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Evaluation of Antimicrobial potential of *Daucus Carota* (Linn.) peels in some polar and non-polar solvents against both bacterial and fungal strains

Shabnum Shaheen, Mehwish jaffer, Tahira Ilyas, Nidaa Haroon, Sidra Younis and Mehreen Jalal

[1]Department of Botany, Lahore College for Women University Lahore, Pakistan

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ABSTRACT

The present study was undertaken to investigate the antimicrobial activity of peels of *Daucus carota* Linn. through experimental trials. The crude extracts of powdered plant material were obtained in various polar and nonpolar solvents viz: Petroleum Ether, chloroform, methanol and distilled water. Well defined zones of inhibition were recorded indicating that *Daucas carrota* peels was potent against pathogenic microbes i.e. *Staphylococcus aureus* and *Aspergillus candidus*. In case of fungal strain the petroleum ether extract of peel showed significant inhibitory zone against *Aspergillus candidus* (11.66 mm).Whereas for the Bacteria *Staphylococcus aureus*, the distilled water extract of peels showed maximum value for the zone of inhibition. Standard antimicrobial discs: Ampicillin A and Ketoconazole against *Staphylococcus aureus* and *Aspergilus candidus* respectively were used to compare the results and thus significant values of the discs were observed against the A. It can be concluded from the present work that overall antimicrobial activity of the plant is quite reasonable against almost all the strains and provides us the ethno botanical data that may be a supportive point about its medicinal value.
INDEX TERMS—Antimicrobial activity, *Aspergills candidus, Stapylococcus aureus*, Fungal strain, Chloroform.

1. INTRODUCTION

Ethnobotanists aim to document, describe and explain complex relationships between cultures and uses of plants that focusing, primarily, on how plants are used, managed and perceived across human societies e.g. as foods, as medicines, in divination, in cosmetics, in dyeing; as textiles, in construction, as tools, as currency, as clothing, in literature, in rituals, and in social life. Ethnobotany (from "ethnology" - study of culture and "botany" - study of plants) is the scientific study of the relationships that exist between people and plants (Deepak and Anshu, 2008).

The use of medicinal plants as the initial treatment agent is a universal phenomenon. Every culture is basically dependent on the use of plants as therapeutic agents. All the plants have some chemical compounds that show their metabolic action against the diseases. The usage of herbal treatment is considered as the top most priority for the medical point of view as they show almost no side effects and 80% of the people in different geographical zone are depending on these resources (Vijayan *et al.,* 2007).

Antimicrobial activity is actually killing microorganisms, or suppressing their multiplication or growth.

An antimicrobial substance kills or inhibits the growth of microbes such as bacteria, fungi, or viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic) and are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to be low.

The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials (Alfonso *et al*., 1985).

Vuksan and Lsievenipper, (2005) reported that Antifungal treatments are frequently sought after to treat mold growth in damp or wet home materials that exhibit mold growth. Most home mold problems are moisture and water caused and the solution for conquering the mold growth is most dependent upon the water and moisture control and removal/discarding of the mold damaged materials.

S. aureus is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. The golden appearance is the etymological root of the bacterium's name; *aureus* means "golden" in Latin. Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks and new strains of *S. aureus*. Recent genetic advances have enabled reliable and rapid techniques for the identification and characterization of clinical isolates of *S. aureus* in real time PCR (Khurana *et al*., 2009).

***Corresponding Author:** Shabnum Shaheen, Department of Botany, Lahore College for Women University Lahore, Pakistan.
Email:shabnum_shaheen@hotmail.com

Aspergillus candidus reported by some authors as a common contaminant of organic dusts. *Aspergillus candidus* possesses white, typically globes, conidial heads producing globose or subglobose, smooth, thin-walled conidia measuring 2.5–3.5 µm in diameter. The fungus is widely distributed in nature and develops upon vegetation in the later stages of decay. It has been reported from grain, flour, hay, compost and a fur processing facility. The optimal conditions for growth of *A. candidus* in barley grain occur at the substrate water content 20–25% and maximal temperature30–40°C.

The study was lead:

- To have an idea about the medicinal value of the plants found in the nearby vacinity and the presence of growth inhibiting substances that can kill the bacteria and fungal strains as well and are pathogenic in nature.
- To make a consideration of the plants that are ethnopharmacologically significant as antimicrobial agents.
- The data thus obtained may be used to discuss the plant chemistry.

