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Screening of Bacteria from selected marketed fishes and their sensitivity spectrum to extracts of spicy indigenous plants

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KEYWORDS

Edible herbs, fishes, antibacterial activity, fish pathogenic bacteria, minimum inhibitory concentration

A B S T R A C T

The study was conducted to investigate the antibacterial properties of five edible herbs against pathogenic bacteria isolated from fishes. Herbs extracts including black pepper (*Piper nigrun*), clove (*Syzygium aromaticum),* curry leaf (*Murraya koenigii),* onion *(Allium cepa)*, coriander (*Coriandrum sativum)* were screened against the bacteria *Staphylococcusaureus,Salmonella typhi, Shigella spp,Esherichia coli, Lactobacillus spp* which was isolated from the fishes Sankara (*Lutjanus campechanus*), Koravai (*Channa striata*), Mathi (*Harengula jaguana*), Jilepi (*Oreochromis nioIticus*) which is obtained from local Arcot market. Methanol and aqueous extracts of clove showed the greatest antibacterial activity among all the herbs extracts tested and curry leaf also showed much activity next to clove. Onion, coriander and pepper showed the least activity towards the herbs.The results of the present study suggest that *Syzygium aromaticum*and *Murraya koenigii* can be used in cooking to treat the respective bacteria.

Introduction

Fish is an important source of food for mankind all over the world from the times immemorial. Fish is a source of animal protein in the diets of man. The importance of fish as source of high quality, balanced and easily digestible protein, vitamins and polysaturated fatty acids is well known. The consumption of fish and fish products is recommended as a means of preventing cardiovascular and other diseases and has greatly increased over recent decades in many European countries (Cahu *et al.*, 2004). Like meat, fish and other seafood may be spoiled by autolysis, oxidation, or bacterial activity or most commonly by combinations of these. Most fish flesh, however, is considered more perishable than meat because of rapid autolysis by the fish enzymes and because of the less acid reaction of fish flesh that favors microbial growth. Also, many of the unsaturated fish oils seem to be more susceptible to oxidative deterioration than are most animal fats. The experts agree that the bacterial spoilage of fish does not begin rigor mortis, when juices are released from the flesh fibers(Banerjee *et al*., 2006).

Most of the bacteria associated with fish diseases are naturally saprophytic organisms, widely distributed in the aquatic environment. Comparatively few species are classified as true obligate pathogens. Both groups of organisms may be present on the external body surface or in the tissues of apparently healthy fish. Food borne diseases are the common problems encountered even in these modern days, which is said to be the periodof scientific development and awareness of hygiene(Bishor and Elizebeth, 1989).The amount of antibiotics utilized in the treatment of fish diseases has increased because of intensive fish production increases. Many studies have documented that faulty and the indiscriminate use of antibiotics in aquaculture has led to an increase of antibiotic resistance in various pathogens of fish and the presence of residual antibiotics in seafood and fish products has increased as well. Furthermore, the emergence of bacterial antibiotic resistance might be transmitting from aquaculture environments to humans and animals.As per the World Health Organization (WHO) report, 80% of the world population presently uses herbal medicine for some aspect of primary health care. With the advancement of modern medicinal technology, it is now easier to identify specific botanical constituents and assess their potential antimicrobial activity (Nakatani, 1994).

Herbs have been extensively utilized in human and veterinary medicine. Currently herbs play a considerable role in aquaculture. Several studies have reported that herbal extracts such as garlic and clove, have potential as antimicrobials against various fish pathogens .The use of herbal extracts is widely expected to become an alternative therapy in aquaculture as a prophylactic and to control fish diseases. Studies concerning antimicrobial properties of herbal extracts against bacteria with fish culture importance in vitro and in vivo are still limited (Muniruzzaman and Chowdhry, 2005). Foods and herbs that contain antibiotic properties in our diet can support immune system and help to defend from certain infectious bacteria. There are many foods and herbs known to have natural antibiotic qualities; and with an increased resistance to pharmaceutical antibiotics in people today, it is wise to eat foods that work in our defends on a daily basis. Uncontrolled use of chemical antimicrobial preservatives has been inducing factor for appearance of microbial strains more and more resistant to classic antimicrobial agents. Difficult to control the microbial survival, showed by isolation of multi-resistant strains, has been reported all over the world. Fifty years of increasing use of chemicals antimicrobials have created a situation leading to an ecological imbalance and enrichment of multiples multi-resistant pathogenic microorganisms (Levy, 1997).

