

ANTIBACTERIAL STUDIES ON *BENINCASA HISPIDA* AND *NIGELLA SATIVA* OIL

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ABSTRACT

Fixed oil of seeds of *Benincasa hispida* and *Nigella sativa* was found active against various resistant pathogens of both gram positive and gram negative bacteria. The maximum zone of inhibition was observed against *S. aureus* (24mm) for *Nigella sativa* but when 1:1 combination of both the plants was used, good activity against all the tested pathogens i.e., *M. luteus*, *E. coli*, *S. aureus*, *P. multocida*, *P. aeruginosa* and *B. subtilis* (20.31 to 18.61mm) was observed proving seeds as a strong candidate for formulations based on these oils to treat various diseases caused by these resistant pathogens.

Key words: seeds oil, *Benincasa hispida*, *Nigella sativa*, antibacterial

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 years¹, and is reported effective in treatment of nervous disorders, ulcer and acidity^{2,3}. The expectorant effect of the *B. hispida* seeds extract due to mucus secretion prevents gastric ulcer reported by⁴ and¹. In addition, *B. hispida* seeds extract could enhance immunoreactions, resulting in histamine secretion inhibition⁵.⁶ Methanolic fruit extract has anti diarrheal activity and reduction in gastro intestinal motility was also reported⁷, while methanolic extract of seeds are reported to be beneficial as natural anti oxidant in the treatment of inflammation and pain⁸.

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 hypertension⁹. Its oil has analgesic and anti neoplastic activity^{10,11} reported the presences 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 rota evaporator and stored in glass vials for further use¹².

Antimicrobial Activity**Test microorganism**

Clinical strains of *Pseudomonas aeruginosa*, *Staph. aureus*, *E. 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 of¹³. 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 10⁶CFU/ mL (standardized by 0.5McFarland 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 24h¹⁴. 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 calculated¹⁵.

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

Samples	<i>Micrococcus luteus</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i> ,	<i>Pasturella multocida</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
<i>Hispidia Benincasa</i>	11.95±0.50	12±0.66	14±0.50	13±1	15±0.98	16±0.98
<i>Nigella Sativa</i>	11±1	9.5±0.707	24.66±0.60	17.5±0.707	15±1.41	15±1
HB+NS (5:5)	18±0.50	20.31±0.8	18.97±0.54	18.61±0.44	11±1.41	15.79±1
ciprofloxacin	24mm	24mm	23mm	22mm	21mm	22mm

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 microbes¹⁶ by Akgul¹⁶ who reported the concentration dependent antibacterial and antifungal activity of *N. sativa* seed. Burits¹⁷ reported the characterization of major component of its oil by GC-MS which include thymoquinone and carvacrol which exhibit antibacterial activity⁹. The antibacterial activity of our sample of *Nigella sativa* L. may be closely related to the high percentage of these compounds. Hayat¹⁸ reported the presences of amino acids, titerpenoids, tannins carbohydrates in the dried seeds of *Benincasa hispida*.

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.

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