



STUDY OF THE EFFECT OF PEPPERMINT EXTRACT ON *SALMONELLA SPP.*, ISOLATED FROM BLOOD SPECIMEN

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Abstract

In the present study, the collection 10 samples from different patients whereas the positive culture were 6/10 (70%) and negative culture were 4/10(30%) and the positive culture appearing all the samples (100%) were *Salmonella spp* depended on routine diagnosis. But the sensitive results Appearing only two samples from bacteria ware sensitive to trimethoprim(10 mg) and carbencillin (25 mg), The inhibitions zones were 6 mm and 4 mm in diameter respectively as the following photo(1). For the Ampicillin given weak effect on three isolation from *Salmonella spp*.

By well diffusion, method was measured the inhibition zone of diluted mint extract. The dilute method of mint extract by distal water from 10^{-1} to 10^{-6} . the bacteria isolates ware resistant for peppermint extract in diluted 10^{-3} – 10^{-6} but the all isolates ware sensitive for the 10^{-1} , 10^{-2} in the well diffusion method after applied extract in wells and measuring of inhibitor zone diameter. The inhibition zone was between from 1.5mm to 2 mm in diameter for all *Salmonella spp* isolates.

Introduction

Typhoid fever is an acute generalized infection of the reticuloendothelial system, intestinal lymphoid tissue and gallbladder caused by *Salmonella enterica* serovar Typhi (*Salmonella typhi*). This communicable disease is restricted to human hosts and humans (chronic carriers) constitute the reservoir of infection. A broad spectrum of clinical illness can follow ingestion of *Salmonella Typhi*, with more severe forms being characterized by persisting high fever, abdominal discomfort, malaise and headache. the late 1980s, strains of *Salmonella typhi* exhibiting resistance to most of the antimicrobials that previously were clinically effective, have spread aggressively throughout the Indian subcontinent, South-East Asia and the Middle East (Gupta, 1994). Whereas, earlier epidemics of antibiotic resistant typhoid fever in Mexico (Gilman *et al.*, 1975) and Viet Nam (Butler, *et al.*, 1973) in the 1970s and Peru (Brenner *et al.*, 2000).

The leafy part of different common herbs, such as thyme, peppermint, sage, black pepper and garlic, has been added to food and food products to improve the flavour. Certain spices and essential oils prolong the storage life of foods by their antimicrobial activity. Being natural food stuffs, they appeal to consumers who tend to question the safety of synthetic additives.

Peppermint is taken internally as a tea, tincture, oil, or extract, and applied externally as a rub or liniment. Herbalists consider peppermint an astringent, antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant, and emmenagogue (Hoffman, 1996; Bove, 1996). Peppermint oil vapor is used as an inhalant for respiratory congestion. Peppermint tea is used to treat coughs, bronchitis, and inflammation of the oral mucosa and throat. It has traditionally been used to treat a variety of digestive complaints such as colic in infants, flatulence, diarrhea, indigestion, nausea and vomiting, morning sickness and anorexia, and as a spasmolytic to reduce gas and cramping. Peppermint is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, gallbladder and

biliary tract disorders, and liver complaints (Fleming, 1998; Tyler, 1992). Peppermint oil is used to relieve menstrual cramps. Peppermint oil is used externally for neuralgia, myalgia, headaches, migraines and chicken pox (Bove, 1996; Fleming, 1998).

Materials and Methods

Bacterial isolate: A ten local isolate being isolated from patients. The symptoms of patients were appeared of typhoid fever according the physician diagnosis. In addition, fully identified and characterized as drugs resistant bacteria was identified according to the routine methods to confirm the stability of its diagnostic characteristics.

Antimicrobial susceptibility testing: Muller-Hinton agar was used for detection the antibiotics susceptibility by disc diffusion test. The following antibiotics were used with known potency (Himedia): ampecillin, carbencillin, chloramphnicol, and trimethoprim. The results of antibiotics susceptibility were interpreted according to CLSI, 2010 (CLSI, 2010). All the results were recorded taking endpoint for compound that was defined as the lowest concentration in which the growth score was zero i.e. optically clear. This criteria was used to define the minimum inhibitory concentration (MIC) of the drug (Chaudhry and Tariq, 2006). The antibacterial activity of the leaf extracts was determined using agar well diffusion method following published procedure with slight modification (Chaudhry and Tariq, 2006) Muller-Hinton agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8 mm diameter) were punched in the agar and filled with plant extracts (Saeed and Tariq 2005). The plates were incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with CLSI, (2010). The resultant clear zones around the discs were measured in mm. The antibacterial activity of plant extracts were indicated by clear zones of growth inhibition. Five replicates were

maintained for each treatment. The data were subjected to statistical analysis.

