Research Article

The Effect of Hydroethanolic Solvents Extracts of *Piper betel* and *Plectranthus amboinicus* on Food Poison Disease Causing Pathogenic Bacteria

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ABSTRACT

The microbial safety of foods continues to be a major concern for consumers, regulatory agencies and food industries throughout the world. The present study studies the hydroethanolic crude extracts of *Piper betle* and *Plectranthus amboinicus* leaves extracts which were prepared and tested for their antimicrobial activity against food of poison that were caused by bacteria *Viz., Salmonella sp* and *E.coli*. And the outcomes showed more antimicrobial activity of *Piper betle* and *Plectranthus amboinicus* leaves extracts towards the *Salmonella sp* and *E.coli*. The crude compound of *Piper betle* and *Plectranthus amboinicus* leaves extracts exhibits the highest level of antibacterial activity on *Salmonella sp* and *E.coli*. Therefore the *Plectranthus amboinicus* leaves extracts expressing the maximum antibacterial activity in the concentration of 2.5mg/ml shown 19 mm zone of inhibition against *Salmonella sp* and 14 mm for *E.coli*. Whereas *Piper betle* leaves extracts expressing maximum activity in the concentration of 2.5mg/ml shown 18 mm zone of inhibition against *Salmonella sp* and 13mm for *E.coli* in the concentration of 5mg/ml. whereas of *Plectranthus amboinicus* and *Piper betle* leaves extracts expressing the Minimum Inhibitory Concentration showing in the concentration of 1mg/ml shown no coagulation and turbidity *Salmonella sp* and *E.coli* respectively.

Keywords: *Piper betle*, *Plectranthus amboinicus,* Antibacterial, Food poison, Contamination.

INTRODUCTION

Food is the ideal vehicle for the dispersion of harmful agents which can cause life frightening food borne illnesses. Food and food products are easily attainable at multiple points in any manufacturing process while they are easily distributed over great distances resulting in a great deal of concern for widespread impact of food borne diseases. Food borne disease is an increasingly serious public health problem all over the world. The main cause is determined to be microorganisms and the control of pathogens may reduce the outbreaks of food borne disease [1].

The previous reports has suggesting that utilization of the fruit and vegetables is beneficial for human health and may help to safeguard from chronic diseases [2 - 4], because of the phenolic compounds present in it [5]. Likewise, some natural substances have been used as seasonings for centuries which have effective antimicrobial properties [6, 7]. In recent years, the secondary plant metabolites have received a great attention due to their various biological functions like cancer prevention [8], as atherosclerosis preventing agents for cardiovascular diseases and in the slowdown of the aging process [9]. In addition, they also have antimicrobial and antioxidant activities [10, 11]. Because of the broad biochemical, nutritional and biological variations existing among the different cultivars/genotypes inside each species of fruit and vegetable, the identification of the best genotypes is important for breeders and consumers to have better quality products [9, 12].

Today, the utilizing of plant drugs is accepted all over the world. Herbs and spices are generally considered to be guarded and proved to be effective against certain ailments. In recent years, use of spices and herbs has been moderately increasing in the developed countries [13]. According to World Health Organization (WHO), 80% of world populace depends on traditional herbal medicine for the treatment of bacterial and fungal infections. Now, it has been established the plants which has naturally posses, synthesize and accumulate the secondary metabolites like phenolic acids, alkaloids, flavonoids, glycosides, tannins, and volatile oils and also has antimicrobial properties.

MATREIAL AND METHODS

Collection and storage of plant materials

Plectranthus amboinicus and *Piper betel* were collected from Karur, Tamil Nadu. The plant parts were rinsed in tap water to remove the dust particles and subjected to shade drying for about one week. Dried plants were pulverized by electric mill. The sample were stored in an airtight container and tested for certain biologically active compounds.

Bacterial strains and culture condition

The pathogenic bacterial strains were acquired from Government hospital Culture Collection Center, GH, Karur, India. *Salmonella spp* and *E.coli* were maintained on Luria–Bertani medium at 37°C.

