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Antimicrobial Activities of *Daucus Carota* Seeds on Selected Pathogenic Micro-organisms

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ABSTRACT

Background: *The antibacterial/antifungal toxicity of Daucus carota (carrot) seeds was evaluated using selected multi-drug resistant bacteria and yeast of clinical origin.* ***Methods:*** *The active constituents of the Daucus carota seeds were extracted using conventional Plant Tissue Homogenization method using cold distilled water, Ethanol and Methanol as solvents. Varying concentrations (5-250 mg/ml) of the three extracts were assayed for antimicrobial activity against the selected isolates- Salmonella typhi, Staphylococcus aureus, Escherichia coli, Klebseilla pneumoniae and Candida albicans; the agar well diffusion method was used. The antibiogram profile of the organisms was also obtained through disc diffusion method.* ***Results:*** *Similar activity was observed in the methanolic and ethanolic extracts while cold distilled water showed no activity on any of the isolates. The antibiotic susceptibility results showed that the isolates used are highly multi-drug resistant. Ofloxacin exhibited the most pronounced activity against all the isolates. Gentamicin and erythromycin both showed activity on Escherichia coli and Salmonella typhi. Lower concentrations of both extracts presented no inhibitory effects on the test organisms, thus resulting in high MIC values recorded for both extracts. Also, the extracts showed no bactericidal action against the isolates.* ***Conclusions:*** *Observations from this research therefore affirm that Daucus carota seeds possess antimicrobial properties that may be explored as a source of future antimicrobial compounds.*

Key words: *Daucus carota, antimicrobial properties, Phytochemicals, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC)*

INTRODUCTION

Medicinal plants contain certain active components, which have been exploited in traditional medical practice for the treatment of various illnesses over the years.[1] Plants of both lower and higher categories are examined to produce active chemical substances with which they defend themselves against foreign agents such as invading microorganisms.[1] Vegetable is an edible component of plants or its part, intended for many purposes of which includes medicinal purposes, commercial purposes.[2]

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Vegetables are an extraordinary dietary source of nutrients, micronutrients, and vitamins, and are thus vital for health and wellbeing. Wild Carrot is the source of the cultivated carrot. This specie is the source of the natural food dye, carotene, which provides it with additional commercial importance. Carrots belong to the Umbelliferae family, an extensive order of the herbaceous plants, and are of great importance to man.[3] Carrots are often eaten cooked, many carrots are also consumed fresh, some are use as dish or stews while several others are poisonous. Carrot has nutritional and health benefits as well as its seeds. *Daucus carota* seeds have a very strong taste and can be used as a seasoning.[4]

Carrot is an outstanding source of phyto nutrient, carrots actually contain a fascinating combination of phyto nutrients, including other carotenoids (especially alpha-carotene and lutein), hydroxycinnamic acids including (caffeic, coumaric, ferulic,) anthocyanins (in the case of purple and red carrots), and polyacetylenes especially falcarinol and falcarindiol.[5] Carrots are an excellent source of vitamin A (in the form of carotenoids). In addition, they are a very good source of biotin, vitamin K, dietary fiber, molybdenum, potassium, vitamin B6, and vitamin C. They are also a good source of manganese, niacin, vitamin B1,

panthothenic acid, phosphorus, folate, copper, vitamin E, and vitamin B2.[6]

The seeds have cardio-protective, hepatoprotective, anti-bacterial, anti-fungal, anti-inflammatory and analgesic effects.[7] *D.* carota seed oil exhibits both smooth-muscle relaxant and vasodilatory action in isolated animal organ studies.[8] This study affirms the antimicrobial potentials of carrot seeds using multi drug-resistant clinical isolates.

METHODS

Collection and Identification of Pathogenic microorganisms

Plant samples were collected and identified from the Herbarium, Plant Biology Department, University of Ilorin, Nigeria.

Source of isolates and maintenance

Pure cultures of selected pathogenic microorganisms were obtained from the Medical Microbiology laboratory, University of Ilorin Teaching Hospital (U.I.T.H), Ilorin, Kwara State. The organisms used in this study were *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus and Candida albicans*. The test organisms were inoculated on sterile agar slants before storing in the refrigerator at 4˚C. Subcultures were done at every 2 weeks' interval to maintain the viability of the test organisms.

Preparation of the plant sample for extraction

The seeds were air dried in a dehumidified room for five (5) days, and pulverized into powdery form using an electric blender. The powdered seeds were then weighed and stored in clean air tight bottles until use.

