2 propan, Cyclohexane, Caryophyllene, 2norpinene,1,4,8,cycloundecatriene, 1,6 cyclodecadiene, Naphthalene, Alphaselinene, 5 oxotricyclo, 2-naphthalenemethanol, Ascorbic acid 2,6dihexa, Haneicossane, Phytol,cis,cis,cis-7,10,13-hexadecattr, Hexacosane, Dotricontane, and Hexatriacontane.

General result of this study has demonstrated that pathogenic *L.monocytogenes* is commonly carried by milk. The pathogens were disseminated in agricultural lands. Therefore, this information is vital to the farmers, the agriculturist, veterinary and medical communites this also concerted efforts and common approach to prevent the spread of fatal foodborne pathogens.

However, there is no doubt that the persistence of poverty (especially in developing countries like India) along with overpopulation, environmental degradation and poor agricultural policies, must have contributed to the persistence of the organisms in the study area environment and farm produce. Until this is checked the incidence of certain food borne organisms like *Listeria* will continue to increase because of changing agricultural, social and economic systems.

The present study clearly indicated the development and use of new drug from antagonistic micro organisms and medicinal herbs to control the pathogenic bacteria especially *L.monocytogenes*. Our final assessment of antilisterial compounds against *Listeria monocytogenes* were identified from *Lactobacillus casei* and *Ocimum sanctum.*

CONCLUSION

From this work, we concluded that the probiotic *Lactobacillus casei* culture filtrate and traditional plant *Ocimum sanctum* extract possess antilisterial activity. The extracts may be used as a food preservative flavoring agent of dairy products.

Table 1: Morphological characterization of *Listeria monocytogenes*

S.NO	Morphological characterization	Results
1	Colony morphology on PALCAM agar	Olive green colonies surrounded by a black halo
2	Brain heart infusion agar	Cream colour
3	Colony shape	Concave colony
4	Colony size	1.7-1 mm
5	Gram's staining	Positive, Rod
6	Motility	28°C, Positive

Table 2: Biochemical characterizations of *Listeria monocytogenes*

S.No	Biochemical test	Results
1	Oxidase test	Negative
2	Indole test	Negative
3	Methyl red test	Positive
4	Voges proskaur test	Positive
5	Citrate test	Positive
6	CAMP test	Positive
7	TSI test	A/A, Positive

Table 3: Morphological characterization *Lactobacillus casei*

S.NO	Morphological characterization	Result
1	MRS Agar	Straw colored colonies
2	Gram's staining	Positive, Rod, spiral
3	Motility	Negative
4	Endospore	Negative
5	Colony size	Smaller

Table 4: Antibiotic sensitivity patterns of *Listeria monocytogenes*

Concentration	*Lactobacilluscasei* Zone of inhibition (mm)	*Ocimum sanctum* Zone of inhibition (mm)	*Ocimum sanctum*+ *Lactobacillus casei* Zone of inhibition (mm)
Control	-	-	-
50µl	50	40	38
100µl	50	44	38
150µl	54	43	40
200µl	54	46	40

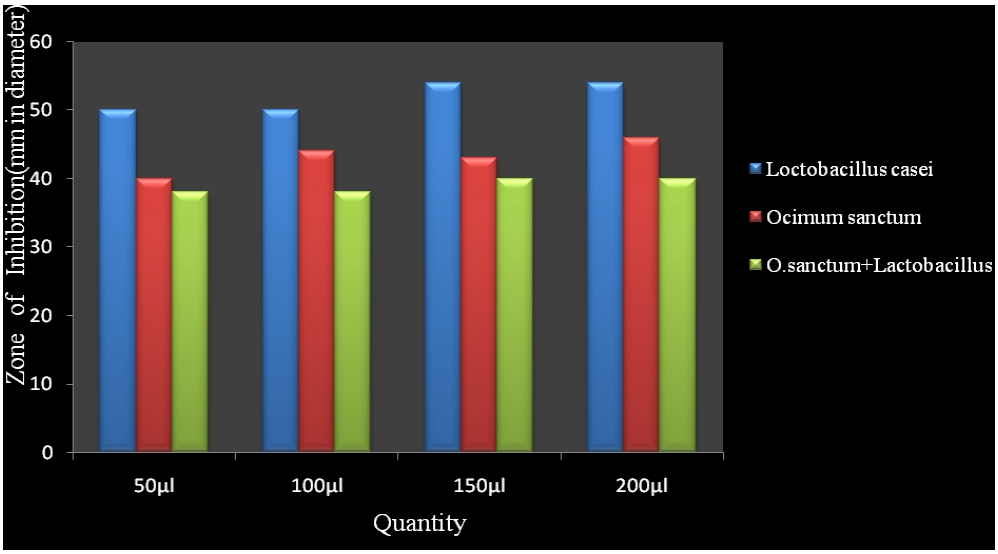

Fig. 1: Antilisterial activity of *Lactobacillus casei*, *Ocimum sanctum*, dual extract of *Ocimum sanctum* and *Lactobacillus casei*

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