INTRODUCTION

Benincasa hispida (B. hispida), commonly known as winter melon, is a popular herb in Asia as well as in other countries. The plant belonging to family of Cucurbitaceae is being cultivated for at least 2000 years, and is reported effective in treatment of nervous disorders, ulcer and acidity. The expectorant effect of the B. hispida seeds extract due to mucus secretion prevents gastric ulcer reported4 and. In addition, B. hispida seeds extract could enhance immunoreactions, resulting in histamine secretion inhibition. Methanolic fruit extract has anti diarrheal activity and reduction in gastro intestinal motility was also reported, while methanolic extract of seeds are reported to be beneficial as natural anti oxidant in the treatment of inflammation and pain.

The seeds of Nigella sativa L. (N. Sativa) commonly known as kalunji, belong to family Ranunculaceae are small, black in color with aromatic odour, found in southern Europe, Asia Minor and northern Africa. It is used in folk medicines as a natural remedy for eczema, asthma, diabetes, fever and gastrointestinal disturbances and hypertension. Its oil has analgesic and anti neoplastic activity reported the presence of Thymoquinone, an active constituent of N. sativa seeds, a pharmacologically active quinone, which has different properties like anti-inflammatory and analgesic actions.

The objective of present research was to evaluate the efficacy of seeds of N. sativa and B. hispida against different microbes that are resistant to various prescribed medicines.

MATERIALS AND METHODS

Collection of sample and oil extraction

Mature seeds of N. sativa and B. hispida were purchased from local market and were washed with water to remove the dust, straw and other particles, and then stored in a desiccator at 25°C for two days. 100grams of seeds of N. sativa and B. hispida was ground in a grinder and extracted for 12 hours in a soxhelt extraction with 500mL n-hexane at 70°C. The fixed oil was then concentrated on rotary evaporator using Heidolph, (Germany) VE-11 rotavaporator and stored in glass vials for further use.

Antimicrobial Activity

Test microorganism

Clinical strains of Pseudomonas aeruginosa, Staph. aureus, Es.coli, B.subtilis, Micrococcus luteus and Pasteurella multocida were obtained from Pakistan Council of Scientific and Industrial Research, (PCSIR); Lahore. The microorganisms were sub-cultured on Brain heart infusion agar and Nutrient agar and incubated aerobically at 37°C.

Combinations of seeds used for efficacy study

Different samples were made to testify their antimicrobial activity, B. hispida, N. sativa and combination of both in 1:1 ratio.

Agar well diffusion method

Antimicrobial activity of N. sativa and B. hispida was determined by agar well diffusion method. Pure isolate of each microbe was sub-cultured on the recommended specific media for each microorganism at 37°C for 24h. A plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10^6 CFU/mL (standardized by 0.5 McFarland standard) and used as the inoculum for performing agar well diffusion assay. 100 μL inoculum was taken on the specified plates of media. Wells of 8mm was made in the plates with the help of cork borer. The dried extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO). 100 μL of the extracts was poured in the wells. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24h. The antimicrobial efficacy, shown by an inhibition zone around the well was measured if it was more than 8 mm. The experiment was performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.
RESULTS AND DISCUSSION
The antibacterial activity of seed oil of N. sativa and B. hispida against selected pathogens are shown in Table 1. Antibacterial activity of the oil was evaluated against gram positive bacteria (M. luteus, S. aureus and B. subtilis) and gram negative bacteria (E. coli, P. multocida and P. aeruginosa) using Ciprofloxacin solution (100μg/ml) as the standard drug. The agar well diffusion method showed variations in zones against different pathogens. Maximum mean zone of inhibition was observed against S. aureus (24.66mm) of N. sativa seed oil and 16mm against B. subtilis of Benincasa hispida oil. When combinations of both seeds oil was used in ratio of 1:1 maximum mean zone of inhibition was observed against E. coli (20mm) and then S. aureus (18.97mm) and P. multocida (18.61mm) respectively. The inhibitory effect of seeds oil of N. sativa L. was previously determined against bacteria and other microbes by Akgul who reported the concentration dependent antibacterial and antifungal activity of N. sativa seed. Burits reported the characterization of major component of its oil by GC-MS which include thymoquinone and carvacrol which exhibit antibacterial activity. The antibacterial activity of our sample of Nigella sativa L. may be closely related to the high percentage of these compounds. 

CONCLUSION
In conclusion, present research was carried out to evaluate the activity of Benincasa hispida and Nigella Sativa seed oil on common disease causing resistant pathogens. Studies should be carried out to find more unknown compounds in various species of B. hispida and N. sativa found in South Asia. As the seeds of B. hispida and N. sativa are locally used to treat various skin, stomach and heart diseases therefore these can be used to form some kind of formulation for their treatment as well.

REFERENCES

Table 1: Antibacterial activity of Nigella sativa and Benincasa hispida against selected pathogens

<table>
<thead>
<tr>
<th>Samples</th>
<th>Micrococcus luteus</th>
<th>Escherichia coli</th>
<th>Staphylococcus aureus</th>
<th>Pasteurella multocida</th>
<th>Pseudomonas aeruginosa</th>
<th>Bacillus subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hispida Benincasa</strong></td>
<td>11.95±0.50</td>
<td>12±0.66</td>
<td>14±0.50</td>
<td>13±1</td>
<td>15±0.98</td>
<td>16±0.98</td>
</tr>
<tr>
<td><strong>Nigella Sativa</strong></td>
<td>11±1</td>
<td>9.5±0.70</td>
<td>24.66±0.60</td>
<td>17.5±0.70</td>
<td>15±1.41</td>
<td>15±1</td>
</tr>
<tr>
<td>HB+NS (5:5)</td>
<td>18±0.50</td>
<td>20.31±0.8</td>
<td>18.97±0.54</td>
<td>18.61±0.44</td>
<td>11±1.41</td>
<td>15.79±1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>24mm</td>
<td>24mm</td>
<td>23mm</td>
<td>22mm</td>
<td>21mm</td>
<td>22mm</td>
</tr>
</tbody>
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