Preparation of *Daucus carota* seed extracts

Three solvents were used for the extraction, that is, distilled water (aqueous), 90% ethanol and100% methanol. Extracts were prepared as described by Doughari, (2012)[9] with slight modifications. Fifty grams (50g) of the powdered seed of *Daucus carota* was added to 200ml of each solvent and were placed on an orbital shaker for 72 hours. The extracts were then separately sieved using muslin cloth and then filtered using No 1 Whatmann filter paper. The filtrates were centrifuged at 3,000 RPM for 10 minutes. The pellets were discarded and the supernatant were sterilized using the membrane filtration unit with type HC filters. The filtrates were collected into sterile bottles and stored in a refrigerator until use.

Determination of Antibacterial activity of the Extracts

The antibacterial activity of the extracts against the selected test organism was determined by a modified agar well diffusion assay method as described by Nouri *et al*., (2009).[10] The organisms were sub-cultured on sterile nutrient agar plates for at 37^{o}C for 24 hours. The sub-cultured test organisms were then standardized according to Da Silva-Diaz, (2014)[11] by aseptically suspending a loopful of the test organisms in sterile peptone water. It was then adjusted to a turbidity of 0.5 Mcfarland standards (10^5 (CFU)/mL).

The solidified MHA plates were then inoculated with 0.5 Mcfarland Standard of the test organisms by dropping a loopful of the broth on the solidified agar plate and then using sterile cotton swab to uniformly spread the inoculum the surface of the agar plate. The plates were left for 20 minutes for the organisms to diffuse into the agar. Five holes were aseptically bored on each of the agar plates, using a sterile 6mm cork bore. The agar wells were then filled with the extracts using sterile micro pipette (one concentration per hole). The plates were then allowed to stand thirty minutes to allow proper diffusion of the extracts into the agar after which they were incubated upright at 37˚C for 18-24 hours. Antibacterial activity was determined by observing, measuring, and recording the diameter of zone of inhibition to the nearest millimetre (mm) using a metre rule.[12]

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined by using the Broth dilution method described by Doughari *et al*. (2012).[9] 0.9ml of the extracts at concentrations of 200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 5mg/ml was added to 9ml of sterile MHB in different test tubes. 1ml of 18hour culture of each of the test organism adjusted to 0.5 Mcfarland Turbidity Standard (1.0 X 10^8) was inoculated into the test tubes. Two control tubes were set up for each batch of organism. These are: one tube containing only 9mls of sterile peptone water, one tube containing 9mls of sterile peptone water and 1ml of respective extract concentrations and, one tube containing 9ml of sterile peptone water and 1ml of respective test organism. After incubation, the tubes were examined for microbial growth by observing the turbidity. The tube with the lowest concentration (highest dilution) with no detectable bacterial/fungal growth or least turbidity when compared to the control tube was taken as the MIC.

Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Samples from the test tubes used in the MIC assays which did not show observable sign of turbidity (or growth) after incubation were streaked out on solidified nutrient agar/PDA plates using sterile cotton swab and incubated at 32/35^{o}C for 16/48hours for the bacterial and fungal plates, respectively. The lowest concentration of the extract that shows no growth on plates after incubation indicates bacterial/fungicidal effect and was taken as the MBC (for the bacterial strains)[12] and as the MFC (for the fungal strain).

Antibiotic Susceptibility Test of organisms

The antibiotic susceptibility testing by a standardized disc method as described by Bauer *et al.,* (1965) was employed. The antibiotics used for this study had been prepared into kit containing multiple discs, each with small discs impregnated with different types of antibiotics. The antibiotics used and their corresponding concentrations are as follows:

Gentamicin (10µg), Cotrimaxazole (25µg), Ofloxacin (30µg), Tetracycline (30µg), Amoxycilin (25µg), Notrofurantoin (300µg), Nalidixic acid (30µg) and Augmentin (30µg) for the gram-positive bacteria and

Ceftazidine (30µg), Cefumoxime (30µg), Gentamicin (10µg), Ceftriaxone (30µg), Erythromycin (5µg), Cloxacilin (5µg), Ofloxacin (5µg) and Augmentin (30µg) for the gram-negative bacteria isolate.

The disc diffusion technique was used for the antibiotic sensitivity test. 18 hour-old broth cultures of the organisms were swabbed on sterile Mueller Hinton agar plates using sterile swab sticks. The multiple antibiotic discs were then placed on the agar surface and pressed using sterile forceps to ensure complete contact with agar. The bacterial isolate plates were incubated at 37^0C for 18hours and the fungus plate at 25^0C for 48hours. The zones of inhibition generated by the antibiotics were measured to the nearest millimetres (mm) [12]. This was intended for comparison of results with those obtained from the potent extracts. Tt he concentrations of the antibiotics as well as the interpretation of zones of inhibition were in accordance with Performance Standards for Antimicrobial Susceptibility Tests.[13]

RESULTS

Sensitivity of test organisms to extracts

Figure 1 shows the sensitivity of the selected pathogenic organisms (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, Candida albicans*) to the three extracts at a stock concentration of 250 mg/ml. All the test organisms were sensitive to ethanol and methanol extracts of *Daucus carota* seeds. However, the aqueous extract had no effect on any of the organisms.

