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# ANTIBACTERIAL ACTIVITIES OF HONEY, SANDAL OIL AND BLACK PEPPER

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**Abstract**

The purpose of the present study was to determine the antibacterial activities of honey, sandal oil and black pepper by taking a selected standard medicine. These products are used for the treatment of various diseases. The bacterial isolates obtained from clinical samples were *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus* and *Bacillus subtilis.* After identification and confirmation the pure culture of clinical bacterial isolates were used to test the sensitivity of bacterial isolates against sample products. Dimethyl sulfoxide and distilled water were used as solvent for sample products. Different types of honey, sandal oil and black pepper were tested against bacterial isolates. Results of all sample products showed zone of inhibition against all selected bacterial isolates. At the dilution of 25µl of stock solution in 75µl and at 50µl: 50µl concentration, the results were similar as obtained from honey and sandal oil. Dimethyl sulfoaxide DMSO and honey 1 showed inhibition zones against all except *Staphylococcus aureus*, whereas honey 2 was effective against all isolates. Sandal 1 oil showed inhibition zones against all isolates except *E.coli* whereas sandal 2 was effective against all. The results obtained from black pepper 1 and 2 did not gave effective results both at 50µ litre: 50µ litre and 25µ litre: 75µ litre concentration, both were found to be effective against *Bacillus subtilus*. Standard medicine showed inhibition zone against all selected bacterial isolates in each test.

## Introduction

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drugs because of the ready availability of chemical diversity (Cos *et al*., 2006). Honey and lemon-honey are traditional remedies in the Middle East and China and for many centuries and have been used in the treatment and prevention of the common cold and various upper respiratory tract infections (Molan, 1992; Zulma & Lulat, 1989).

Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae. This fruit is also used to produce white pepper and green pepper. Black pepper is native to India and has been a prized spice since ancient times. Similarly it has been shown to have antimicrobial activity (Dorman & Deans, 2000). Black pepper (*Piper nigrum*) is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever (Ravindran, 2000), intermittent fever, cold extremities, colic, gastric ailments and diarrhoea (Ao *et al*., 1998). Wilkinson (2005) determined the activity of 13 honeys, including three commercial antibacterial honeys, against *Escherichia coli* and *Pseudomonas aeruginosa*. Antibacterial activity of the honeys was assayed using standard well diffusion methods. JirovetzIn *et al*., (2005) concluded that the sandal wood from different sources and mixture of of α- and β-santanols were highly effective against gram negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and as well as yeast *Candida albicans*. The present work gives strong evidence of antibacterial activities of honey, sandal oil and black pepper.

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## Material and Methods

The products chosen were locally sold honey (marhaba and wild variety), sandal oil of haqplanter and unknown brand, grounded black pepper. Media used in experimentation were nutrient agar, blood agar and mcconkey agar. Identification of isolates was done after standard isolation techniques including gram staining, streaking and biochemical tests (TSI, molility test and Indole test). The solvent used for stock solution preparation and for further dilutions were Dimethylsulphoxide and distilled water. Disc diffusion method was used to measure zone of inhibition and imepeneme used as standard medicine.

Bacterial identification and characterization were carried out by comparing the results obtained with the characterization definitions of Monica Cheesbrough's manual of District laboratory practice in Tropical Countries part 2. The zones of inhibition were measured at two dilutions 25µl of stock solution and 75µl of DMSO and 50µl of stock solution and 50µl of DMSO. The imepeme was used as standard and zones of inhibition were measured at these concentration.

## Results and Discussion

In the present study natural products were checked against bacterial isolates. Results of identification and confirmation of selected bacterial isolates obtained from biochemical tests are shown in Table 1. These results showed that the isolates were *Staphylococcus aureus*, *Salmonella* sp., *Bacillus subtilis* and *E. coli*. Differential media like Mcconkey agar and blood agar were also used. The zones of inhibition were found to be almost constant for standard, but the zones decrease in diameter as the dilution of product sample decreases. The results showing zone of inhibition are presented in Table 2.