2. MATERIALS AND METHOD

For the determination of antimicrobial activity the utilized organisms were two kinds of pathogenic bacteria namely:-

Staphylococcus aureus

Aspergillus candidus

Collection and preservation of the plant materials

In the presence study an effort was made to find out the antimicrobial potential of a locally available plant, *Daucus carota.* The peels were collected from a juice corner during the month of February.

Before extraction all the plant material was dried at room temperature and then ground to make powder which was preserved in the amber colored specimen jars, until required.

2.3 Solvent Extraction by Maceration Method

150g of ground plant material was extracted in sequence with polar and non-polar solvents .The extraction was carried out by soaking the powder in each of the solvent for the period of 8 days. e.g. P. ether, Chloroform, Methanol and Water. The residue was filtered and the filtrate was preserved in the labeled amber colored glass jars, whereas the residue was further soaked in the next solvent in series.

2.4 Medium Preparation

Each of the bacteria were cultured on the nutrient agar medium (Cruickshank *et al.,* 1975). The medium for each bacteria was prepared as follows:

Nutrient broth =0.8 gm , Agar =1.4 gm, Distilled water =100 ml

The medium for each fungal strain was prepared as follows (Johnssen1940)

PDA=3.5gm, Malt extract =2gram, Agar=1.8gram , Distilled water=100ml. All the ingredients were mixed in distilled water on hot plate and sterilized by autoclaving at 121°C with 15 lb/sq inch pressure for 15 minutes. The bacteria cultured on this medium were used for preparing inoculums.

2.5 Preparation of potato dextrose agar medium

The fungi were cultured on potato dextrose agar medium, which was prepared according to Johnnsen (1940). For the fungus culture 3.9 grams of PDA (potato dextrose agar was dissolved in 100 ml of distilled water in 250 ml of Erlenmeyer flask, gently heated on hot plate and then sterilized by autoclaving at 121 degree centigrade with 15 lb/sq inch pressure for 15 minutes, the pH was adjusted at 5.5.

Slants for bacteria

About 5.o ml of agar was added in each test tube .the tubes were cotton plugged and then sterilized by autoclaving at 121°C with 15 lb/sq inch pressure for 15 minutes. After this test tubes were placed in slanting position and medium was allowed to solidify at room temperature. Bacteria were transferred into these slants with the help of inoculating needle, in laminar flow. Each slant was labeled with the name of bacterium present in it and was incubated for 24 hours. These bacterial cultures were used for preparing innocula. *Slants for fungi*

About 5.oml of potato dextrose medium was added in each test tube and was cotton plugged. These test tubes were then sterilization by autoclaving it. These test tubes were placed in slanting position and the medium was allowed to solidify at room temperature. Fungi were transferred into these slants with help of inoculating needle, in laminar flow. each slant was labeled with the name of fungus present in it and incubated for 5 days at 30°C. These fungal cultures was used for preparing inocula (Qadeer *et al .,*1990).

Preparation of inoculums

A 24 hours old culture of each bacterium and 5 days old culture of each fungus was as an inoculums for antimicrobial screening.. the culture were prepared on slants. About 25ml distilled water was added in 250ml Erlenmeyer flasks and was sterilized in autoclave. After appearance of colonies on slants 10ml sterilized distilled water from each flask was added in to these slants, with the help of sterilized pipettes. Colonies were gently scratched over the surface of slant with the help of inoculating needle. This suspension in slant was again added into 250 ml Erlenmeyer flasks which were kept in shaker for 30 minutes to break the clumps of conidia in case of bacteria or spores in case of fungi and to form a homogenized mixture.

Preparation of plates

Petri plates were sterilized at 180°C for two hours. The sterilized melted nutrient agar and potato dextrose agar was poured into these sterilized Petri plates at a temperature of 45°C. 10ml of inocula suspension was added into flask containing nutrient medium and the temperature of medium was kept between 47-52°C. After pouring the medium in plates, it was then allowed to solidify at room temperature.

The prepared Petri plates containing the solidified and inoculated medium subjected for the measurement of anti microbial activity of crude extract of given plant. For this purpose the Petri plate was marked into four sections and marked these sections as 1, 2, 3 and 4 and labeled accordingly. In each section the uniform hole was made with the help of cork borer No. 2. In hole of first section the crude extract was poured, in the second hole pure solvent was added, in the third hole commercially available standard antibiotic disc was placed, as Ampiccilin disc (25 ug) **Ketoconazole** disc (25ug), Augmentin disc (30ug). The anti fungal and anti bacterial disc was utilized in same manner a d whole process was carried out in the aseptic conditions. a zone of inhibition became prominent after the incubation time, that is 24 hours for bacteria and 48 hours for the fungi .Some of plates showing zone of inhibition were also photographed. The zones were also produced by pure solvent and the standard discs, but the actual measurement of zone of inhibition was obtained after subtracting the value produced by pure solvent. The zones of inhibition were measured with the help of Vernier caliper in millimeters.

