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# *In vitro* evaluation of herbal decoctions in reducing *Vibrio cholerae* on *Chicoreus ramosus* meat

**P. Ashok Kumar[1]*, P. Karpagam[2] and Jamila Patterson[2]**

[1]Department of Microbiology, Sokoto State University, Sokoto, Nigeria.
[2]Sri Kaliswari College, Sivakasi, Tamil Nadu, India.
[3]Suganthi Devadason Marine Research Institute, Tuticorin – 628 001, Tamil Nadu, India.

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**India is rich in its plants diversity, a number of plants have been documented for their medicinal potential, which are in use by the traditional healers, herbals folklorists and in Indian systems of medicine. Contaminated water with free-living *Vibro cholerae* cells are probably the main origin of epidemics, followed to a lesser extent by contaminated food, especially seafood products like oysters, crabs, and shellfish. Thirteen potent medicinal herbs were finally chosen from all the 45 medicinal plants collected from Thiruvannamalai mountain hills, for the preparation of decoctions. Studies were done to determine the effectiveness of herbal decoctions in killing *V. cholerae* inoculated onto raw *Chicoreus ramosus* meat. Preliminary studies were done to screen aqueous decoctions of herbs commonly used to soften meat for their ability to inhibit the growth of *V. cholerae* before doing immersion treatments of inoculated *C. ramosus*. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these medicinal herbs were more effective than traditional antibiotics to combat the pathogenic microorganism studied. The effect of herbal decoctions on the bacterial populations of *V. cholerae* in *C. ramosus* meat may be attributed to the higher degree of antimicrobial activity exhibited by these herbal decoctions.**

**Key words:** Herbal decoctions, *Vibrio cholerae*, *Chicoreus ramosus,* medicinal plants, sea foods.

## INTRODUCTION

Plants have been an integral part of human society since the start of civilization. India has diversity plant species amongst which a great number of plants possess medicinal properties, used by the traditional healers, herbals folklorists and in Indian systems of medicine namely, Ayurveda, Unani, Siddha apart from Homeopathy and Electropathy. A number of pharmaceutical companies, both national and international, are utilizing the plant-based formulations/extractions against various diseases and disorders (Kirtikar and Basu, 1935; Singh and Gautam, 1997; Khan et al., 2002). Plant based components were the original therapeutic interventions used by man to control diseases in humans and livestock. Nearly all cultures from ancient times to the present day have used plants as a source of medicines (Lino and Deogracious, 2005). Much interest has been centered on utilization of plant-derived compounds as antimicrobials in foods (Davidson and Naidu, 2000). These naturally occurring compounds have potential to expand the narrow spectrum of activity afforded by the traditional, regulatory-approved antimicrobial food preservatives. The use of herbal medicines in developed countries is also growing and 25% of the UK population is dosed with herbal medicines regularly (Canter et al., 2005). Natural products of higher plants may offer a new source of antibacterial agents for external use e.g. compresses,

*Corresponding author. E-mail: drpashokkumar@gmail.com. Tel: +234 8113512595

cataplasm, gargles and ointments (Brantner and Grein, 1994).

Contaminated water with free-living *Vibro cholerae* cells are probably the main origin of epidemics, followed to a lesser extent by contaminated food, especially seafood products like oysters, crabs, and shellfish (DePaola, 1981; Kaysner and Hill, 1994). It was shown that toxigenic O1 *V. cholerae* can survive refrigeration and freezing in food supplies shipped internationally, therefore an epidemic strain may travel far from its original endemic location (Centers for Disease Control, 1991).

*Chicoreus ramosus* is one of the large sized gastropods and forms a major fishery along the coast of Gulf of Mannar. The *Chicoreus* meat is delicious and proteinaceous, and the demand for this gastropod has increased considerably (Jamila et al., 1994). The meat gets contaminated to a larger extent due to the unhygienic practices followed by the local fisher folks.