Antibiogram profile of the test organisms

The bacteria used in this study are generally multi-drug resistant. The isolates were completely resistant to eight of the eleven antibiotics used (Fig 2). Ofloxacin inhibited the growth of all the bacteria while erythromycin and gentamicin inhibited *E. coli* and *S. typhi.* The highest activity recorded was 33.5 mm for Ofloxacin on *S. aureus* and the least being 14 mm for gentamicin on *E. coli*.

Effects of varying concentrations of ethanol and methanol extracts

The test organisms were subjected to varied concentrations of the ethanol and methanol extracts ranging from 5 – 200 mg/ml. All the organisms except *C. albicans* showed considerably high resistance to lower concentrations of the ethanolic extract. *S. typhi* and *E. coli* were completely resistant to all the concentrations. *S. aureus* and *K. pneumoniae* were sensitive to extract concentrations at 150 mg/ml and/ or 200 mg/ml. *C. albicans*, however, showed susceptibility to all the concentrations of the ethanolic extract with inhibition zones ranging from 12 – 19 mm (Fig 3). For the methanolic extract, a slightly higher activity was observed for the lower concentrations compared to the ethanolic extracts. As observed in ethanolic extract, *S. typhi* and *E. coli* were resistant to all concentrations of the extract. *S. aureus* was sensitive to all concentrations with zones of inhibition ranging from 11 – 19 mm (Fig 3). *K. pneumoniae* and *C. albicans* were also sensitive to higher concentrations (100 – 200 mg/l, 150 and 200 mg/ml, respectively).

Minimum Inhibitory Concentration and Minimum Bactericidal concentration

Minimum inhibitory concentration (MIC) values obtained were 200 mg/ml, 200 mg/ml and 250 mg/ml for *K. pneumoniae, C. albicans* and *S. aureus,* respectively (Table 1). Both extracts, however, had no MBC value on any of the test organisms (Table 1).

DISCUSSION

The ethanolic and methanolic extracts of *Daucus carota* seeds at stock concentration of 250 mg/ml showed inhibitory effects against selected bacteria and yeast of clinical origin. Similar trends were also observed in the zones of inhibition of both extracts. However, the aqueous (cold) extract failed to show any inhibitory activity on any of the isolates. The variations in the antibacterial activity of the three extracts, in particular the aqueous extracts could have a nexus with the nature of the solvents. Ethanol and methanol are both organic solvents with higher polarity compared to what. Due to this, bioactive compounds in the plants (phytochemicals) are extracted and dissolve more readily in these solvents than water.[14,15]

Besides the higher zones of inhibition displayed by few (three) of the standard antibiotics used, it could not be assertively concluded that the antibiotics were more effective on the test organisms than the plant extracts. The four bacteria used in the study were all strictly resistant to eight out of the eleven antibiotics used. The eight ineffective antibiotics include second and third generation cephalosporins, aminoglycosides, quinolones and penicillins. The organisms showed sensitivity to at most three antibiotics out of the eleven used, thus indicating mass extensive drug resistance (XDR). Ofloxacin was the most effective antibiotic by inhibiting the growth of all the bacteria (17.5 - 33.5 mm). This appreciable activity could be due to the broad-spectrum mechanism of action of ofloxacin.

Lower concentrations (5 - 200 mg/ ml) of both ethanolic and methanolic extracts did not seem to have much effect on the test organisms. *S. typhi* and *E. coli* were completely resistant to all the concentrations for both extracts. Consequently, *C. albicans* and *S. aureus* were sensitive to all concentrations of the ethanolic and methanolic extracts, respectively. *K. pneumoniae* was also sensitive to concentrations ranging from 100 - 200 mg/ ml. The resistance of these organisms to lower concentrations of the extracts may indicate that higher concentrations of the seed extracts are needed to cause an inhibitory effect or cidal effect on pathogenic organisms. Following the trend of results from the effects of varying concentrations, the least MIC value obtained was 200 mg/ ml for *K. pneumoniae* and *Candida albicans* both in ethanolic extract. The stock concentration 250 mg/ml was taken as the MIC value for the isolates in methanolic extract and *S. aureus* in ethanolic extract. Despite the bacteriostatic actions observed at high concentrations, the extracts did not show bactericidal action against any of the isolates.