Fish oil is sometimes used after heart transplant surgery to prevent high blood pressure and kidney damage that can be caused by the surgery itself or by drugs used to reduce the chances that the body will reject the new heart. Fish oil is sometimes used after coronary artery bypass surgery. It seems to help keep the blood vessel that has been rerouted from closing up. When fish oil is obtained by eating fish, the way the fish is prepared seems to make a difference. Eating broiled or baked fish appears to reduce the risk of heart disease, but eating fried fish or fish sandwiches not only cancels out the benefits of fish oil, but may actually increase heart disease risk (Nayak, 2010).

Materials and methods

Plant materials

The herbs (pepper, curry leaves and clove) collected were shadow dried and powdered well in mixer grinder whereas, onion bulb and coriander leaves were freshly taken washed in running tap water and fresh extract was taken using mixer grinder.

Extraction procedure

One gram of dry powder was mixed up in 10ml of distilled water and for fresh extracts 1ml of fresh extract is mixed in 9 ml of distilled water. Methanol was also used as a solvent following the same procedure as that of water.

Isolation and Identification of Bacteria

Standard Bacteriological methods were followed to isolate and identify the bacteria from fish samples

Antibacterial assay

Agar well diffusion method

The antibacterial activity of bacterial strains against edible herbal extracts were assayed by Agar well diffusion method at different concentrations of well 20µl, 40µl, 60µl, 80µl and 100µl respectively and crude extract in aqueous medium has done in 20µl concentration. The results were tabulated in table-I,II,III,IV, V and VI. The minimum inhibitory concentrations were also determined by broth dilution method at 520 nm in colorimeter. Based on the sensitivity among the plant materials in the antibacterial activity, the MIC has done for cloves and curry leaves for which the organisms showed much sensitivity and their results were tabulated in the following tables- VII and VIII.

Result and Discussion

Antibacterial assay

Agar well diffusion method

In curry leaves S. aureus showed sensitivity in 40µl and above concentrations, Lactobacillus spp showed sensitivity in 60µl and above concentrations, S.typhi showed sensitivity in 80 and 100µl concentrations, whereas Shigella spp and E. coli showed sensitivity only at 100µl concentrations. The data were depicted in table-II.

In cloves S.aureus, S.typhi and Shigella spp showed senstivity at 60µl and above concentrations, whereas E.coli and Lactobacilus spp showed sensitivity at 40µl concentrations itself.The data were depicted in table-III.

In antibacterial assay of pepper all the strains showed sensitivity towards 100µl concentrations.The data were depicted in table-IV.

In onion S.typhi and Lactobacillus spp showed sensitivity in 100µl concentrations.The data were depicted in table-V.

In coriander Lactobacillus spp alone showed sensitivity in 100µl concentrations.The data were depicted in table-VI.

Table.I Antibacterial activity against herbal extracts(Aqueous medium)

MICRO ORGANISM	PEPPER	CLOVE	CURRY LEAVES	CORIANDER	ONION	CHLORAMPHENICOL
S.aureus	No zone	18mm	No zone	No zone	No zone	19mm
Escherichia coli	No zone	18mm	26mm	No zone	No zone	20mm
Salmonella typhi	No zone	10mm	14mm	No zone	No zone	7mm
Shigella spp	No zone	15mm	No zone	No zone	No zone	15mm
Lactobacillus spp	No zone	17mm	5mm	No zone	No zone	11mm

Fig.1 Antibacterial activity against herbal extracts (Aqueous medium)