Spoilage of fresh and refrigerated poultry meat is attributed by growth and metabolic activities of bacteria (Barnes and Impey, 1968; Barnes, 1976; Jackson et al., 1997). In earlier studies, pathogenic bacteria such as *Vibrio parahaemolyticus, Escherichia coli* and *Salmonella* associated with *C. ramosus* were also reported (Jamila et al., 1996). Essential oils and aqueous extracts (decoctions) of other culinary herbs are known to inhibit the growth of yeasts (Conner and Beuchat, 1984; Beuchat, 1994; Sofos et al., 1998).

Aqueous extracts, the most frequent form of application in traditional medicine, were tested for their antibacterial activity to determine whether the empirical application of plant drugs could be supported by scientific examination. In the present study, forty-five higher plants which have been described in ancient herbal books and medicinal folklore reported as vulneraries were studied for antibacterial activity.

## MATERIALS AND METHODS

The authenticated plant materials (Table 1) used in this study was collected from Thiruvannamalai mountain hills. Preliminary studies were done to screen decoctions of herbs for their ability to inhibit the growth of *V. cholerae* before doing immersion treatments of inoculated *C. ramosus*. The plant extracts were prepared using the modified method of Alade and Irobi (1993). The powdered plant materials such as leaves, flowers, seeds, fruits, barks and subterranean parts (roots, rhizomes) (100 g) were soaked in 500 ml of distilled water for 72 h. Then, the mixture was refluxed followed by agitation at 200 rpm in a rotary shaker for 1 h. The filtrate obtained was concentrated under vacuum at 40°C to obtain the dry extract. The modified agar well diffusion method was employed for screening the antimicrobial activity (Perez et al., 1990).

Thirteen potent medicinal herbs were finally chosen from all the 45 medicinal plants for the preparation of decoctions. Herbs were coarsely chopped and 100 g were soaked in 200 ml of boiling water. The submerged herbs were boiled for 30 min with frequent mixing, and then allowed to set at room temperature for 1 h and filtered through sterile glass wool in a funnel. Filtrates were considered as 100% herb decoctions (Ismail et al., 2001).

Raw *C. ramosus* meat was purchased from the local retailer and brought to the laboratory. The meat was cleaned and kept in deep freezer (-18°C) until used for the experiments. *V. cholerae* strain used in this study was obtained from Christian Medical College (CMC), Vellore, India. A loopful of *V. cholerae* was transferred to fresh nutrient broth and was incubated at room temperature for 24 h followed by adding 2 ml of the *V. cholerae* to 100 ml of sterile Alkaline Peptone Water (APW) and thoroughly mixed. The meat surface inoculation was carried out by using the standard procedure described by Ismail et al. (2001). Raw *C. ramosus* meat (10 g) was submerged in the *V. cholerae* suspension and gently mixed for 1 min. Meat was drained on sterile wire screen for 10 to 15 min and sealed separately in polythene bags. They were stored in refrigerator for 0, 48 and 96 h.

Studies were done to determine the effectiveness of herbal decoctions for bactericidal activity towards *V. cholerae* inoculated onto raw *C. ramosus* meat. The efficacy of treatments in reducing populations of naturally occurring aerobic microorganisms on raw *C. ramosus* meat was evaluated. Four meat samples were analyzed for each treatment and two replicate trials were done. To each inoculated raw *C. ramosus* meat in the polythene bag, 100 ml of APW (control) or 100% herbal decoctions were added. The Alkaline Peptone Water (APW) and all herbal decoctions were stored at room temperature. The bags were sealed and vigorously shaken by hand for 1 min. The treated meat was transferred to sterile plastic bags and stored in the refrigerator. The inoculated raw *C. ramosus* meats stored in the refrigerator were analyzed for populations of *V. cholerae* and total aerobic microorganisms within 1 h of treatment (0 day storage) and after storage for 24, 48 and 96 h. The serially diluted samples were pour-plated on Thiosulphate Citrate Bile Sucrose (TCBS) Agar for *V. cholerae* (Karunasagar et al., 1986). The plates were incubated at room temperature for 24 h and the colonies were counted.

