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ANTIBACTERIAL ACTIVITY OF *DAUCUS CAROTA* (CARROT) PEEL EXTRACT ON SOME SELECTED CLINICAL ISOLATES FROM MARYAM ABACHA WOMEN AND CHILDREN HOSPITAL, SOKOTO

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ABSTRACT

The antibacterial activity of *Daucus carota* (Carrot) peel extract was evaluated using clinical bacterial isolates obtained from Maryam Abacha Women and Children Hospital, Sokoto. Active constituents of *Daucus carota* peel were extracted using aqueous and methanol as solvents, varying concentrations of the extracts (25-200mg/ml) were assayed against *S. aureus*, *E. coli*, and *P. aeruginosa*; the method used was agar well diffusion method and considerable zones of inhibition were recorded against *S. aureus* and *E. coli* while *P. aeruginosa* showed no zone of inhibition at 200mg/ml concentration, aqueous extract showed maximum zone of inhibition. Ciprofloxacin was used as standard antibiotic to compare zone of inhibition with that of extract. This study showed that *S. aureus* and *E. coli* were sensitive to both extracts. Minimum Inhibitory Concentration (MIC) values against all the organisms were also recorded, but no Minimum Bactericidal Concentration (MBC) was recorded. It can be concluded from this study that overall antibacterial activity of *Daucus carota* peel extracts was quite reasonable against *S. aureus* and *E. coli* and provides data that may be supportive point about its medicinal values.

KEYWORDS: Daucus Carota; Antibacterial Activity; Bacterial Isolates; Zone Of Inhibition.

INTRODUCTION

Aromatic and medicinal plants, such as those found in Lamiaceae and Apiaceae families, have been widely used in folk medicine to treat several ailments. Their effects are particularly associated with the essential oils, which are widely described as having several bioactive properties such as antioxidant, anti-inflammatory, antifungal, and antibacterial ones.[1] Plants of the genus Daucus L. (Apiaceae) grow mostly in temperate regions of Europe, West Asia, and Africa. Nevertheless, some species have been found to grow in North America and Australia. The species *Daucus carota L.*, commonly known as Carrot, is recognized worldwide due to its roots widely used for both food and medicinal purposes. In addition, the seed essential oil has also been described as antihelmintic, antimicrobial, hypotensive, and diuretic, amongst other biological properties.[2]

Nevertheless, only a few studies identify the subspecies used, a very important aspect to consider bearing in mind the high variability mentioned. For example, D. carota subsp. halophilus essential oil has been reported for its antifungal properties against several human pathogenic fungi. In turn, besides the antifungal activities, D. carota subsp. gummifer essential oil has also been described as an anti-inflammatory agent while that of D. carota subsp. maritimus has been pointed out as exhibiting a potential antibacterial effect.[3]

Vegetables and some fruits yield between 25% and 30% of nonedible products, which include skins and seeds that normally have no further usage and are commonly wasted or discarded. The byproducts of plant food processing represent a major disposal problem for the industry concerned, but they are also promising sources of compounds which may be used because of theirfavourable technological or nutritional properties.[4]

Fruits and vegetables are rich sources of nutrients that are directly or indirectly associated with homeostasis in human beings.[5] They contain a variety of phytochemicals (also known as bioactive compounds) recognised for their nutraceutical effects and health benefits.[5] These chemicals aid in the prevention of cancer and cardiovascular diseases due to their antioxidant and anti-inflammatory action[6], plasma lipid modification[6,7], and anti- tumor properties.[8] In addition, phytochemicals are also responsible for the smell, flavour, and colour of agricultural commodities.[6] The peels and pomace of fruits and vegetables are a