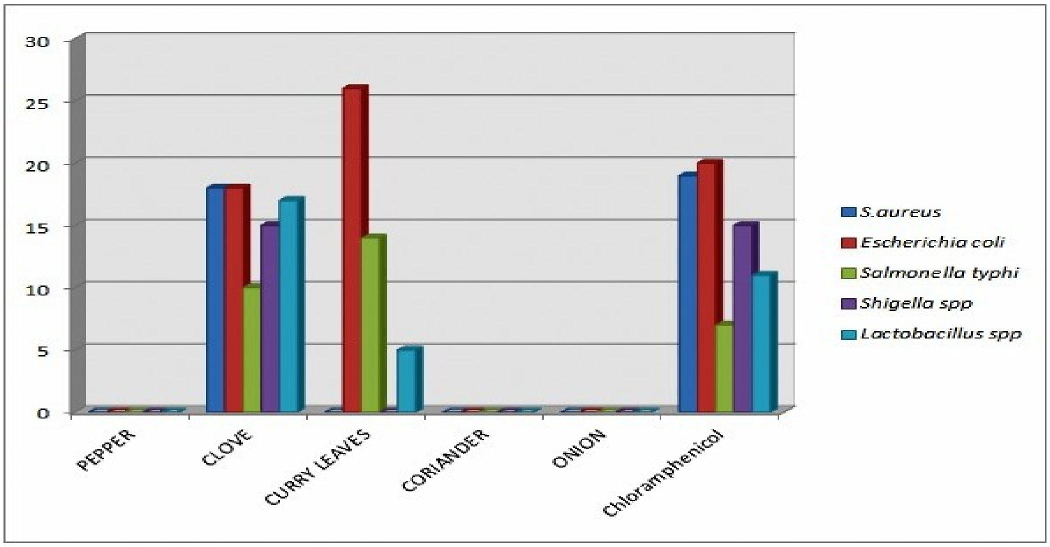


Table.II Antibacterial activity of curry leaves

ISOLATED ORGANISMS	ZONE OF INHIBITION (mm)					CHLORAMPHENICOL
	20 µl	40 µl	60 µl	80µl	100 µl	
S.aureus	15	19	21	25	27	19
Escherichia coli	13	13	13	15	17	20
Salmonella typhi	7	13	15	18	21	7
Shigella spp	7	12	13	15	17	15
Lactobacillus spp	5	13	19	19	20	11

Fig.2 Antibacterial activity of curry leaves

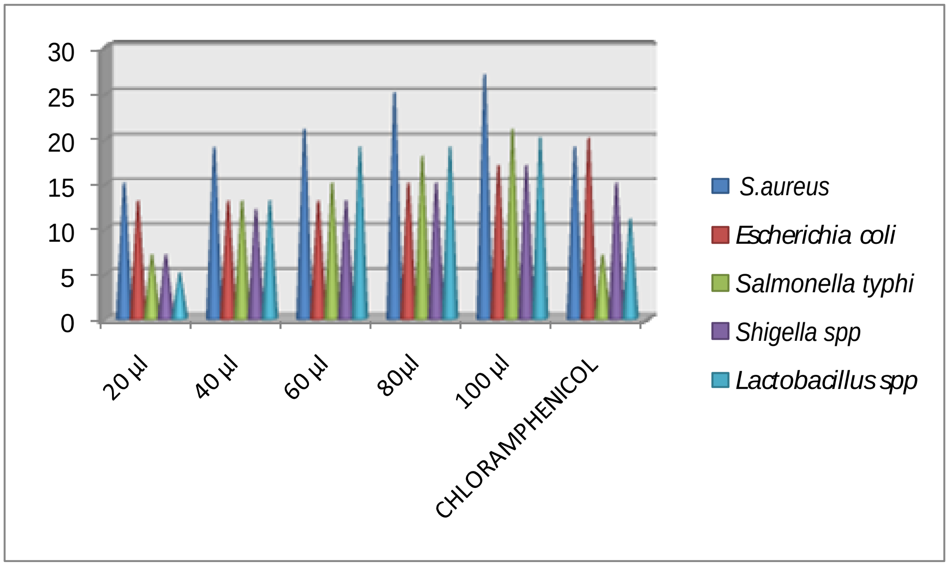


Table.III Antibacterial activity of cloves

ISOLATED ORGANISMS	ZONE OF INHIBITION (mm)					CHLORAMPHENICOL
	20 µl	40 µl	60 µl	80µl	100 µl	
S.aureus	7	19	20	22	22	19
Escherichia coli	10	20	22	23	25	20
Salmonella typhi	10	15	20	21	25	7
Shigella spp	7	11	20	22	22	15
Lactobacillus spp	5	16	18	20	25	11