Organoleptic evaluation of plant samples

The evaluations of pharmacognostic character of the medicinal plants were performed to test the quality. The plant parts were organoleptically evaluated for various sensory parameters like the color, appearance of the plant parts mainly size, shape and sound. The external texture, fracture (granular, splintery, smooth) and external marking (furrow, wrinkles, ridges, annular, out growth) of the plant part were examined. The fragrance, test for odour (aromatic, balsamic, camphoraceous, spicy, pleasant, irritating) and taste (sweet, bitter, sour, astringent, pungent, acidic, alkaline) were evaluated [14].

Hydroethanolic Extraction Method

The Phytochemical components were extracted using hydroethanolic extraction method. About 20g of pulverized samples were dissolved in hydroethanol in the ratio of 1:1 v/v (ethanol: distilled water, 50:50 [50%]) and incubate for 48 hours at ambient temperature with random shaking. After incubation the extracts were filtered and evaporated at 40°C [15].

Preliminary Phytochemical Screening

This has been attempted in the dry leaves of *Plectranthus amboinicus* and *Piper betel* to find out the presence or absence of certain bioactive compounds. The existence of alkaloids, saponins, treponoids, glycosides, flavonoids, sterols and steroids, tannins and phenolic compounds, carbohydrates, in the solvent extracts were detected by simple qualitative chemical analysis [16].

Agar Well Diffusion Method

The agar well diffusion method [17] is used to determine the growth inhibition. The plants extracts were prepared at a concentration of 10, 5, 2.5 mg/mL dissolved in Dimethyl sulphoxide (DMSO). The sterile Muller Hinton Agar plates were prepared and punctured 6mm diameter wells with uniform spacing for various concentrations for each extracts. The log phase culture broth was swabbed over the agar surface using sterile cotton swab to obtain uniform lawn of culture. The wells were filled with 10µl of the above mentioned concentration of plant extracts respectively. The plates were then incubated at 37^{0}C for 24 hours.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations [18] of the plant extract were found using broth dilution assay method. For assaying plant extracts, the starting concentration of the plant extracts were kept at 4 mg/mL in the first tubes which containing 1mL of broth. Tubes were vortex mixed thoroughly to make the initial standard concentration. This was serially diluted to other tubes and finally 1mL is discarded. To all these test tubes, 0.1mL of the cultures of target microorganism were added separately and incubated at 37^{0}C for 24-48 hours. The tubes were analyzed for visible turbidity after incubation and plated on to the nutrient media and incubated for 24 hours at 37^{0}C.

RESULTS

Natural products are in great need for their extensive biological properties and bioactive components (phenols, flavonoids, saponins, glycosides, terpenoids etc.,) which had been proved to be useful against large number of causative agents of diseases. Many researchers have been showed isolation and purification of phytoactive compounds from natural source in recent decades. These phytoactive compounds are 100% natural, less side effect and fight against wide range of disease alignment. Extraction of Phytochemicals from Hydroethanolic extract of *Plectranthus amboinicus* and *Piper betel* were already reported. However, this is the first scientific report deals the extraction of Active Phytochemicals from *Plectranthus amboinicus* and *Piper betel leaves* by using 50% Ethanol and 50% Aqueous as Hydroethanolic as a extracting agent. Present study, Active phytochemicals was extracted from Hydroethanolic extract of *Plectranthus amboinicus* and *Piper betel*. Active phytochemicals contains from crude sample was concentrated and further extracted (Table 2).

In agar well diffusion method, both the *Plectranthus amboinicus* and *Piper betel* shows higher activity at lower concentration against the *Salmonella* when compared with *E.coli.* The extracts of both *Plectranthus amboinicus* and *Piper betel* show higher activity at concentration of 5mg/mL against *E.coli.* The extract of *Plectranthus amboinicus* has maximum activity of 14mm of zone of clearance and *Piper betel* leaves extracts has 13mm of zone of clearance at the concentration of 5mg/mL (Fig 1 and 3). When comparing the both plant extracts, *Plectranthus amboinicus* shows higher efficacy to inhibit the growth of the food poisoning pathogens (Table 3&4).

The extract of *Plectranthus amboinicus* has maximum activity in the concentration of 2.5 mg/mL shows 19mm diameter of zone of clearance against *Salmonella* (Fig 2 and 4). Whereas in case of *Piper betel* leaves extracts also exhibit the highest activity in the lower concentration of 2.5mg/mL shows 18mm of zone of inhibition against the *Salmonella* (Table 3&4).