source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which includes antioxidants, antimutagenic, cardio preventive, antibacterial and antiviral activities.[9] Carrot (*Daucuscarota*) is classified as vitaminized food as it is rich in β-carotene, ascorbic acid and tocopherol6. Carrot is also a significant source of phenolic compounds such as hydroxycinnamic acids and derivatives[10], para-hydroxybenzoic acids and polyacetylenes.[11] Carrot (*Daucus carota*) is one of the most global well-liked root vegetables. It is an important crop of Apiaceae family with small, mostly white flowers set in umbrella-like inflorescence.[12] A long time ago, carrots were applied for health care purposes and regularly used in human nourishment[13], Plants naturally are an affluent supply of active ingredients with healing potential to improve human fitness with controlled hostile effects.[14] Such ingredients has an imperative pharmacological effects and consequently a broaden global market. Many anti hepatotoxic, cardio tonic, nutraceuticals, sweeteners, food additives and animal feed.[15] The studies on evaluating the phenol content of carrot revealed the peel had higher content of phenolics than the flesh. Kähkönen *et al.*[16] found that carrot peel and flesh contained 6.6 and 0.6 mg gallic acid equivalent/g dry weight respectively. Phenolic content in different tissues decreased from peel, phloem to xylem, similarly antioxidant and radical scavenging activities in different tissues decreased in same order as the phenolic content and correlated well with total phenolic contents.

MATERIALS AND METHODS

Study Design: The study was cross sectional and comparative.

Study Area: This study was carried out in sokoto state which is located within the North-Western geopolitical zone of Nigeria. According to the National Population Commission (2010), population figures stand at 3,7026,76 persons with a land area of 33,776.89 square kilometres. The population mainly consists of the Hausa/Fulani ethnic groups; the major occupation of the people is farming and animal husbandry. The two major seasons in the State are the dry (October to May) and wet seasons (May to October). Majority of its indigenes are Muslims. Sokoto state is located between latitude 9^{O} Nand 4^{O}N and between 3^{O}E and 8^{O}E in the Northern Nigeria. It has the sudan savannah type climate and vegetation. Most of the acgriculturist here practice irrigation due to the nature of rain and harshness of the sunlight.[17] The study area was Maryam Abacha Women and Children Hospital sokoto.

Study Population: Study population was patients attending Maryam Abacha Women and Children Hospital located at the old market area of sokoto north local government sokoto.

Ethical Approval: The ethical approval was obtained from the ethical committee of Maryam Abacha Women and Children Hospital Sokoto.

Sample Collection

Plant collection and identification: *Carrot (Daucus carota)* was obtained commercially at local garden at Ruggar Liman of kware local government area sokoto state, Nigeria and the Authentication and Identificationof plant materials was carried out by a Botanist at Herbarium Laboratory of the Department of Botany Usmanu Danfodiyo University, Sokoto ID (UDUTH/ANS/0183).

Test Bacteria: Clinical bacterial Isolates used for this study were *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* and were obtained at Maryam Abacha Women and Children Hospital Sokoto. *Staphylococcus aureus* isolate was subcultured on Muller Hinton agar and colonial appearance was large, round, opaque, golden yellow colonies and the identity was confirmed by gram stain, catalase and coagulase biochemical test; *Escherichia coli* isolate was subcultured on Mac Conkey agar and produced lactose fermenting colonies and identity was confirmed by Gram stain, Citrate, Urease and KIA biochemical test and *Pseudomonas aeruginosa* isolate was subcultured on Muller Hinton agar and the identity was confirmed by production of pigment and oxidase biochemical test.[18]

Media Preparation: The media used were Muller Hinton Agar, Nutrient Agar, Nutrient broth, MacConkey agar and were prepared according to the manufacturer's instructions and aseptic procedure as described by Cheesbrough, 2003.

Preparation of Crude Plant Materials

Healthy, disease free and mature carrots was purchased from the local garden. The carrot was cleaned and washed, and it was manually peeled using a sterile peeler and the peels were then shade dried at room temperature until it dried completely. The shade dried carrot peels was powdered in a pestle and mortar and kept in airtight bottles until further use. The powdered carrot peel was soaked in methanol and Water for 24 hours by maceration technique. The supernatants were filtered through Whatman No.1 filter paper and concentrated using hot air oven and water bath respectively. The dry residue was preserved at 5°C in airtight bottles until further use.[4]