Statistical analysis

First, mean value and standard value was calculated. The data was presented as Mean ± S.E.(M± standard error). The treatment effects were compared after Snedecor and Cochran (1980) and significant differences among replicates was presented as Duncan's multiple range tests, in the form of probability <p> values, using the computer software Costat, cs6204 W.exe. and SPSS software was used for the formation of Anova Tables. The Duncan's Multiple Range test was also applied to know the significant result. The results were ultimately presented in the Tables.

3. RESULTS

Daucus carota distilled water peel extract produced the maximum value of zone of inhibition ,i-e. 14.83 ± 0.76^{a} against *Staphylococcus aureus* and minimum value of inhibitory zone were observed by methanol extract ,i-e.11.5 ± 0.5^{c} against *Staphylococcus aureus* respectively as given in table 4.1.*Daucus carota* petroleum ether peel extract produced the maximum value of zone of inhibition,i-e 11.66 ± 0.76^{a} against *Aspergillus candidus* and minimum value of inhibitory zone were observed by distilled water extract i-e.10.5 ± 0.5^{a} against *Aspergillus candidus* respectively as given in table 4.2.

4. DISCUSSIONS

The use of herb and medicinal plants as the first medicine is a universal phenomenon. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plant for their therapeutic properties .All drugs of the past were the chemical substance with a particular therapeutic extracted from plants. The results indicated that all the plants and their portion are antimicrobial in nature some of them showed very highly antimicrobial against bacteria and fungi, while some of them were comparatively less antimicrobial in nature. A variety of standard antimicrobial discs were run to compare the zones of inhibition against *staphylococcus aureus* and *aspergillus candidus*. *Daucus Carota* peel water extract showed the maximum value for zone of inhibition 14.83 ± 0.76^{a} against *Staphylococus aureus*, this ovary high antimicrobial value may be due to the compound having strong antibiotic potential present in the extract. The same work was done by Meriqa *et al* 2012 in which he used the bulb extracts and determined the antimicrobial activity against different strains including the *Staphylococcus aureus.* It can be due to the presence of antimicrobial compounds present in both the plant extracts.

The maximum antifungal value was observed in peel petroleum ether extract(11.66 ± 0.76^{a}) against *Aspergillus candidus* .Antifungal value of the extract may be due to the presence of such compounds in the extracts.in the same way Dash *et al*., 2011 determined the antifungal activity of the *Centella asiatica* plant against some fungal strains in different extracts and observed the remarkable zones of inhibition.

No significant difference between the type of fungi was obtained when analyzed by analysis of variance, and the significant differences were further evaluated by Duncan's Multiple Range Test. The significant difference was observed among the plant parts and as well as among the solvents used. The significant difference was observed between the plant parts and the fungal species as well as reported in Anova table.

5. CONCLUSION

It can be concluded from the present work that overall antimicrobial activity of the plant is quite reasonable against almost all the strains and provides us the ethno botanical data that may be a supportive point about its medicinal value

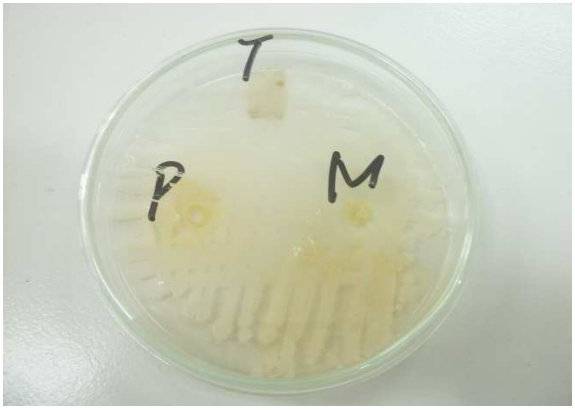

Plate no 1; Zone of inhibition (mm) produced by various extracts of *Daucus carota* peels against *Staphylococcus aureus*.