Peppermint extraction: This method was employed according to Hayyan, I, Al-Taweil (2014) as the followings steps the firstly step, Plant leaves suspension (water and alcoholic) was preparation by Water extracts 10 g of mint leaf \ 100 ml DW in flask steeped for 7 days and boiling for 20 minutes with cool before blander. Secondary step, ultra distraction the peppermint leaves by blander. Thirdly, Plant suspension was Filtration by filter papers. Finally, the extract was Sterile by microfiltration..

Results

Isolation and Identification of bacteria

In the present study, the collection 10 samples from different patients whereas the positive culture were 6/10 (70%) and negative culture were 4/10(30%). Such as the following figure (1)

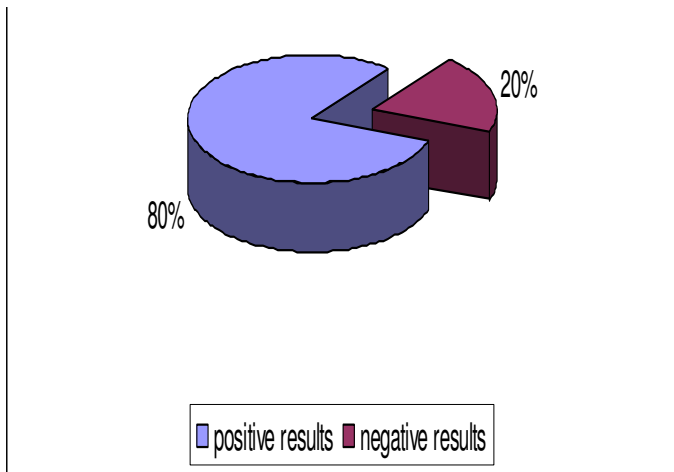


Fig. 1 : Percentages of isolation

In positive culture appearing all the samples were (100%) of *Salmonella spp* depended on routine diagnosis.

The sensitivity of *Salmonella spp* to the antibiotics

Disc diffusion test (DD test)

This test included six isolates of *salmonella spp*. Appearing results resistance for antibiotics in disc diffusion test but only two samples from bacteria ware sensitive to trimethoprim(10mg) and carbencillin (25mg), The inhibitions zones were 6 mm and 4 mm in diameter respectively as the following photo(1). For the Ampecillin given weak effect on three isolation from *Salmonella spp* isolates as the following table (1)

Table 1 : Inhibition zone diameter for antibiotics

BACTERIAL ISOLATE	INHIBITION ZONE DIAMETER (MM) FOR ANTIBIOTICS			
	Ampecillin	Carbencillin	Trimethoprim	Chloramphnicol
1	0.5	2	R*	R
2	0.5	4	6	R
3	R	R	R	R
4	R	4	6	R
5	4	R	R	R
6	R	R	R	R

* *Salmonella spp* resistance for antibiotics

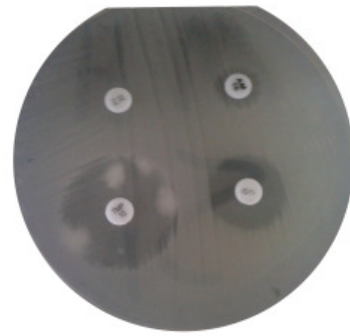


Photo (1) the inhibitions zones of sensitive *salmonella spp* Peppermint extracts sensitive method:

In this experiment used six isolate in present study that appearance resistance for antibiotics. By well diffusion, method was measured the inhibition zone of diluted mint extract. The dilute method of mint extract by distal water from 10⁻¹ to 10⁻⁶. the bacteria isolates ware resistant for peppermint extract in diluted 10⁻³ – 10⁻⁶ but the all isolates ware sensitive for the 10⁻¹, 10⁻² in the well diffusion method after applied extract in wells and measuring of inhibitor zone diameter as the show photo (2). The inhibition zone was between from 1.5mm to 2 mm in diameter for all *Salmonella spp* isolates.



Photo (2) inhibit zone diameter of 10⁻¹ peppermint extract.

Discussion

Because of differences in the chemical composition of peppermint essential oil from different parts of world we analyzed the chemical composition of peppermint oil and its antimicrobial effect against a large number of microorganisms. The antibacterial activity of peppermint leave’s juice against Gram negative bacilli was higher than that of its stem juice (Yadegarinia *et al.*, 2006). The antimicrobial activity of peppermint extract was against *Salmonella spp*. This oil has good antioxidant activity in two systems of DPPH free radical scavenging and carotene/linoleic acid systems (Kerman and Kucera, 1967). Other pharmacological activities, such as antiviral activity against *Influenza*, *Herpes* and other viruses (Girme *et al.*, 2006) and its anthelmintic effect is confirmed, The anthelmintic effect of peppermint methanol extract is comparable with Albendazole with mechanism of paralysis and death of worms (Avendano *et al.*, 1986).

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