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Combined Effect of Ginger and Honey Against Salmonellosis

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ABSTRACT

Salmonellosis is an infectious disease caused by bacteria called Salmonella. Researchers have shown cockroaches as reservoirs and vectors of Salmonella sp. Ginger (Zingiber officinale) and honey are one of the nature's gifts to mankind and have been used to prevent and control many microbial infections. The aim of this study was to evaluate the antibacterial effects of Z.officinale and honey on Salmonella sp. isolated from cockroaches. Disc diffusion and tube dilution methods were used to determine the antibacterial activity of the ginger extract, honey and combination of both against Salmonella sp. The results indicated that the ginger extract, honey and its combination inhibited the growth of Salmonella typhi, Salmonella paratyphi A and Salmonella paratyphi B. The ginger - honey mixture had noticeable effect on examined species compared to the individual effects of ginger and honey, thus justifying their combined use in treatment for enteric infection.

KEYWORDS : Ginger, Honey, Synergistic effect, Salmonellosis, Antibacterial activity

Introduction

Salmonellosis is a type of food poisoning caused by Salmonella bacterium. Cockroaches, reptiles, baby chicks, ducklings and small rodents act as a carrier of Salmonella sp. Researchers have identified Salmonella (Black colonies on SS agar) from about 70% of the cockroaches collected and Salmonella survives in cockroaches for more than 10 months thus serve as reservoirs and vectors of Salmonella (1). The onset of infection is reported to appear within 12 to 72 hrs and the symptoms include diarrhoea, fever and abdominal cramps. Infants, older people and adults with weaker immune system are at a highest risk. The increasing failure of chemotherapeutics antibiotics resistance exhibited by many pathogenic infectious agents has led to the screening of alternative remedies (2,3,4). Honey has been used as medicine since ancient times and still used in folk medicine. The therapeutic use of honey has been rediscovered and is also gaining acceptance as antimicrobial agent (5). Ginger (Zingiber officinale) is a native plant of Southeast Asia. It is reported to have number of pharmacological activities. Its efficacy is due to its aromatic, carminative and absorbent properties (6). The aim of this study was to evaluate the antibacterial activity of ginger, Indian mountain honey and its combinations and to determine the minimum inhibitory concentration against the Salmonella sp. isolated from cockroaches.

Materials and Methods

Sample collection:

Native mountain honey was procured from IMCOPS, Chennai, India. The sample was stored in dark at 4°C until use. The rhizomes of ginger was obtained from Koyambedu market, Chennai, India. They were cut into small pieces shade dried and ground to fine powder. Known quantities of the ground material was extracted with hexane using Soxhlet apparatus for 16 hrs and the solvent was evaporated to dryness. The residues were weighed and stored at 4°C until use.

Bacterial Isolates and Antibacterial activity:

Bacterial strains of Salmonella typhi, Salmonella paratyphi A and Salmonella paratyphi B isolated from cockroach were obtained from the Department of Microbiology, JBAS College for Women, Chennai. The antibacterial activities of the ginger, honey and its combination were carried out using the agar disc diffusion method described by Kirby-Bauer,1996 (7) and Broth tube dilution method described by Natta et al.,2008 (8).

Preparation of inoculum:

Active cultures for experiment was prepared by transferring a loop full of cells from the stock cultures to test tubes containing Muller-Hinton broth and was incubated for 24hrs at 37°C and the turbidity was matched to Mc Farland standard 0.5.

Agar Disc Diffusion method:

Antimicrobial activity was evaluated by using disc diffusion technique.The inoculum was spread evenly on the Mueller-Hinton agar (MHA) surface using a sterile cotton swab and was allowed to dry for 5-10mints. The disc impregnated with 20 µl of sample [concentration : 1000µg, 500 µg, 250 µg, 125 µg, 62.5 µg] was placed on MHA and the plates were incubated at 37°C for 24 hrs. After incubation, a clear zone around a disc indicated antibacterial activity. Diameter of the zones of inhibition was measured in millimeters.

Broth dilution method:

The minimum inhibitory concentration (MIC) was determined by the method of Natta et al., (2008) after slight modifications. Serial dilutions (volume and concentration in the first tube 2ml of 10mg/ml) of the sample was made in a liquid medium which was inoculated with test organisms and incubated at 37°C overnight. The turbidity of the samples was examined (OD at 620nm was measured) for bacterial growth. The lowest concentration (highest dilution) of sample inhibiting the growth of test organisms was considered to be the MIC. Ginger- honey mixture was prepared in different volumes (1:1), (1:4) and (4:1).

Results

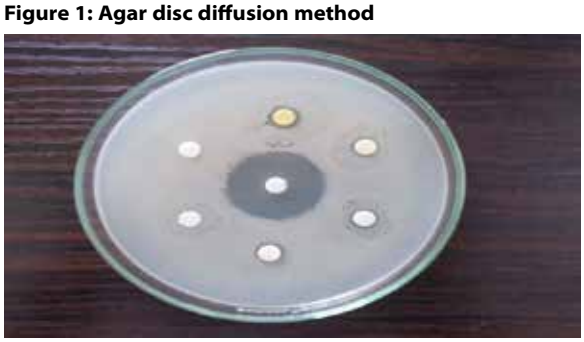
The combined effect of ginger and honey showed high inhibitory effect on all the test organisms used in this study giving a zone size of 17mm (concentration of 1000µg) by disc diffusion method. This is evidenced by the increase in the zone of inhibition size. The disc diffusion method of ginger extract (zone size: 9-13mm) and honey (zone size: 7-12mm) had varying degree of activity on all the test organisms. The highest inhibitory and synergistic activity was found on Salmonella typhi, followed by S. paratyphi A and S. paratyphi B. (Table 1 & Figure 1).