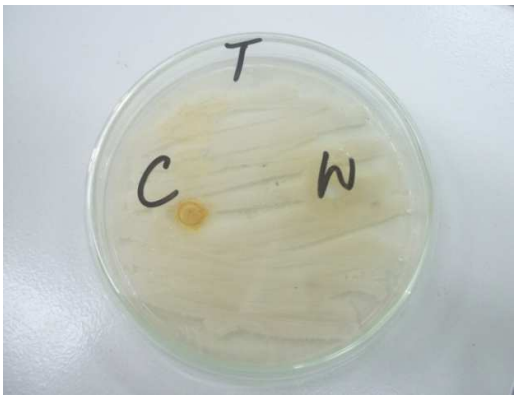

Plate no 2; Zone of inhibition (mm) produced by various extracts of *Daucus carota* peels against *Staphylococcus aureus*.

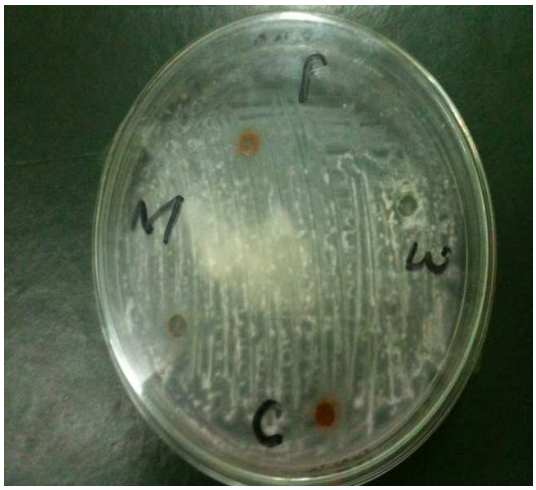

Plate no 3; Zone of inhibition (mm) produced by various extracts of *Daucus carota* peels against *Aspergillus candidus.*

Table I. Zone of inhibition (mm) produced by various extracts of *Daucus carota* against *Staphylococcus aureus*.

Petroleum ether	Chloroform	Methanol	Water
12.8±0.76^{b}	11.8±0.76^{c}	11.5±0.5^{c}	14.8±0.76^{a}

Each value is an average of three replicate± denotes standard deviation among replicates And number followed by different letters at 0.05 level of significance (P≤0.05) whereas the value of LSD 0.57.

Table II. Zone of inhibition (mm) produced by various extracts of Daucus Carota against *Aspergillus candidus*

Petroleum ether	Chloroform	Methanol	Water
11.6±0.76[a]	10.83±0.76[a]	10.83±0.76[a]	10.5±0.5[a]

Each value is an average of three replicate± denotes standard deviation among replicates and number followed by different letters at 0.05 level of significance (P≤0.05) whereas the value of LSD 1.57.

Table III. Zone of inhibition (mm) produced by various standard discs against Bacteria and Fungi.

No. Of Observations	Zone of inhibition(mm)	
	Ampicillin A	Ketoconazole
S.aureus	23.7mm 24.9mm 22.7mm	- - -
A.candidus	- - -	44.2mm 45.7mm 47.5mm
Mean	23.6mm	46.5mm

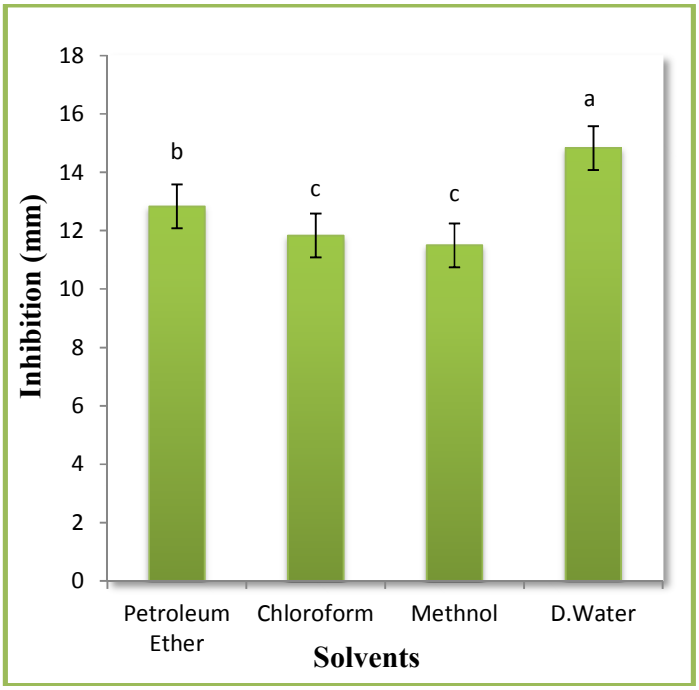

Fig. 1: Zone of inhibition (mm) produced by various extracts of *Daucus carota peels* against *Staphylococcus aureus*