**Table 1. Results of biochemical tests on each selected bacterial isolates.**

| Bacterial isolates | Biochemical tests | | | | |
|---|---|---|---|---|---|
| | **Gram staining** | **Indole test** | **TSI** | **Motility test** | **Coagulase test** |
| *E. coli* | Gram -ive rods | + | Y No gas, no hydrogen sulfide | + | - |
| *Salmonella* sp. | Gram -tive rods | - | Hydrogen sulfide + | + | - |
| *S. aureus* | Gram +tive clusters | × | × | × | + |
| *B. subtilis* | Gram +tive rods | - | Hydrogen Sulfide - | + | × |

**Table 2. Results showing zone of inhibition.**

| Isolates | Sample products | | | | | | | | | | | | Standard | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Honey 1** | | **Honey 2** | | **Sandal. oil 1** | | **Sandal. oil 2** | | **Black. pep1** | | **Black. pep2** | | | |
| | **25µl** | **50µl** | **25µl** | **50µl** | **25µl** | **50µl** | **25µl** | **50µl** | **25µl** | **50µl** | **25µl** | **50µl** | | |
| *E. coli* | 12mm | 12mm | 10mm | 11mm | Nil | Nil | 12mm | 8mm | Nil | Nil | Nil | 10mm | 39mm | 37mm |
| *Salmonella* sp. | 10mm | 18mm | 12mm | 14mm | 12mm | 8mm | 14mm | 10mm | Nil | Nil | 14mm | Nil | 29mm | 28mm |
| *S. aureus* | Nil | Nil | 10mm | 14mm | 14mm | 14mm | 10mm | 10mm | Nil | Nil | Nil | Nil | 40mm | 43mm |
| *B. subtillus* | 13mm | 12mm | 10mm | 16mm | 10mm | 10mm | 13mm | 18mm | Nil | Nil | 15mm | 12mm | 41mm | 46mm |

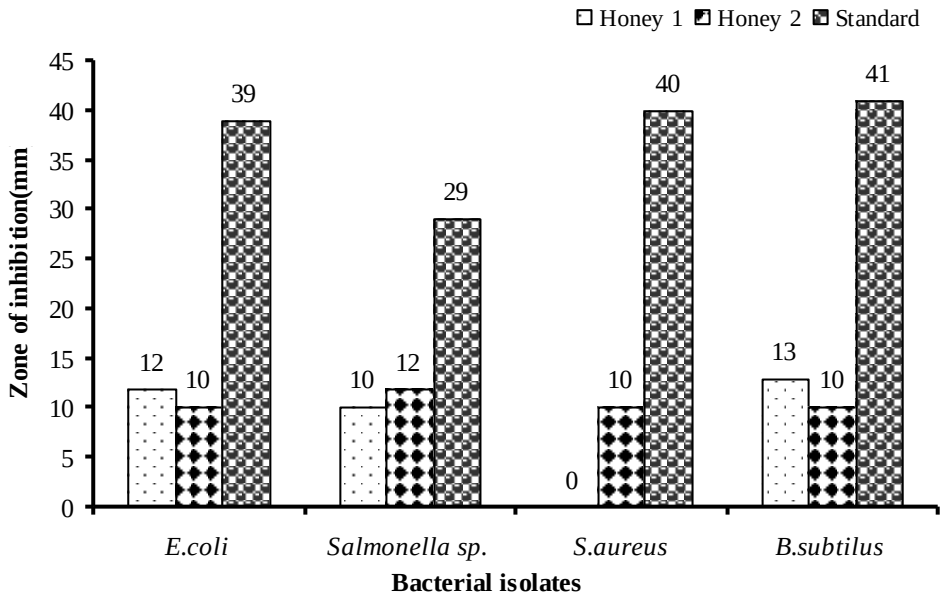


Fig. 1. Inhibition zones in mm by Honey (1), Honey (2) and standard against bacterial isolates at 25:75 concentration.