Table 1- Zone of inhibition of ginger, honey and ginger- honey combination by Agar disc diffusion method

Sl.No.	Microorganism	Zone of Inhibition in mm					
		1000µg	500µg	250µg	125µg	62.5µg	DMSO
I	Ginger						
1	S. typhi	13±0.02	12±0.05	12±0.02	12±0.04	10±0.01	-
2	S. paratyphi A	12±0.02	11±0.01	10±0.04	10±0.02	10±0.04	-
3	S. paratyphi B	11±0.04	11±0.02	10±0.01	09±0.02	09±0.01	-
II	Honey						
1	S. typhi	12±0.01	11±0.02	11±0.01	10±0.02	10±0.01	-
2	S. paratyphi A	10±0.01	09±0.02	08±0.01	07±0.02	07±0.02	-
3	S. paratyphi B	09±0.00	08±0.01	08±0.01	07±0.02	07±0.02	-
III	Ginger + Honey						
1	S. typhi	17±0.00	16±0.02	15±0.03	13±0.01	10±0.00	-
2	S. paratyphi A	13±0.01	12±0.03	11±0.01	11±0.02	10±0.01	-
3	S. paratyphi B	12±0.01	11±0.02	10±0.01	09±0.00	09±0.01	-

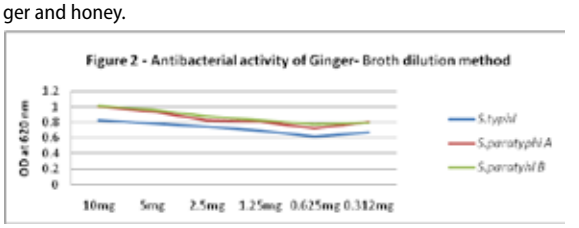
All values are expressed as Mean±S.D zone of inhibition against the test isolates

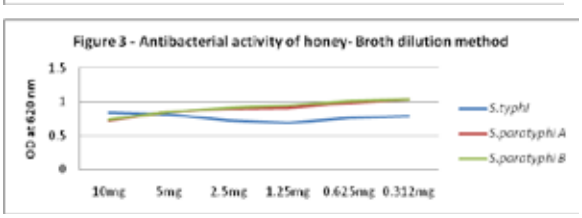
Figure 1: Agar disc diffusion method

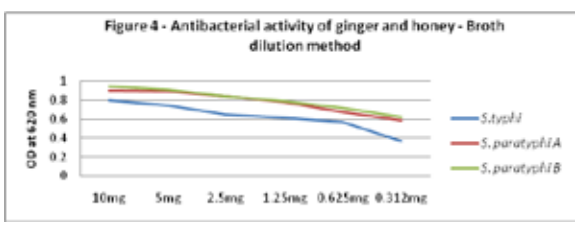


Antibacterial effect of Ginger Honey combination against test organism

The MIC of the samples against the test organism are illustrated in Figure 2, 3 and 4. Despite the appearance of growth at a concentration of 0.625mg (Figure 2) and 10mg (Figure 3) for ginger and honey respectively the growth was significantly less than the control. For the combined effect of ginger–honey (1:1), the growth was observed to be very low at a concentration of 0.312mg. Whereas the values were observed to be fluctuating in other ginger honey combination of 1:4and 4:1. A synergistic effect was well observed with 1:1 ginger honey combination which can be evidenced with the decrease in the MIC value (Figure 4) when compared to the individual effects of ginger and honey.







Discussion

The study revealed the antimicrobial effect of ginger, honey and combination of ginger –honey in 1:1 ratio on the organisms tested. The antibacterial activities of the ginger extracts are due to the compounds like flavonoids and sesquiterpenoids. The results of antimicrobial effect of ginger (Table 2) in the study are in accordance with the reports of Akoachere et al.,(2002), Malu et al.,(2008), Gao and Zhang ,(2009) and Sebiomo et al.,(2011) (9,10,11,12).

The present study has also demonstrated individual activity of honey at different concentrations against the selected Salmonella sp. Honey is known to contain phenol, fatty acids, lipids, amylases, ascorbic acid, peroxidases and fructose and has high osmolarity and low pH. These elements acting alone or synergistically may contribute significantly to the antimicrobial activity of honey as reported by Al-Jabri et al.,2005 (13). The results obtained in this study was found to be in agreement with Abdul Hannan et al.,2009 (14).

The highest zone of inhibitions and the least MIC values was observed with ginger and honey combination (1:1) than compared to the individual effects of ginger hexane extract or honey. The difference in the zones of inhibition to varying degrees and the lowest MIC recorded may be attributed to the secondary metabolite (inhibins) or phytochemicals (gingerol and flavonoids) (15) in ginger and peroxidases, polyphenols or ascorbic acid present in honey (16). Such combination of medicinal plants like G. kola and V. amygdalina extracts suspended in honey were earlier studied by Mboto et al.,2009(17) and was found to inhibit the growth of selected microbes very effectively than honey or medicinal plants individually. The results obtained in this study was in accordance with the work of Yahaya et al.,2012(18).

Conclusion

The present study shows high antimicrobial effect of the combined effect of ginger-honey extract. Therefore this combination can show much promise in the development of phytomedicines with great antimicrobial properties especially in treatment of enteric infections.

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