Fig.3 Antibacterial activity of cloves

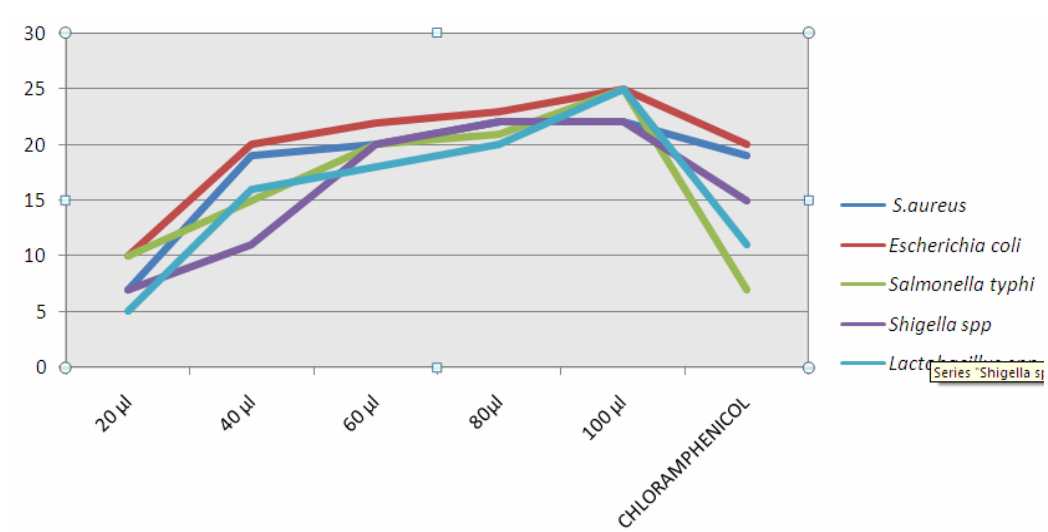


Table.IV Antibacterial activity of pepper

ISOLATED ORGANISMS	ZONE OF INHIBITION (mm)					CHLORAMPHENICOL
	20 µl	40 µl	60 µl	80µl	100 µl	
S.aureus	No zone	5	10	10	19	19
Escherichia coli	No zone	10	12	13	16	20
Salmonella typhi	No zone	7	15	16	16	7
Shigella spp	7	7	11	12	18	15
Lactobacillus spp	5	10	10	15	20	11

Fig.4 Antibacterial activity of pepper

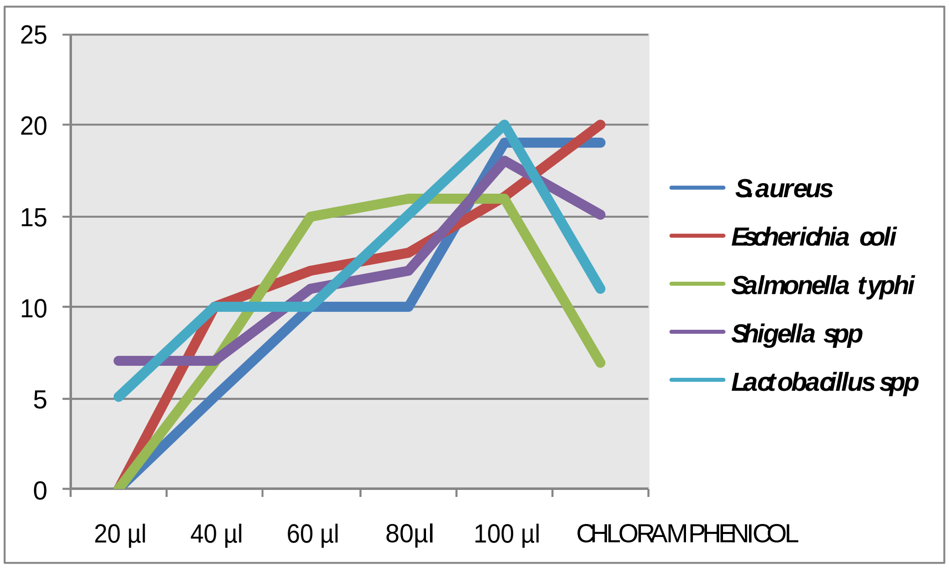


Table.V Antibacterial activity of onion

ISOLATED ORGANISMS	ZONE OF INHIBITION (mm)					CHLORAMPHENICOL
	20 µl	40 µl	60 µl	80µl	100 µl	
S.aureus	No zone	No zone	10	11	13	19
Escherichia coli	No zone	No zone	No zone	No zone	No zone	20
Salmonella typhi	No zone	8	12	15	19	7
Shigella spp	No zone	7	10	12	15	15
Lactobacillus spp	No zone	8	11	11	15	11