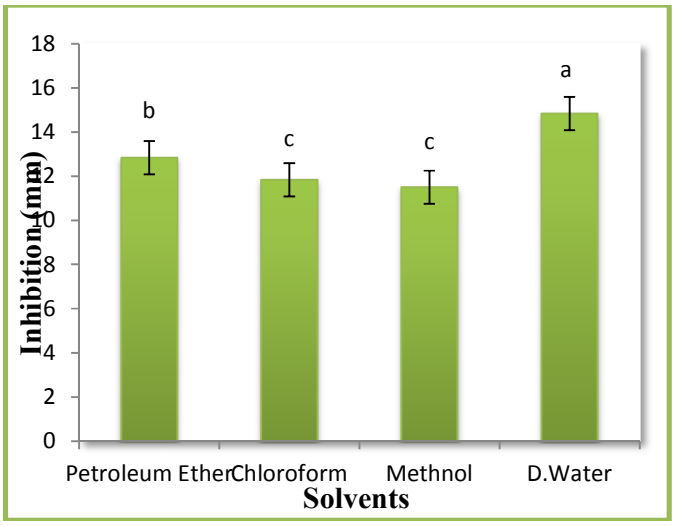

Fig 2. Zone of inhibition (mm) produced by various extracts of *Daucus carota peels* against *Staphylococcus aureus*

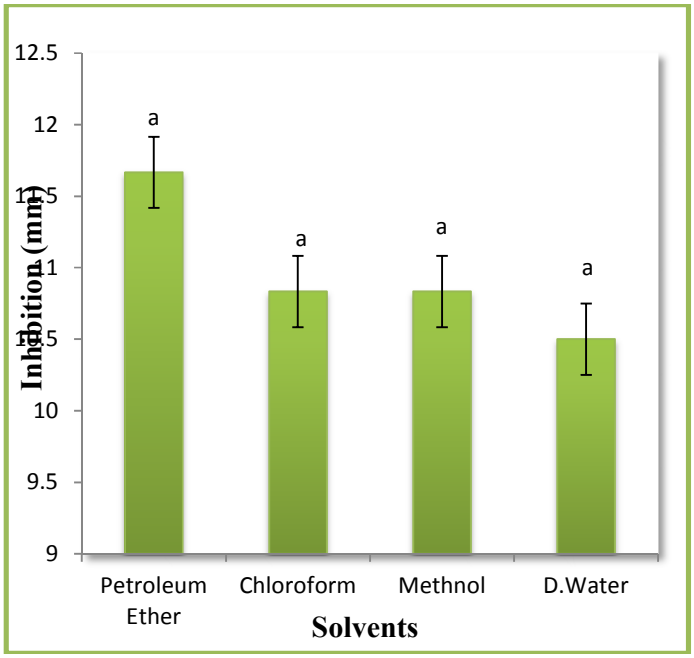

Fig 3. Zone of inhibition (mm) produced by various extracts of *Daucus carota peels* against *Aspergillus candidus*

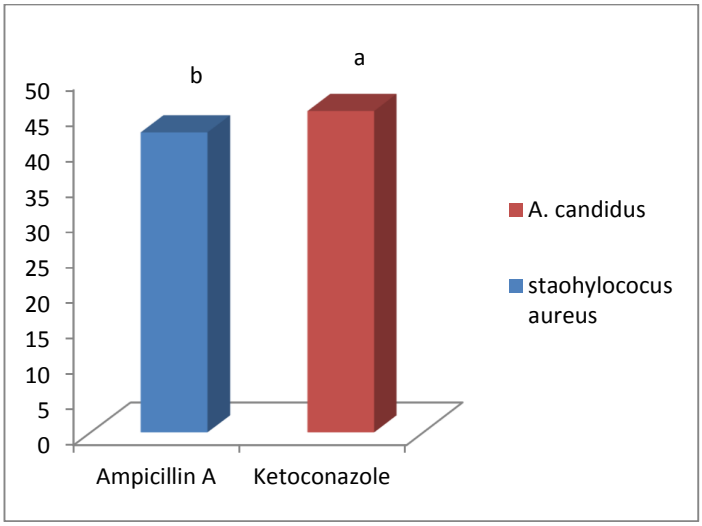

Fig 4. Zone of inhibition (mm) produced by various standard discs against Bacteria and Fungi.

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