Preparation of Aqueous Crude Extracts: The extraction was carried out using maceration method at the department of pharmacognosy and ethnomedicine. Fifty seven grams (57g) of powdered extract was weighed and dispensed in500mls of distilled water in a conical flask and the crude preparation was left in a shaker at room temperature for 24hours and mixture was filtered using funnel and No 1 Whatman filter paper. The filterate was transferred into pre-weighed beaker and

concentrated by evaporating the solvent at 60^{O}C in water bath and the aqueous extract was stored until further use.[19]

Preparation of Methanollic Crude Extract: The extraction was carried out using soxhlet method at the department of pharmacognosy and ethnomedicine. Sixty two point six nine grams (62.69g) of powdered extract was weighed and dispensed in 400mls of Methanol in a conical flask and the crude preparation was left for 24hours in a shaker at room temperature and mixture was filtered using funnel and non adsorbent cotton wool which serve as stopper. The filterate was transfered into pre-weighed beaker and concentrated by evaporating the solvent at 60^{O}C in water bath and the methanollic extract was stored in sample bottle at 40^{O}C prior to use.[19]

Phytochemical Screening: The aqueous and methanollic extracts was subjected to phytochemical screening using standard tests to detect the presence or absence of the different types of phytochemical constituent. Analytical methods described by Trease and Evans[19], were employed in carrying out the tests.

1. Test for flavonoids

1.1 Sodium hydroxide test: 1ml of 10% sodium hydroxide solution was added into 2mls of both extract. A yellow color indicated the presence of flavonoids.

1.2 Ferric chloride test

The extract was boiled with water and filtered. To 2mls of the filtrate, 2 drops of ferric chloride solution was added. A blue green or violet color indicated the presence of a phenolic nucleus.

1.3 Shinoda test: Small quantity of extract dissolved in water. Concentrated hydrochloric was added and magnesium chips. Cherry red precipitate indicated the presence of flavonoid.

2. Test for alkaloids: About 0.5g of the extract was stirred with 5ml of 1% aqueous hydrochloric acid in a water bath and filtered. 3ml of the filtrate was divided into three test tubes.

2.1 Dragendroffs test: To the first test tube, few drops of freshly prepared Dragendroff's reagent was added and observed for formation of an orange to brownish precipitate which indicated the presences of alkaloids.

2.2 Mayer's test

To the second test tube few drops of Mayer's reagent was added and observed for the formation of white to yellowish or cream precipitate which indicated the presences of alkaloids.

2.3 Wagner's test

To the third test tube, one drop of Wagner's reagent was added and observed for brown-reddish or brown precipitate which indicated the presences of alkaloids.

3. Test for Saponins

3.1 Frothing Test

Small amount of each extract of was added into a test tube and about 10mlsof water was added to it. It was thoroughly shaken for 30 seconds.Honeycomb froth persisting in the test tube for 10-15 minutes indicated the presence of saponins.

4. Tests for Carbohydrates

4.1 Molisch's Test

A little amount of each extract was added into a test tube and a little amount of Molisch's reagent was added. Few drops of concentrated sulphuric acid were added. Appearance of a reddish colored ring indicated the presence of carbohydrates.

4.2 Fehling's Test

A little amount of extract was added into a test tube and a few drops of Fehling's reagents A and B were added. It was then heated. Presence of brick red precipitate indicated the presence of carbohydrates.

5. Test for Tannins

5.1 Lead Sub acetate Test

A little amount of extract was added into atest tube and dissolved with chloroform. Then a few drops of lead sub acetate were added. Presence of a heavy precipitate indicated that tannins are present in the plant extract.

5.2 Ferric Chloride Test

A little amount of extract was dissolved in a small amount of water and then few drops of ferric chloride were added. Presence of blue black color indicates that tannins are present.

6. Test for Cardiac Glycosides

6.1 Kella – Kelliani Test

A small amount of extract of was dissolved in glacial acetic acid. Few drops of ferric chloride were added, shaken andfew drops of concentrated sulphuric acid were added. Appearance of a purple-brown ring at the interface indicated the presence of cardiac glycosides.