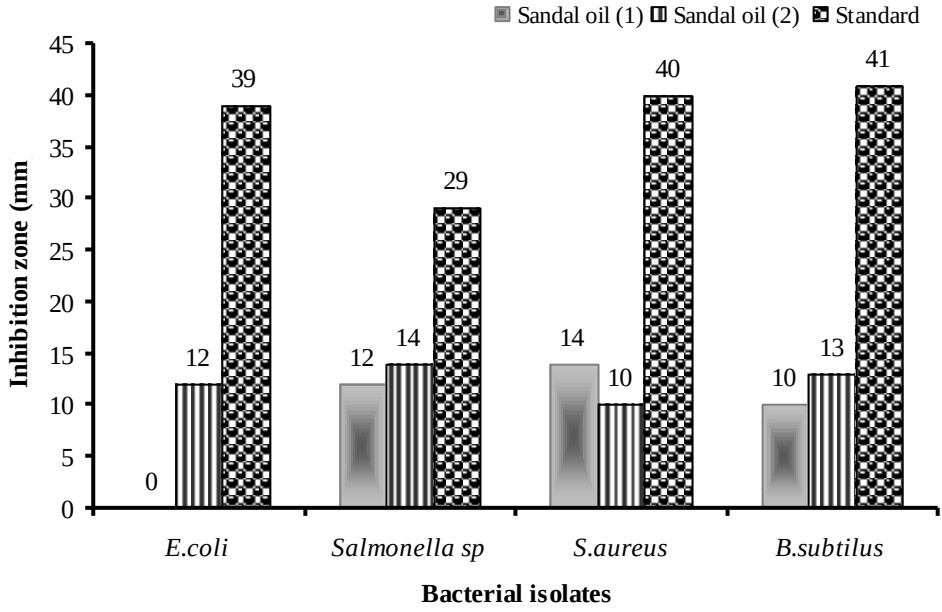


Fig. 2. Inhibition zones in mm by Sandal oil (1), Sandal oil (2) and Standard against bacterial at 25:75 concentration.

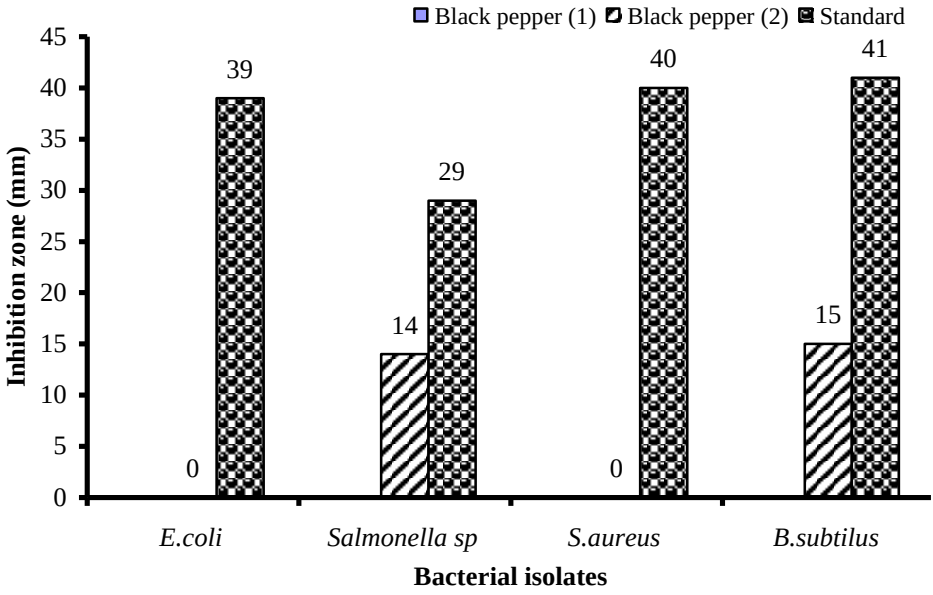


Fig. 3. Inhibition zones in mm by Black pepper (1) and Black pepper (2) and Standard against bacterial isolates at 25:75 concentration.

