PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ANALYSIS OF CARROT LEAVES EXTRACT

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Abstract. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported to be well tolerated when compared with synthetic drugs. In view of this, the phytochemical screening and antibacterial activity of Daucus carota leaves was investigated. The extraction of the leaves shows the carrot leaves has percentage moisture content of 67.00% with mass and percentage yield of 3.16 g, 6.32%. 0.90 g, 2.895, and 0.40 g, 1.35% for the hexane, ethyl acetate and ethanol fractions respectively, while the phytochemical screening of different extracts revealed the presence of alkaloids, flavonoids, saponins and steroids but no tannins, cardiac glycoside and anthraquinones. The antibacterial activity showed that the drug used as positive control (amoxicillin) and various Daucus carota leaves extract possesses antibacterial activity against E. coli, S. aureus and Shigella at prepared concentrations of 0.25 mg/mL, 0.50 mg/mL, 1.00 mg/mL and 2.00 mg/mL. The lowest minimum inhibitory concentrations observed were 0.25 mg/mL for almost all the extract. The minimum bactericidal concentration against S. aureus for all the extract was 0.25 mg/mL 0.50 mg/mL, 1.00 mg/mL and 2.00 mg/mL The results of both the phytochemical screening and antibacterial activity shows that Daucus carota leaves has good potentials for development of new antibacterial drugs.
Keywords: *phytochemical screening, antibacterial analysis, antibiotics, carrot leaves, drug resistance*

Introduction

Herbaceous plants have long been used in traditional medicine in various cultures throughout the world (Velavan, 2015). Despite the continuous improvements in modern medicine in recent decades, plants still make an important contribution to health care (Velavan, 2015). In recent years, attention has been shifted towards the use of natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables in phytotherapy (Agomuo et al., 2016). Medicinal plants contain some organic compounds that produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids and flavonoids. Fruits and vegetables are rich sources of nutrients that contain phytochemicals (also known as bioactive compounds), which are recognised for their pharmaceutical effects and health benefits (Agomuo et al., 2016). Even though the knowledge of how these substances provide medicinal value to humans reflects a relatively scientific understanding, but still some part of fruits and vegetables such as carrot leaves are considered as waste in most part of the world (Agomuo et al., 2016). Carrots are one of the most widely used and enjoyed vegetables in the world, partly because they are relatively easy to grow, and are very resourceful in a number of dishes and cultural delicacies (FAO, 2001). Carrots are scientifically classified as Daucus

carota and it is categorised as a root vegetable (Yelne et al., 2000). They contain many medicinal and health benefits, and not to mention the taste which makes it an important vegetable in cultural cuisines across the globe (Yelne et al., 2000). Most of the health benefits of carrots that include prevention of heart diseases, property of reducing cholesterol, blood pressure, immune booster, digestion, prevents cancer, macular degeneration, improves eyesight and many other can be attributed to their beta-carotene and fibre content (Bahl and Bahl, 2012; Ahmadua et al., 2007; Cushnie and Lamb, 2005; Yelne et al., 2000; El-Olemy et al., 1994). However, majority of the studies on carrots have focused on cultivation, breeding, nutrient content and other pharmacological potentials of the root tubers and essential oil (Galeotti et al., 2008; Salunkhe and Chavan, 1989; Herborne, 1973), but the leaves are consider being a waste and hence their potentials as a source of medicine has not yet been scientifically explored. In view of the above, this study aimed at investigating the phytochemicals present in the leaves of carrot and, evaluates the antibacterial potentials of the leaves extract using Echerichia coli, Staphyloccus aureus and Shigella for the possibility of new drug development as represented in *Figure 1*.

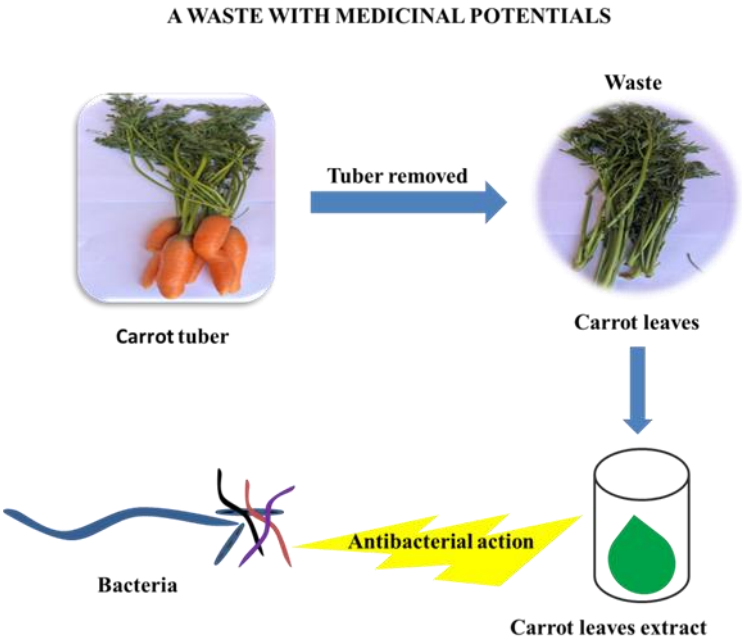

Figure 1. *Antibacterial action of carrot leaves extract.*

Materials and Methods

All solvents and chemicals used were of analytical grade and were obtained from sigma aldrich and BDH Chemicals England respectively. Nutrient Agar and Nutrient Broth were obtained from Titan Biotech L.T.D India.

Sample collection

The sample of carrot leaves was purchased in February, 2021 from Kasuwar daji market in Sokoto State, Nigeria.