Table 1: MIC and MBC of ethanol and methanol extracts

Organism	Conc. of ethanol (mg/ml)						MIC	MBC	Conc. of methanol (mg/ml)						MIC	MBC
	5	25	50	100	150	200			5	25	50	100	150	200		
S. aureus	+	+	+	+	+	+	250	-	+	+	+	+	+	+	250	-
K. pneumoniae	+	+	+	+	+	-	200	-	+	+	+	+	+	+	250	-
C. albicans	+	+	+	+	+	-	200	-	+	+	+	+	+	+	250	-

'+' indicates growth '-' indicates no growth

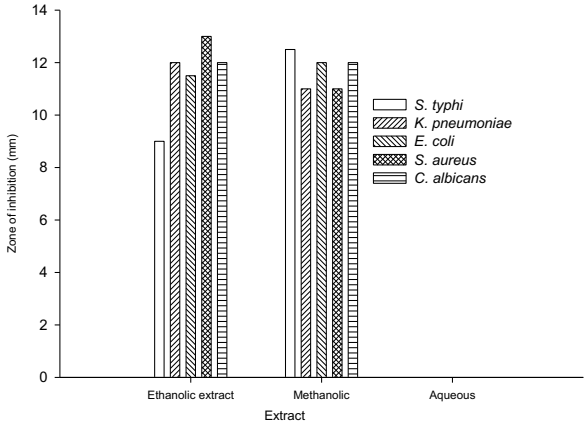


Fig 1: Antibacterial activity of the extracts of *Daucus carota* seed

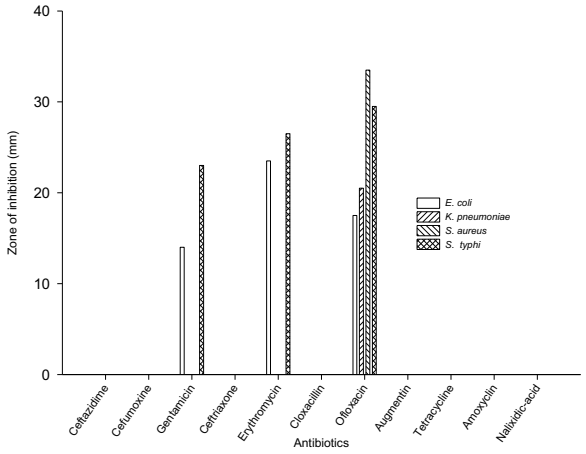
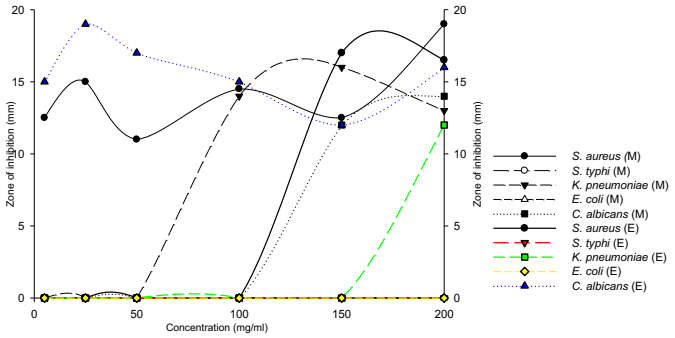


Fig 2: Antibiotic susceptibility test of the organism

Evidently, seeds of *Daucus carota* possess antimicrobial properties but its efficacy will depend on the nature of solvent and type of extraction of the active ingredients. The high MIC observed could be because of the crude nature of the extracts; other compounds which are irrelevant in the antimicrobial activity may be present in the extracts thus suppressing the active ingredients. More defined activities maybe observed by using a purified form of the bioactive ingredients. According to reports on several researches on essential oil from *Daucus carota* seeds, a more pronounced level of antibacterial activity is observed in the oil compared to the grounded seed. Antibacterial activities of oils have been attributed to the presence of high concentrations of thymol, thymoquinone and P - cymene as well as the ability of the essential oil to permeate the cell membrane.[16,17] Phytochemicals such as flavonoids, phenols and various glycosides have been identified in the seeds of *Daucus carota*.[18] These phytochemicals have all been confirmed to possess pharmacological properties which support their potential use as antimicrobial agents.

The alarming rate of increase of resistance to antimicrobials is a major public health concern. More worrisome is the decline in the production of new antibiotics and the constant evolution of resistance to existing ones. The search for new sources of effective antimicrobial compounds with little or no chances of cross resistance is therefore very crucial.[19]



'E' represents ethanol 'M' represents methanol

Fig 3: Activity of the various concentrations of ethanolic and methanolic extracts on the organisms

CONCLUSION

Seeds of *Daucus carota* have proven to possess biologically active components that can be channelled into producing prophylactic or therapeutic antimicrobial agents. It has also presented possible uses in the treatment of fatal or persistent infections involving multi drug-resistant bacteria or yeast. Further research is required to advocate its efficacy at lower concentrations.

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