Fig.5 Antibacterial activity of onion

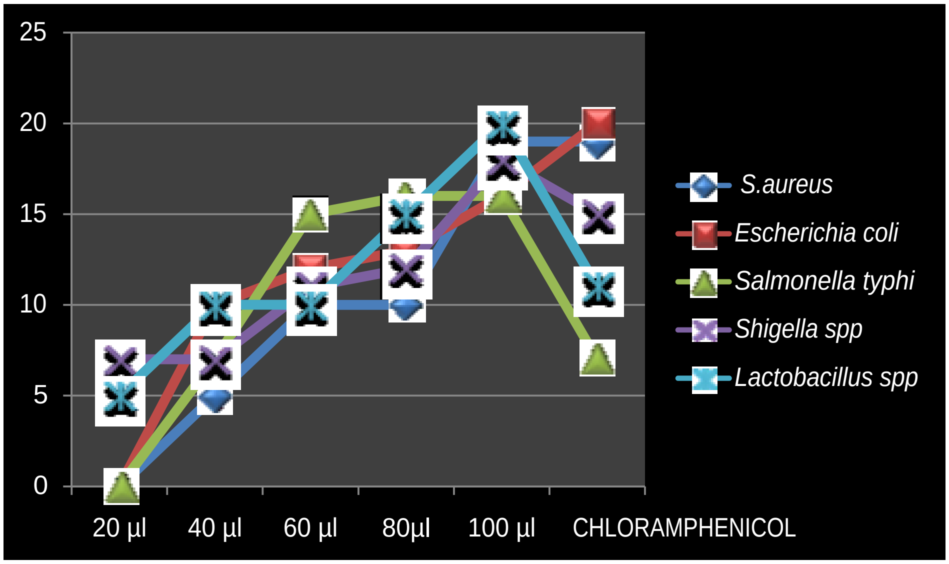
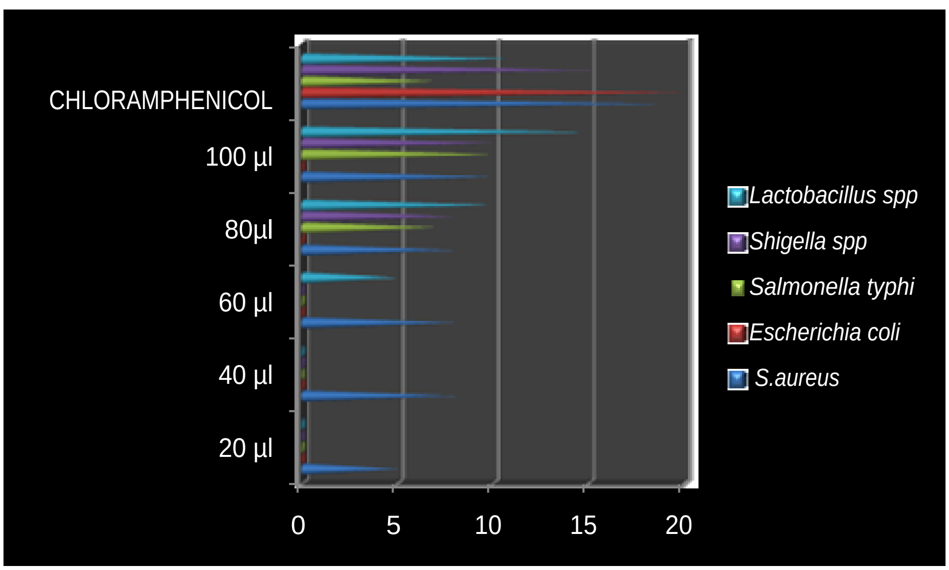


Table.VI Antibacterial activity of coriander

ISOLATED ORGANISMS	ZONE OF INHIBITION (mm)					CHLORAMPHENICOL
	20 µl	40 µl	60 µl	80µl	100 µl	
S.aureus	5	8	8	8	10	19
Escherichia coli	No zone	No zone	No zone	No zone	No zone	20
Salmonella typhi	No zone	No zone	No zone	7	10	7
Shigella spp	No zone	No zone	No zone	8	10	15
Lactobacillus spp	No zone	No zone	5	10	15	11

Fig.6 Antibacterial activity of coriander



Minimum Inhibitory Concentration
(Broth dilution method)

Table.VII Minimum inhibitory concentration of curry leaves

Bacteria	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Control
S.aureus	0.02	0.02	0.03	0.07	0.20	0.27	0.03
Escherichia coli	0.03	0.03	0.35	0.41	0.42	0.46	
Salmonella typhi	0.03	0.03	0.09	0.11	0.28	0.33	
Shigella spp	0.02	0.03	0.19	0.20	0.34	0.50	
Lactobacillus spp	0.02	0.03	0.07	0.20	0.43	0.51	