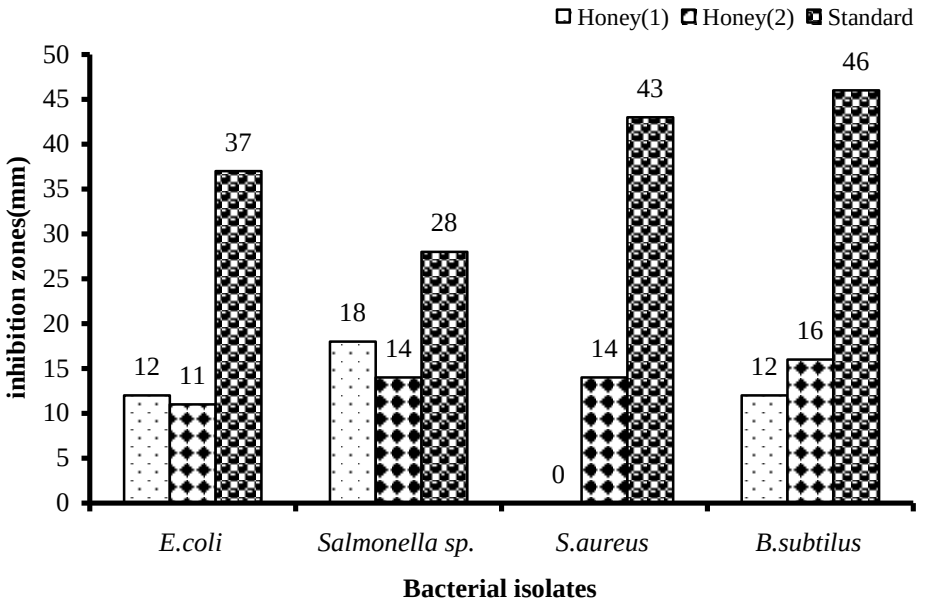


Fig. 4. Inhibition zones in mm by Honey (1), Honey (2) and standard against bacterial isolates in 50:50 concentration.

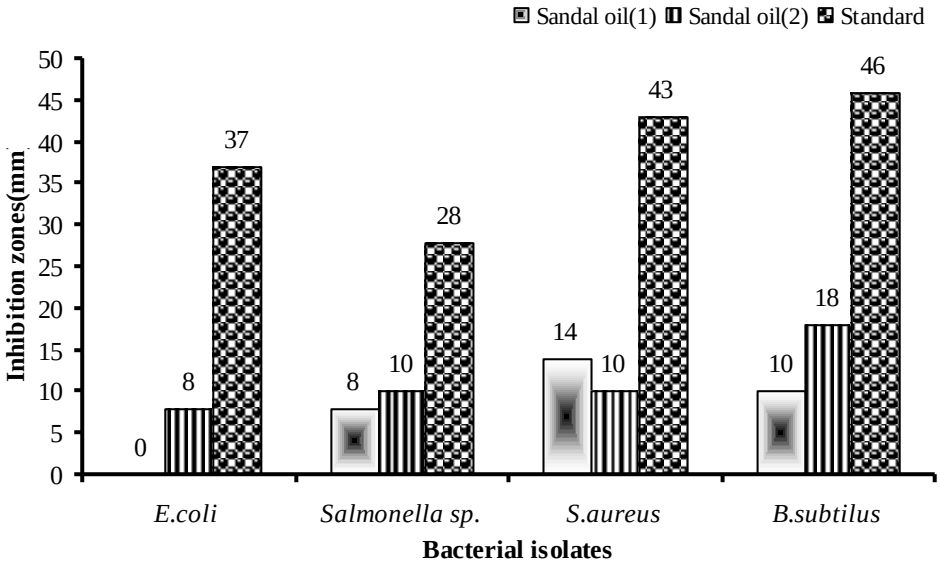


Fig. 5. Inhibition zones in mm by Sandal oil (1), Sandal oil (2) and Standard against bacterial isolates at50:50 concentration.