## RESULTS

Among the 45 medicinal herbs (belonging to thirty different families), only 30% (13 plants) of the medicinal herbal decoctions of different plant parts tested exhibited a pronounced antibacterial effect against *V. cholerae*. *Cleom gynandra* (leaf), *Launaea sarmentosa* (leaf), *Cordia obliquawillel* (leaf), *Cassia angustifolia* (leaf), *Abrus precatorius* (leaf), *Smilax china* (leaf), *Sida cordifolia* (leaf), *Tinospora cordifolia* (leaf), *Moringa oleifera* (leaf), *Aegle marmelos* (fruit), *Alpinia galangal* (leaf) and *Lippia nodiflora* (rhizome) produced outstanding antibacterial effects against *V. cholerae* (Table 1). *Alternanthera sessilis, Gymnema sylvestra, Pergularia extensa, Berberis aristata, Cadaba tarinosa, Terminalia belerica, Eclipta prostrate, Ipomoea obscura, Melothria maderaspatana, Phyllanthus niruri, Acalypha indica, Taraktogenous kurzii, Enicostimma littorale, Piper longum, Mucuma prurita, Metia azedarach, Cocculus hirsatus, Myristica fragrans, Morinda coreia, Trachyspermum ammi,* and *Centella asiatica* showed no activity against *V. cholerae*.

Significant reduction in the population of *V. cholerae* occurred when the *C. ramosus* meat was dipped into the potent herbal decoctions. Based on the results from the effectiveness of herbal decoctions in killing *V. cholerae* inoculated onto raw *C. ramosus* meat, *A. marmelos, A. galangal, C. gynandra, C. obliquawillel, M. oleifera, S.*

**Table 1.** Antibacterial screening of 45 medicinal plant decoctions against *V. cholerae* by means of agar diffusion method.

| S/N | Family name | Botanical name | Part | *V. cholerae* |
|---|---|---|---|---|
| 1 | Amaranthaceae | *Alternanthera sessilis* | Leaf | - |
| 2 | Asclepiadaceae | *Gymnema sylvestra* | Leaf | - |
| 3 | | *Pergularia extensa* | Leaf | - |
| 4 | Berberidaceae | *Berberis aristata* | Root | - |
| 5 | Capparaceae | *Cleom gynandra* | Leaf | ++++ |
| 6 | Capparidaceae | *Cadaba tarinosa* | Leaf | - |
| 7 | Combretaceae | *Terminalia belerica* | Fruit | - |
| 8 | Compositae | *Eclipta prostrate* | Plant | - |
| 9 | | *Launaea sarmentosa* | Leaf | ++++ |
| 10 | Convolvulaceae | *Ipomoea obscura* | Leaf | - |
| 11 | Cordiaceae | *Cordia obliquawillel* | Leaf | ++++ |
| 12 | Cucurbitaceae | *Melothria maderaspatana* | Leaf | - |
| 13 | Euphorbiaceae | *Phyllanthus niruri* | Plant | - |
| 14 | | *Euphorbia hirta* | Leaf | +++ |
| 15 | | *Emblica officinalis* | Fruit | +++ |
| 16 | | *Acalypha indica* | Leaf | - |
| 17 | Flacoureaceae | *Taraktogenous kurzii* | Seed | - |
| 18 | Gentianaceae | *Enicostimma littorale* | Plant | - |
| 19 | Gramineae | *Vetiveria zizanioides* | Root | +++ |
| 20 | Labiatae | *Ocimum basilicum* | Leaf | +++ |
| 21 | | *Piper longum* | Fruit | - |
| 22 | Leguminaceae | *Cassia angustifolia* | Leaf | ++++ |
| 23 | | *Mucuma prurita* | Seed | - |
| 24 | Leguminosae | *Abrus precatorius* | Root | ++++ |
| 25 | | *Trigonella foenum graecum* | Seed | ++ |
| 26 | | *Agati grandiflora desv* | Leaf | ++ |
| 27 | Liliaceae | *Asparagus racemosus* | Leaf | +++ |
| 28 | | *Smilax china* | Root | ++++ |
| 29 | Malvaceae | *Abutilon indicum* | Leaf | +++ |
| 30 | | *Sida cordifolia* | Leaf | ++++ |
| 31 | Marsileaceae | *Marsilea quadrifolia* | Leaf | ++++ |
| 32 | Meliaceae | *Metia azedarach* | Leaf | - |
| 33 | Menispermaceae | *Tinospora cordifolia* | Leaf | ++++ |
| 34 | | *Cocculus hirsatus* | Leaf | - |
| 35 | Moringaceae | *Moringa oleifera* | Leaf | ++++ |
| 36 | Myristicaceae | *Myristica fragrans* | Aerial | - |
| 37 | Rubiaceae | *Morinda coreia* | Bark | - |
| 38 | Rutaceae | *Aegle marmelos* | Fruit | ++++ |
| 39 | | *Murraya koenigii* | Leaf | ++ |
| 40 | Solanaceae | *Solanum nigum* | Leaf | ++ |
| 41 | | *Withania somnifera* | Root | ++ |
| 42 | Umbelliferae | *Trachyspermum ammi* | Seed | - |
| 43 | | *Centella asiatica* | Leaf | - |
| 44 | Verbenaceae | *Lippia nodiflora* | Leaf | ++++ |
| 45 | Zingiberaceae | *Alpinia galanga* | Rhizome | ++++ |