In Indian scenario, many researchers have showed the antimicrobial activity for various crude plant extract and few of them only established antimicrobial activity against Crude and purified bioactive compounds from natural source. It has been already proved that various extract of *Plectranthus amboinicus* and *Piper betel* leaves and its isolate have antimicrobial activity against some common pathogens. Moreover, this is the first scientific report deals the antimicrobial activity of Crude active phytochemicals shown highest activity against food poison causing pathogens. In this study, Antimicrobial activity of *Plectranthus amboinicus* and *Piper betel* against *Salmonella* and *E.coli* were determined by MIC. *Plectranthus amboinicus* and *Piper betel* exhibited an MIC

range of 4mg/ml to 1mg/ml against selected food poison pathogens such as *Salmonella* and *E.coli*, the highest Inhibition occurred in 1mg/ml for both designated crude extracts *Viz,. Plectranthus amboinicus* and *Piper betel*.in Table 5, this antimicrobial activity is due to the inhibition of cell wall growth proteins of *Salmonella* and *E.coli* by active phytochemical extracts *Plectranthus amboinicus* and *Piper betel*. Recent studies have shown that many plant derived phenolic compounds and related polyphenols contribute [19] significantly to the inhibition of cell wall growth proteins of many pathogenic microbes.

In many cases, *Campylobactor, Listeria and Lactobacilli* are the most causative microbes for Food poison diseases. In which, human food poison microbes, ferment the fermentable carbohydrates in the human consuming food produce toxin and poison environment and form food poison infection in the human body. This herbal crude active phytochemicals may eradicate the food poison causing bacteria multiplication as well as toxin production denaturation.

Present study also suggests that the Crude active phytochemical extracts of *Plectranthus amboinicus* and *Piper betel* has potent antibacterial activity. Similarly, many of the researches have reported that *Plectranthus amboinicus* and *Piper betel* contains phenolic compound such as hydroxycavicol, quercetin, Eugenol, carotenoids etc., effectively inhibit the growth of common food pathogens [20].

Table 1: Organoleptic evaluation of plant samples

Parameters	*P.amboinicus*	*P.betel*
Colour	Light Green	Green
Odour	Raw, Pungent when crushed	Aromatic odour
Appearance	Dark green when powdered	Dark brown color when powdered
Taste	Spicy and mint	Sweet to pungent

Table 2: Phytochemical screening

Phytochemical constituents	*P.amboinicus*	*P.betel*
Alkaloids	+	+
Saponins	-	-
Terpenoids	+	+
Glycosides	+	-
Flavanoids	+	+
Steroids and sterols	+	+
Tannins and phenols	+	+

"+" indicates presence of phytochemical constituents; "-" indicates the absence of phytochemical constituents

Table 3: Antimicrobial Activity of *P.amboinicus and P.betel against food borne disease causing bacteria*

Sample	Organism	Zone of inhibition (diameter in mm)			
		10mg/mL	5mg/mL	2.5mg/mL	Control
P.amboinicus	***E.coli***	19	14	≤10	No zone
	Salmonella	28	22	19	No zone
P.betel	***E.coli***	16	13	No zone	No zone
	Salmonella	21	19	18	No zone

Table 4: Minimum Inhibitory Concentration of *P.amboinicus and P.betel against food borne disease causing bacteria*

Sample	Organism	Dilution (plating method)					
		T1 (4mg/mL)	T2 (2mg/mL)	T3 (1mg/mL)	T4 (0.5mg/mL)	T5 (0.25mg/mL)	T6 (0.125mg/mL)
P.amboinicus	***E.coli***	NG	NG	NG	G	G	G
	Salmonella	NG	NG	NG	G	G	G
P.betel	***E.coli***	NG	NG	NG	G	G	G
	Salmonella	NG	NG	NG	G	G	G