Fig.7 Minimum inhibitory concentration of curry leaves

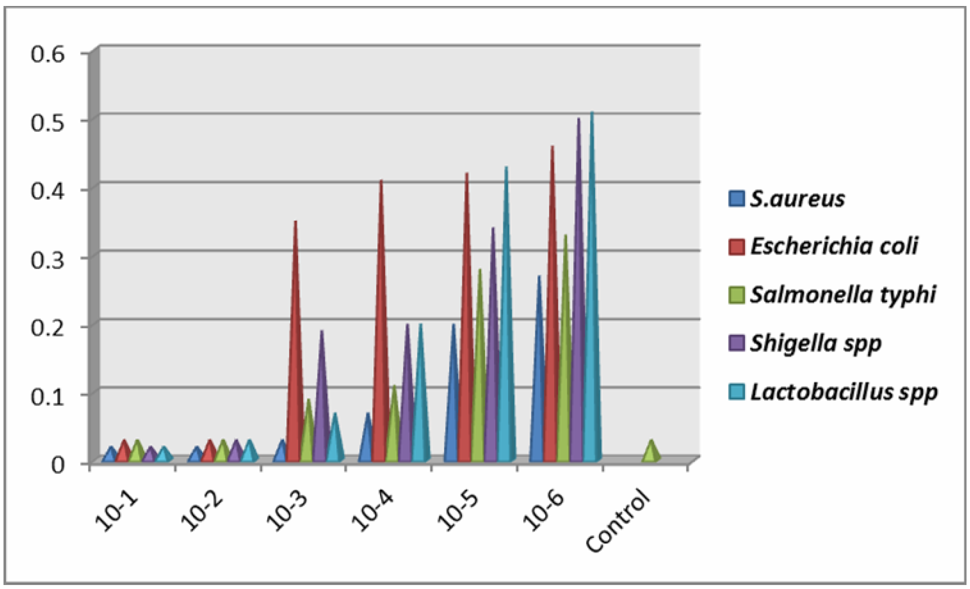
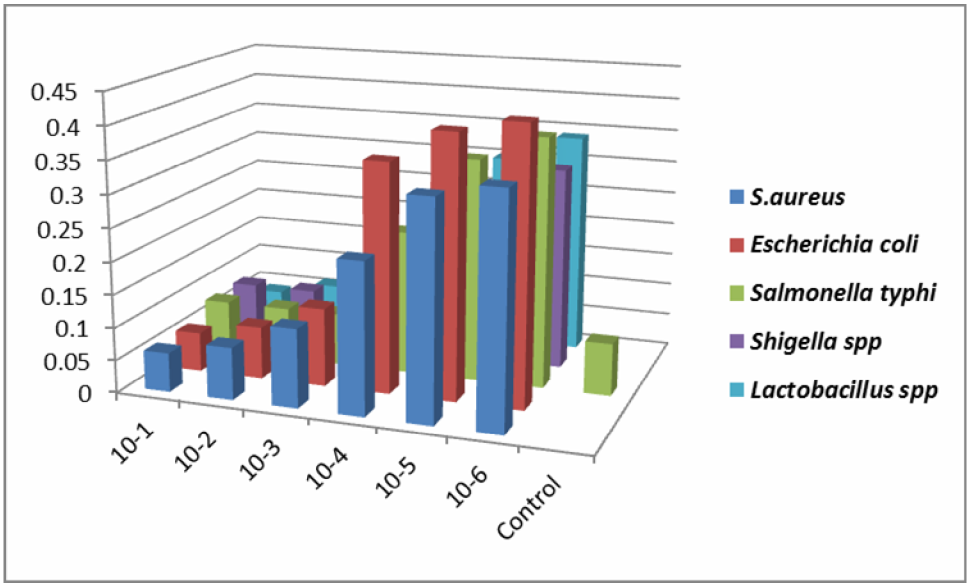


Table.VIII Minimum inhibitory concentration of cloves

Bacteria	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Control
S.aureus	0.06	0.08	0.12	0.23	0.33	0.35	0.08
Escherichia coli	0.06	0.08	0.12	0.35	0.40	0.42	
Salmonella typhi	0.08	0.08	0.08	0.22	0.34	0.38	
Shigella spp	0.08	0.08	0.08	0.24	0.26	0.31	
Lactobacillus spp	0.04	0.06	0.08	0.20	0.30	0.34	

Fig.8 Minimum inhibitory concentration of cloves



Food poisoning caused by different kinds of microorganism are well studied worldwide. As fishes are considered the major food items used by majority of the population, their quality and their contribution to food infections and intoxications must be considered. Many pathogens isolated in this study not necessarily come straight away from the aquatic environment for the fishes tested. Fishes may be spoiled after the entry in to the market area were spoilage causing organism, become permanent inhabitant, because of the poor environmental hygiene and human fecal pollution. Contaminants and other spoilage-causing agent would have entered from the fishes transported earlier or in the past to the marketed area.