7. Tests for Steroids

7.1 Liebermann-Burchard's Test

Small amountof extract was dissolved in chloroform and equal volume of acetic anhydride and then few drops of concentrated sulphuric acid were added. The extract is positive for steroids if the upper layer is blue-green in colour while the lower layer is red.

7.2 Salkowski's Test

A small amount of extract of was taken and dissolved in some chloroform. Few drops of concentrated Sulphuric acid was added. Appearance of a brown ring indicated the presence of steroids.

Preparation of 0.5% Mac Farland Turbidity Standard

MacFarland turbidity standard solution was prepared bymeasuring(0.05mls) of 1% Anhydrous Barium chloride and (9.95mls) of 1% Sulphuric acid to give 0.5 MacFarland Turbidity standard solution.[20]

Preparation of Bacterial Inoculum

The test bacterial isolates were inoculated into Nutrient broth and incubated overnight at 37^{O}C,the turbidity of the resulting suspension was further diluted with Nutrient broth until it's comparable with MacFarland turbidity standard.[21]

Preparation of Standard Antibiotics: Ciprofloxacin antibiotic was used as standard antibioticand was prepared by dissolving 5mg in 5mls of sterile distilled water giving a concentration of 1mg/mls.[21]

Screening for Antibacterial Activity of the Crude Extracts

The antibacterial activity of the carrot peel extract against test bacteria was analyzed by well diffusion method. The carefully adjusted inoculum suspension was allowed to stand for 15 minutes and a sterile cotton swab was dipped into the adjusted suspension, rotated several times and press firmly on the inside wall of the tube above the fluid to remove the excess fluid from the swab (CLSI, 2012). There after the swab was streaked over the entire sterile surface of the dried Mueller Hinton agar plate. This procedure was repeated twice by rotating the plate at approximately 60° each time to ensure an even distribution of the inoculum (CLSI, 2012). Six wells of six millimeter (6mm) diameter were punched using cork borer and filled with the different concentrations (25mg/mls,50mg/mls,100mg/mls, 200mg/mls, 1mg/mls of stanadard and methanol as control) of each plant extract for each plate with aqueous and methanollic extracts respectively. One milligram per milliliter (1mg/mls) of Ciprofloxacin was prepared and used as standard to determine sensitivity of the isolates. Finally the plates were incubated for 20 hours at 37ºC. The zones of inhibition were measured and noted.[22]

Determination of Minimum Inhibitory Concentrations (MIC): The minimum inhibitory concentration was determined according to the National Committee for Clinical Standard (1999).Thirteen (13) test tubes were set up and 1mls of nutrient broth was aseptically pipetted into test tube 1-11. Two milliliter (2mls) of nutrient broth was added into test tube 12 and one milliliter (1mls) of extract was added into test tubes 1 and 13. Doubling dilution was done from tube 1 up to 10 and 1mls was discarded from tube 10. Then one (1mls) of the test organisms inoculums was pipetted into test tube 1-11, test tube 11,12 and 13 served as growth, broth and antibiotic controls respectively and they were all incubated at 37^{O}c for 24 hrs overnight. At the end of the incubation, the lowest concentration of the extracts showing no growth was taken as the MIC. Same procedure was adopted for the determination of the MIC of the ciprofloxacin used as the standard antibiotic for comparison with the extracts.

Determination of Minimum Bactericidal Concentrations (MBC)

The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing on solid media (Muller Hinton agar) 0.01ml (10µL) of the highest concentrations of the dilutions which showed visible growth and all the tubes showing no visible sign of growth in the MIC tube dilution test.[18] MBC was the lowest concentration that results in killing 99.9% of the test organisms.[20]

RESULTS

The phytochemical screening of the *Daucus carota* peel extract revealed the presence of Alkaloids, Carbohydrates, Flavonoids, Phenols, Proteins, Saponins and Triterphenoid as presented in Table 1.

Percentage (%) yield of both aqueous and methanollic extract was shown in Table 2. Aqueous extract yielded 22.51% and Methanollic extract yielded 11.28%.