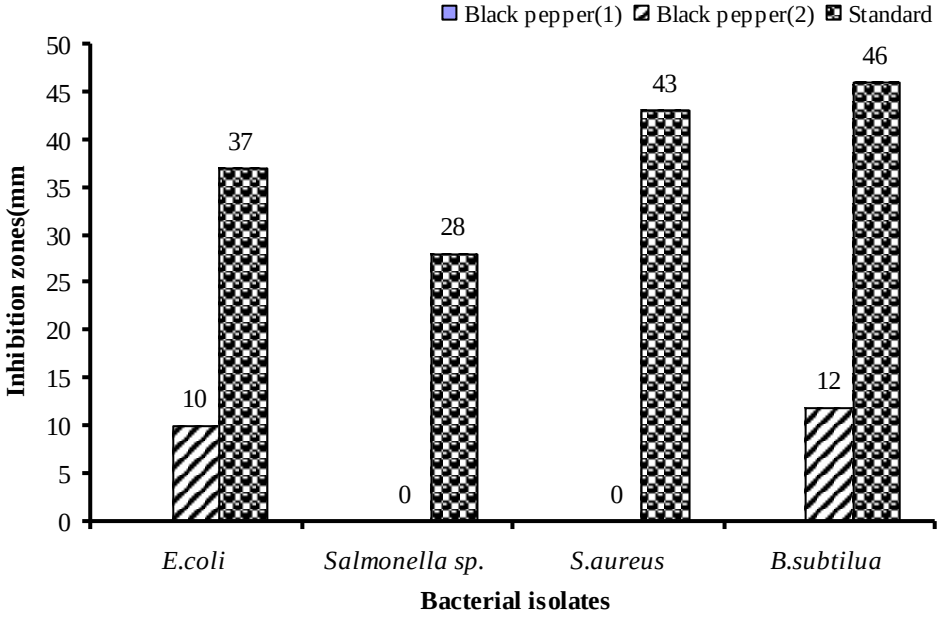


Fig. 6. Zone of inhibition in mm by black pepper (1), black pepper (2) and standard against bacterial isolates at 50:50 concentration.

The present study showed that all the sample products have bactericidal activity. It was found that the stock solution of all the sample products inhibited the growth of all the bacterial isolates, but when the dilutions were made the efficacy reduced. Honey 1 and honey 2 at the same concentration gave different zones, this is inconsistent with the reported of Taormina *et al*., (2001) which states that the zones depend on the type and concentration of honey. Sandal oil 1 on second dilution had no effect on *E. coli* growth whereas sandal oil 2 was effective against all the four strains, suggesting it is highly effective against pathogenic bacteria. Jerovitz *et al*., (2005) also reported that the antimicrobial activity of different sandal oil is different for *E.coli*. Antibacterial activity of black pepper dissolved in distilled water was found to be better than black pepper dissolved in DMSO. Black pepper 2 was found to be effective against *Salmonella* and *Bacillus subtilis*. Similar report have been by Reddy *et al*., (2001) about antibacterial activity of the pure isolates from black pepper (Pipelonguminine) against *Bacillus subtilis* and piperine against *Staphylococcus aureus.* However, further research is needed to optimize the effective use of this agent in clinical practice (Molan, 2001).

This study is a qualitative assessment of selected products against some identified bacterial isolates to whether these products which are commonly used in our daily life, local herbal medical system and ayuvedic system have an activity against different strains of bacteria. Among the natural products used honey is found to be most potent having broad spectrum antibacterial activity. Hence it is recommended that the sensitivity of other bacterial strains should be checked.

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