Two edible fishes, Bulls eye, *Priacanthus hamur* and Hard tail scad, *Megalaspis cordyla* from the waters of Royapuram coast, Chennai, Tamil Nadu were chosen for isolation of bacterial human pathogens in their gills, intestine, muscle and skin. Based on their growth characteristics on specific culture media, the following human bacterial pathogens, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Shigella dysenteriae* were isolated in both the fishes. The medium of the fishes and the sediments of their habitat were furthermore collected and examined for pathogens. Different bacterial species were found in the medium that was analyzed, including the human pathogens isolated from the fishes. Surprisingly the five human bacterial pathogens harbored in the fishes were found in the medium but not in the sediment. This research is primarily to highlight the quality of these two edible fishes in the coastal waters of Royapuram, Chennai and to create awareness amid fish eating population (Sujatha *et al*., 2011).

In this study bacterial pathogens from fishes of (edible fishes like Sankara, Koravai, Mathi and Jilepi) Vellore fish market.were selected for the isolation of bacteria. *E. coli*, *Salmonella typhi*, *Shigella spp*, *Staphylococcus aureus*, *Lactobacillus spp* were the predominant organisms isolated from those fishes. These clearly indicated the possibilities of involvement of human being in the transmission of above organism as well as other pathogenic and non-pathogenic forms.They were identified and confirmed bymorphological, cultural, and biochemical characterization.

The antibacterial activity of the two plant extracts and their synergism were studied against some MDR strains. The tested organisms particularly *Klebsiella pneumoniae* were found to be inhibited by *Murraya koenigii* extract and also the combination of *Murraya koenigii* and *Coriandrum sativum* in comparison to *Coriandrum sativum* alone. The combinations of the two extracts in various ratios were found to be effective towards MDR strains. On increasing the proportion of the *Murraya koenigii* and *Coriandrum sativum* the inhibitory effect increased. The extracts were found to be effective against all the test organisms except *Salmonella typhi*. MIC was determined by using broth dilution method. The results supported the notion that Synergism of extracts in various ratios is inhibitory towards Multi-drug resistant strains.

The extracts of ten culinary spices were screened to identify their antimicrobial activities against*Streptococcus agalactiae* by using disk diffusion assay. Only *Cinnamomum verum*, *Allium sativum Linn*, *Eugenia caryophyllus* and *Thymus vulgaris* displayed antimicrobial activity. The bark *C. verum* extract displayed the highest antimicrobial activity with a 18 mm inhibition zone. The minimum inhibitory concentration (MIC) values for spice extracts were determined by utilizing the agar diffusion method. The lowest MIC value with high efficacy against *S. agalactiae* was 0.15 mg\mL, which was obtained from *C. verum* extract. The median lethal dose (LD) of *S. agalactiae* to tilapia fingerlings was measured to be 1.56×10 CFU/mL (Hoshino *et al.*,1996).

The antibacterial activity of locally available plant extracts against pathogenic bacteria of aquatic animals namely, *Aeromonas hydrophila, Aeromonas sobria, Vibrio paraharmolyticus.* Five plants viz: *Terminalia arjuna*(Arjuna), *C. asiatica* (Thankuni), *Ziziphus mauritiana* (Kul), *Murraya koenigii* (Kari), *Ocimum sanctum* (Tulsi) were selected for in vitro antibacterial activity assay. The Minimum Inhibitory Concentration of the methanolic extracts of the leaves of the said plants was determined by Disc Diffusion method. The plant extracts except tulsi showed antibacterial activity against all the three bacteria. Significant inhibitory activity was found in *C. asiatica, T. arjuna, M. koengii, Z. maurtitiana* respectively. Methanol was used as positive control and Tetracycline hydrochloride was used as negative control. Higher concentration of methanolic extract showed better result consistently for all the plants than lower concentration. The most effective inhibitory activity was observed in *C. asiatica* against *A. sobria*(Banerjee, 2010).

In the global food industry more priority is given to natural preservatives as there is increase occurrence of resistance in pathogenic strains against chemical food preservatives (George, 2009). It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists (Lewis *et al.*, 2006).The presence of saponins, tannins, alkaloids, flavonoids, steroids and glycosides provoked for an in-depth study on the plant. The metabolites are of various pharmacological importances. Many triterpene saponins and their aglycones have been reported to have varied uses as anti ulcerogenic, anti-inflammatory, fibrinolytic,antipyretic,analgesic and anti-edematous in action.