++++: ≥19 mm inhibition zone; +++: ≥17mm inhibition zone); ++: ≥12 mm inhibition zone; -: ≤5 mm inhibition zone.

**Table 2.** Mean populations of *V. cholerae* ($log_{10}$ cfu/g) on *C. ramosus* meat dipped in herbal decoctions.

| Botanical name | 1 h | 24 h | 48 h | 96 h |
|---|---|---|---|---|
| Control | 3.39 ± 0.02 | 3.26 ± 0.21 | 3.19 ± 0.34 | 2.76 ± 0.63 |
| *Abrus precatorius* | 3.36 ± 0.01 | 2.84 ± 0.23 | 0.82 ± 1.43 | Nil |
| *Aegle marmelos* | 3.36 ± 0.09 | 1.99 ± 1.73 | 0.68 ± 1.17 | Nil |
| *Alpinia galanga* | 3.36 ± 0.03 | 3.01 ± 0.1 | 0.76 ± 1.32 | Nil |
| *Cassia angustifolia* | 3.31 ± 0.05 | 2.79 ± 0.25 | 0.82 ± 1.43 | 0.76 ± 1.32 |
| *Cleom gynandra* | 3.35 ± 0.04 | 2.88 ± 0.16 | 0.92 ± 1.6 | Nil |
| *Cordia obliquawillel* | 3.36 ± 0.09 | 2.82 ± 0.3 | 0.76 ± 1.32 | Nil |
| *Launaea sarmentosa* | 3.36 ± 0.03 | 2.05 ± 1.77 | 0.98 ± 1.7 | 0.76 ± 1.32 |
| *Lippia nodiflora* | 3.39 ± 0.03 | 1.98 ± 1.72 | 0.86 ± 1.5 | 0.68 ± 1.17 |
| *Marsilea quadrifolia* | 3.37 ± 0.05 | 2.92 ± 0.27 | 0.98 ± 1.7 | 0.76 ± 1.32 |
| *Moringa oleifera* | 3.34 ± 0.03 | 2.94 ± 0.22 | 0.89 ± 1.55 | Nil |
| *Sida cordifolia* | 3.40 ± 0.04 | 2.82 ± 0.22 | 0.76 ± 1.32 | Nil |
| *Smilax china* | 3.39 ± 0.03 | 2.08 ± 1.8 | 1.73 ± 1.5 | 0.68 ± 1.17 |
| *Tinospora cordifolia* | 3.39 ± 0.04 | 2.76 ± 0.32 | 0.86 ± 1.5 | Nil |

Data are presented with mean ± standard deviation.