The antimicrobial screening of Aqueous extract (200mg/ml) against *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* indicated 15.5mm, 14.0mm and 0.0mm respectively, at 100mg/ml the activities showed 11.0mm, 10.5mm and 0.0mm respectively. At 50mg/ml the activities showed 8.0mm, 7mm and 0.0mm Respectively. And At 25mg/ml the activities were 5.5mm, 5.0mm and 0.0mm respectively, as shown in Table 3.

The antimicrobial screening of methanol extract at (200mg/ml) against *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* showed 12.0mm, 11.5mm and 0.0mm respectively. At 100mg/ml the activities were 9.5mm, 9.5mm and 0.0mm respectively. At 50mg/ml the activities showed 7.0mm, 6.5mm and 0.0mm respectively. And at 25mg/ml the activities were 4.5mm, 4.0mm and 0.0mm respectively as shown in Table 4.

Antibacterial activity of standard against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* ranged from 28.0mm - 30.0mm.

Minimum inhibitory concentration (MIC) of both Aqueous and Methanollic extract against *Staphylococcus aureus* were 150mg/ml and 200mg/ml, that of *Escherichia coli* were 100mg/ml and 150mg/ml and that of *Pseudomonas aeruginosa* were 250mg/ml and 300mg/ml respectively. The MIC of standard antibiotic against *Staphylococcus aureus* was 0.5mg/ml, *Escherichia coli* was 0.015mg/ml and *Pseudomonas aeruginosa* was 0.5mg/ml (table 5). Minimum Bactericidal Concentration (MBC) was not recorded for both aqueous and methanollic extracts.

Table. 1. Qualitative phytochemical screening of *Daucus carota* Aqueous and Methanol extracts.

PHYTOCHEMICAL CONSTITUENT	AQUEOUS	METHANOL
Alkaloids	+	+
Carbohydrates	+	+
Flavonoids	+	+
Tannins	-	-
Phenols	+	+
Proteins	+	+
Saponins	+	+
Cardiac glycosides	-	-
Triterphenoids	+	+

Table 2. Percentage (%) yield of both aqueous and methanollic extract.

Extract	Yield (%)
Aqueous	22.51
Methanol	11.28

% = Percentage

Table 3. Diameter of zones of inhibition of standard andaqueous extract.

Bacterial isolates	Concentration (mg/ml) / Zone of inhibition (mm)				
	Std (mg/ml)	Aqueous Extract (mg/ml)			
	1	200	100	50	25
S. aureus	29.5	15.5	11.0	8.0	5.5
E. coli	29.5	14.0	10.5	7.0	5.0
P. aeruginosa	28.0	0.00	0.00	0.00	0.00

Mg/ml = milligrams per milliliter, mm = millimeter

Table 4. Diameter of zones of inhibition of standard and methanol extract.

Bacterial isolates	Concentration (mg/ml) / Zone of inhibition (mm)				
	Standard (mg/ml)	Methanolic Extract (mg/ml)			
	1	200	100	50	25
S. aureus	29.0	12.0	9.5	7.0	4.5
E. coli	30.0	11.5	9.5	6.5	4.0
P. aeruginosa	29.0	0.00	0.00	0.00	0.00

Mg/ml = milligrams per milliliter, mm = millimeter

Table 5. Minimum Inhibitory Concentrations (MIC) of Aqueous and Methanollic Extract.

Bacterial isolates	Concentration (mg/ml) / Zone of inhibition (mm)		
	Standard	Aqueous	Methanolic
S. aureus	0.5	150	200
E. coli	0.015	100	150
P. aeruginosa	0.25	250	300

Mg/ml = milligram per milliliter

DISCUSSION

The antimicrobial activity of plant extract is due to different secondary metabolites in the extract known as phytochemicals. Phytoconstituents of plant determine its medicinal values. The functions of phytochemicals in plants broaden our knowledge of their usefulness to humans.[23]

Phytochemical screening of Daucus carota showed the presence of; Carbohydrates, Alkaloids, Flavonoids, Phenols, Proteins, Saponins and Triterphenoids and among are bioactive compounds known posses antioxidant and antimicrobial activities as well as valuable sources of dietetic fibre.[23]

From this study, both aqueous and methanol extracts of Daucus carota had antibacterial activity against *staphylococcus aureus* and *Escherichia coli* at 200mg/ml, the antibacterial activity of both aqueous and methanol extract increased linearly with increased in the concentration of the extract in mg/ml. This is due to the fact that lower concentrations gave no or least antibacterial activity while high concentrations gave higher activity. Findings of this study are in line with that of Anibijuwan et al.[24], which also reported that lower concentrations give lesser activity than higher concentrations.