"NG" indicates No growth, "G" indicates Growth

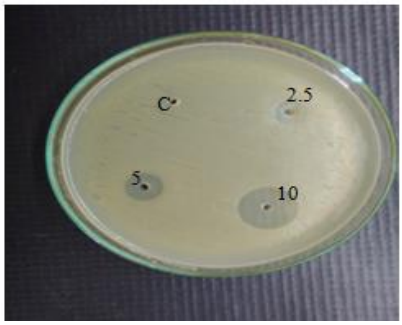

Figure 1: Antimicrobial Activity of *P.amboinicus* on *E.coli*

C – Control, 10 -10mg/mL, 5-5 mg/mL,2.-2.5 mg/mL

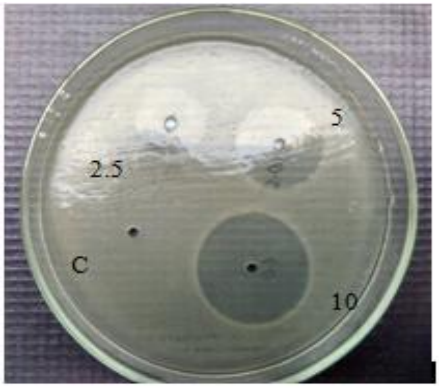

Figure 2: Antimicrobial Activity of *P.amboinicus* on *Salmonella*

C – Control, 10 -10mg/mL, 5-5 mg/mL,2.-2.5 mg/mL

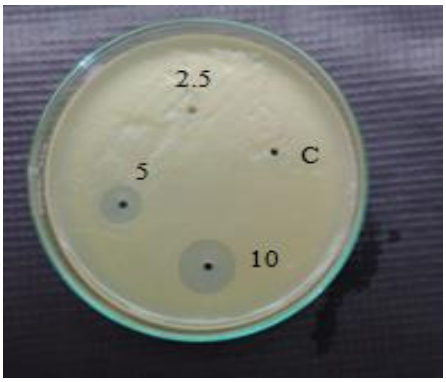

Figure 3: Antimicrobial Activity of *P.betel on E.Coli*

C – Control, 10 -10mg/mL, 5-5 mg/mL,2.-2.5 mg/mL

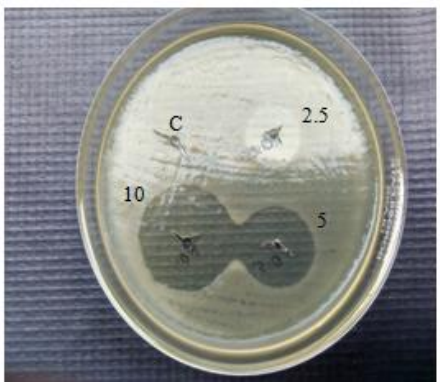

Figure 4: Antimicrobial Activity of *P.betel* on *Salmonella*

C – Control, 10 -10mg/mL, 5-5 mg/mL,2.-2.5 mg/mL

DISCUSSION

In this study hydroethanolic of *Piper betle*, and *Plectranthus amboinicus* extracts proved to be the most effective against two food-borne bacteria tested. A number of studies have reported on the antimicrobial activities of plant extracts and essential oils against food-borne pathogens [21]. However, direct comparison of results obtained in various studies is problematic due to a number of factors such as variability in composition of plant extracts as a result of environmental conditions, two number of herb samples tested, differences in experimental design including MIC , MBC, but in many publications the agar disc diffusion method was used to determine the antimicrobial efficacy of plant extracts or essential oils, which should be treated rather as preliminary, qualitative data only [9]. A number of previous studies have proved that *Piper betle* and *Plectranthus amboinicus*, extracts or essential oils have strongest inhibitory effects against food-borne bacteria among many herbs tested[9]. The hydroethanolic extracts of *Piper betel* and *Plectranthus amboinicus* displayed significant inhibitory properties against *Escherichia coli*, and *Salmonella* spp.

CONCLUSION

Due to consumer awareness and negative perception of artificial preservatives in food, in recent years, the attention is shifting towards alternatives that the consumers recognize as natural. Thus, the herbal extracts, particularly *Piper betel* and *P.amboinicus*, are getting more space in the food industry to prevent the bacteria that are responsible for the food contamination and spoilages. However, if the herbal extracts are to be more widely applied as an antibacterial agent in foods, the cytotoxic and organoleptic properties are extremely important issues to consider. They may vary according to extract composition where the method of extraction plays an important role. The plant component can be formulated as a food preservative and the shelf life has to be evaluated.

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