*cordifolia* and *T. cordifolia* were chosen to be evaluated as dip treatments for killing *V. cholerae* on *C. ramosus* meat. All these herbal decoctions inhibited completely the growth of *V. cholerae* on *C. ramosus* meat at 96 h. However, all the thirteen herbal decoctions experimented exhibited some reduction in the *V. cholerae* load on 48th hour. No complete inhibition rather than a reduction in the population of *V. cholerae* was noticed from the following herbal decoctions, namely, *C. angustifolia, L. sarmentosa, L. nodiflora, Marsilea quadrifolia* and *S. china* (Table 2).

## DISCUSSION

All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these medicinal herbs were more effective than traditional antibiotics to combat the pathogenic microorganism studied. The effect of herbal decoctions on the bacterial populations of *V. cholerae* in *C. ramosus* meat may be attributed to the higher degree of antimicrobial activity exhibited by these herbal decoctions.

Organisms causing dysentery were also inhibited by the extract. Since acute diarrhoeal diseases have different modes of pathogenesis, it is very useful to have demonstrated, through the present and previous reports, that the antidiarrhoeal action is multifaceted, just like the pathophysiology of these diseases.

The study also showed that *V. cholerae* is highly susceptible to the extracts. In the seafood, especially fish and shellfish, *Vibrio* species increase in number due to biological concentration (Donovan and Netten, 1995). Toda et al. (1991) have found that black tea extract had vibriocidal activity like that of these herbal decoctions used for the present study. Extracts from the kernel of *Brucea javanica* have also been reported to possess antibacterial activity against *Shigella shiga, Shigella flexneri, Shigella boydii, Salmonella lexington, Salmonella derby, Salmonella typhi* type II, *V. cholerae* serotype Inaba and *V. cholerae* serotype Ogawa (Wasuwat, 1971). Alcoholic extracts of *Ocimum sanctum* showed wider zones of inhibition for *V. cholerae* (Geeta et al., 2001). Such an inhibitory property would prevent the organism from producing the *Vibrio* toxin, and this would imply that, when administered to the sea foods and cholera patients, it should significantly lower the morbidity and mortality, especially in children in remote places without hospital facilities.

Generally, chlorinated water is used to remove bacterial pathogens. However, studies have shown that washing with chlorinated water has a limited bactericidal effect (Lin et al., 2000). Beuchat et al. (1998) reported that most of the chemicals would not be suitable for application at the household level. Consumers are increasingly avoiding, consuming foods treated with preservatives of chemical origin and so natural alternatives are needed to achieve high-degree safety with respect to food borne pathogenic microorganisms (Rauha et al., 2000). The study of Sengun and Karapinar (2004) reported that, treatment with lemon juice was most effective in eliminating viable *Salmonella typhimurium* cells than treatment with vinegar. Beuchat (1994) and Sofos et al. (1998) showed similar results with relatively low antimicrobial activity of herbal decoctions due to insolubility of the essential oil components which are known to inhibit the growth of yeasts and a wide range of bacteria, and they also suggested that it may well be attributed to the

attributed to the present high antibacterial activity exhibited by the leaf decoctions of *C. gynandra*, *L. sarmentosa, C. obliquawillel, C.angustifolia, A. precatorius, S. china, S. cordifolia, T. cordifolia, M. oleifera*, *A. marmelos*, *A. galangal* and *L. nodiflora*.

In recent years, there has been growing demand for improved ways of disinfecting seafoods. This is driven, amongst other things, by concerns over the frequency and extent of contamination of fresh seafoods by human pathogens such as *V. cholerae*, which is able to grow and survive even at refrigeration temperatures. Contamination of produce arises in the harvest area as well as during storage, transport and packaging, where conditions can favour the growth of pathogenic bacteria. Recent interest has been shown in treatment with herbal decoctions, a strong protectant, for the surface decontamination of sea foods. The bactericidal action of herbal decoctions is well established, and the decoctions have been used world-wide for over decades for the disinfection of seafoods and various other food commodities. Hence, these plants could be a source of new antibiotic compounds. The millenarian use of these medicinal plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. The results of the present study revealed that plant extracts could be effective antibiotics.

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