Growth inhibition zones measured for aqueous extract ranged from 5.5 - 15mm and that of methanol extract

ranged from 4 – 13.5mm and standard antibiotic ranged from 28 – 30mm. Besides higher zones of inhibition noted for both extract, Standard antibiotic showed higher zones of inhibition than the extracts, but this could not be assertively concluded that the antibiotic was more effective to the organism than the plant extract. This is because the isolates used might be strictly multidrug resistant.

The antibacterial potential was dose dependent against *S.aureus*, *E.coli* and Pseudomonas aeruginosa. Zones of inhibition for aqueous extract against; *S.aureus* at 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml were 15.5mm, 11mm, 8mm and 5.5mm respectively, For *E.coli* at 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml were 14mm, 10.5mm, 7mm and 5mm respectively, Pseudomonas aeruginosa did not show any inhibition zone at 200mg/ml.

Zones of inhibition for methanol extract against; *S. aureus* at 200mg/ml, 100mg/ml, 0mg/ml and 25mg/ml were 12.0mm, 9.5mm, 7.0mm and 4.5mm respectively, for *E. coli* at 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml were 11.5mm, 9.5mm, 6.5mm and 4.0mm respectively and P. aeruginosa did not show any zone of inhibition.

There were little differences in antibacterial activity of both extracts as presented in Table 3 and 4. And this might be attributed to the polarity of the solvents used. A research by Anibijuwon et al.[24], revealed that aqueous extract illuminated maximum zone of inhibition in their study conducted with Aspergillus and *Staphylococcus aureus*. This is consistent with findings of this study where aqueous extract showed maximum zone of inhibition against bacteria than the methanol. However, findings of this study contradict that of Fatmir et al.[25], which indicated that both aqueous and methanollic extracts showed the same zones of inhibition against *S. aureus* and *E. coli*. This may be due to the lower concentrations of extract used in their study (3mg/ml).

Minimum inhibitory concentration (MIC) values against *S. aureus*, *E. coli*, P. aeruginosa for aqueous extract were 150mg/ml,100mg/ml and 250mg/ml respectively, and for methanol were 200mg/ml, 150mg/ml and 300mg/ml respectively, as presented in Table 5.

The result also illuminated sensitivity of Gram positive organisms to carrot peel extract than Gram negative organisms, this is in line with findings of Tian et al.[26], that investigated the antibacterial activity of both aqueous and methanol extracts of Galla chinensis plant and reported that plants extract are more sensitive to Gram positive organisms than Gram negative.

A research carried out by Al-Baarri et al.[27], showed that addition of hypothiocyanite enhances the activity of carrot peel extract against *S. aureus* and *E. coli*.

Anibijuwon et al.[24], indicated that aqueous extract of Daucus carota seeds showed no antimicrobial activity on any isolates in their study conducted on *S. aureus*, S. typhi, Candida. albicans and K. pneumonia.

CONCLUSION

In addition to nutritional value of Daucus carota, it has also been proven to possess biologically active components like phenolics, alkaloids, flavonoids that are responsible for its antibacterial activity and can be used into producing prophylactic and therapeutic antimicrobial agents. All the concentrations used showed reasonable bacteristatic action against *S. aureus* and *E. coli*.

RECOMMENDATIONS

Further studies on Daucus carota peel extract are warranted to validate its antimicrobial benefits, creating awareness among consumers about side effects and toxicity of the synthetic compounds in Daucus carota are recommended.

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