The presence of tannin in most of plant extract could be responsible for possible antitumor and antioxidant activities (Hostettmann, 1995).

In Unani medicine, black pepper has been described as an aphrodisiac and as a remedy to alleviate colic. A preparation called 'jawa rishai thurush' is composed of pepper, ginger, salt, lemon juice and the plants vidanga (*Embelia ribes*) and mint (Menthaspecies). It has been prescribed to alleviate indigestion and stomach acidity (Ensminger andEnsminger, 1986).

Piperine present in black pepper acts as a thermogenic compound. Piperine enhances thermogenesis of lipid and accelerates energy metabolism in the body also increases the serotonin and beta-endorphin production in the brain. The disc diffusion techniques was employed by using the aqueous decoction of black pepper , bay leaf, ani seed, coriander against the 176 bacterial isolates belonging to 12 different genera of bacterial population isolated from oral cavity of 200 individual. The antibacterial activity of aqueous decotion of black pepper exhibited 75% bay leaf 53.4% and ani seed 18.1% at the concentration of 10µl/disc (Nazia and Parween, 2006).

The extracts of several herbs and spices (honeysuckle, scutellaria, forsythia suspense, cinnamon and rosemary with 75% ethanol from clove oil dissolved in 75% ethanol) in agar media to observe their inhibitory effects on the g5rowth of *Escherichia coli, Pseudonamas fluorescence* and *Lactobacillus plantarum.* All the extracts suppressed the growth of these bacteria *Scuttellaria* exhibited the strongest effect against *Escherichia coli.* The inhibitory activity of mixtures of cinnamon and clove oils against important spoilage microorganisms and *Staphylococcus aureus* and *Pediococcus halophilus* were found to be sensitive (Matan *et al.*, 2006).

The effectiveness of cardamom, anise, basil, coriander, rosemary, parsley, dill and angelica essential oil for controlling the growth and survival of pathogenic and saprophytic microorganisms was studied. The results showed inhibitory property for oregano, basil and coriander essential oil, which presented minimum lethal concentration (v/v) ranging between 8 and 50ppm for *Pseudomonas aeruginosa, Stahylococcus aureus* and *Yersinia enterocolitica*. The antimicrobial activity of garlic, ginger, clove, black pepper and ground green chilli and their aqueous extracts on human pathogenic bacteria including *Bacillus sphaericus, Staphylococcusaureus,S. epidermidis, Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa,Salmonella typhi* and *Shigella flexneri* and found that all tested bacteria were sensitive to ground garlic and its extract (Elgayyar *et al*., 2001).

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Alfolayan, 2003). Similarly, in another study clove oil was found active against food borne Gram positive bacteria and Gram-negative bacteria. Futhermore, active constituents of clove (biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanoic acid) possess antibacterial activities against Gram-negative anaerobic periodontal oral pathogens, including *Streptococcus mutans,Actinomycetes viscosus, Porphyromonas* and *Prevotella intermedia* (Cai and Wu, 1996).

The results of this present investigation has clearly indicated that the above said plants, though already used as ingredient of food in one way or another, paves a scientific way of proving their effectiveness as good antibacterial agents. Hence, they could be safely used as food preservative to prevent contamination and spoilage of different type of food and their raw materials and also to prevent, control and treat human and animal diseases caused by those bacterial species. Further investigation is needed to find the actual ingredient responsible for antimicrobial activity and also the real toxicity of the extracts as well as those ingredients to various organs such as liver kidney, heart and lungs.

In the present study, Antibacterial activity of plant materials such as curry leaves, clove-flower bud, coriander leaf, onion bulb, and pepper seed was done against the bacterial isolates. The organisms showed sensitivity towards all the herbs and the organisms showed more sensitivity towards curry leaves and cloves. Based on the sensitivity among the plant materials in the antibacterial activity, the MIC has done for cloves and curry leaves and there results were tabulated with its OD value at 520 nm. In Curry leaves *S.aureus* showed minimum inhibition on 250µg/ml concentrations whereas *E.coli*, *Lactobacillus spp*, *S.typhi* and *Shigella spp* showed inhibition on 500µg/ml concentrations. In MIC, of cloves *S. aureus*,and *E.coli* showed minimum inhibition on 500µg/ml concentrations, Whereas *Shigella spp*,*Shigella spp* and *Lactobacillus spp* showed minimum inhibition on 250µg/ml concentration towards the herb.

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