Sample treatment

The leaves was first washed thoroughly with distilled water to remove dirty and other impurities and was allowed to dry at room temperature for 5 days the dried leaves was ground to fine powder using mortar and pestle. The powdered samples were stored in an air tight water free polyethene bag and used for the analysis.

Extraction of leaves

The leaves powder (50 g) was extracted by maceration in a 1000 cm^3 glass stoppered conical flask. N-hexane (200 cm^3) was first used to defat (extract) the leaves. The marc was allowed to dry and then extracted with ethyl acetate, and then to ethanol. The extraction was done according to the order of increasing polarity of the solvents. In each case the leaves was left in contact with the solvent for 48 hours with intermittent stirring to ensure maximum extraction. After 48 hours, the extracts were filtered using Whatman filter No.1 and the filtrates were transferred to evaporation dishes and concentrated using air circulated oven at temperature between 80-100 ^oC.

Qualitative phytochemical screening of extract

The tests for flavonoids, tannins, saponnins, steroids/triterpenoids, alkaloids, cardiac glycosides and anthraquinones were carried according to the methods described by Khameneh et al. (2019), Halilu et al. (2013), Rhen and Cidlowski (2005), as well as Garrod et al. (1973).

Antibacterial studies

Test organisms

The test organisms were obtained from the Department of microbiology, Faculty of Sciences, Sokoto State University, Sokoto, Nigeria. The microorganisms are standard laboratory strains of Staphylococcus aureus (gram +ve), Shigella (gram -ve), Escherichia coli (gram -ve).

Susceptibility test

The antibacterial test was conducted using the petri dish plate method described by Sule et al. (2011). The Nutrient Agar plates prepared according to manufacturer's instruction were allowed to solidify for 15 minutes at room temperature and incubated without inoculum for 24 hours at 37 °C to ensure the sterility of the medium. The Nutrient Agar plates were flooded with 1 ml of the inoculum and the excess was removed using Pasteur pipette. Five wells (cups) of about 6 mm in diameter were cut on each Nutrient Agar plate using a sterile cork borer and the agar plugs were removed using sterile ampoule file. The extract solution (0.1 mL) was placed in each of the wells and were allowed to settle for two hours at room temperature and then incubated for 24 hours at 37 °C. The inhibition zone was observed and then recorded in millimeters using a transparent ruler. Standard antibiotic was used.

Minimum inhibitory concentration (MIC)

This was carried out as described by Sule et al. (2011). Minimum inhibitory concentration (MIC) was defined as the lowest concentration where no visible turbidity would be observed in the test tubes. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test extracts. In this test, the micro-organisms

were prepared using the broth dilution technique. The stock solution (20 mg/mL) of the leaves extract was prepared by dissolving 0.2 g of the leaves extract in 10 mL Dmso. Four concentration were prepared (0.25mg/mL, 0.5 mg/mL, 1.0 mg/mL and 2.0 mg/mL), from the stock solution using serial dilution technique and later inoculated with 0.2 mL suspension of the test organisms. After 24 hours incubation at 37 °C, the tubes were observed for turbidity. The lowest concentration where no turbidity was observed was determine and noted as the Minimum Inhibitory Concentration (MIC).

Minimum bactericidal concentration (MBC)

The minimal bactericidal concentration was determined from broth dilution test resulting from the MIC tubes as described previously by inoculating the content of each test tube on a nutrient agar plate. The plates were then incubated at 37 °C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration.

Results and Discussion

Extraction and qualitative phytochemical screening

Table 1 and *Table 2* give the details of the percentage moisture content, percentage yield and mass of the fractions obtained from carrot leaves respectively. The leaves had a percentage moisture content of 67.00 % and the mass of 3.16 g, 0.90 g, 0.40 g, with percentage yield of 6.32%, 2.89% and 1.35%, obtained for n-hexane ethyl acetate and ethanol respectively. The results indicate that n-hexane fraction contains more of the extract. *Table 3* shows the results of the qualitative phytochemical screening of the fractions obtained. Alkaloids, saponins, flavonoids and steroids were detected in all the three fraction of carrot leaves while antharaquinones, tannins and cardiac glycoside were not detected in all the fractions.

***Table 1**. Percentage moisture content of carrot leaves (%).*

S/N	Weight of empty dish (W0)	Weight of empty dish + sample (W1)	Weight of empty dish + dry sample	Moisture content (%)
1	2.83	3.83	3.16	67.00

***Table 2**. Mass of fractions (g) and percentage yield of carrot leaves extract (%).*

Extract	Original mass (g)	Mass (g) of extract	Percentage yield (%)
N-hexane	50.00	3.16	6.32
Ethyl acetate	31.10	0.90	2.89
Ethanol	29.62	0.40	1.35

***Table 3**. Qualitative phytochemical screening of carrot leaves extracts.*

Phytochemicals	N-hexane	Ethyl acetate	Ethanol
Alkaloids			
(a) Mayer's test	+	+	+
(b) Wagner's test	+	+	+
(c) Dragendorff's test	+	+	+
Flavonoids			
(a) Sodium hydroxide	+	+	+
(b) Ethyl accetate test	+	+	+
(c) Shinoda's test	+	+	+
Steroids			
(a) Salkowski's test	+	+	+
(b) Lieberman-Buckard's test	+	+	+
Saponins			

(a) Foam test	+	+	+
Tannins			
(a) Iron (III) chloride test	-	-	-
(b) Lead acetate	-	-	-
Antharaquinones			
(a) Borntrager's test	-	-	-
Cardiac glycoside			
(a) Keller-Killiani's test	-	-	-

Note: (+) detected; (-) not detected.

Antibacterial activity of carrot leaves extract

Table 4(a) to *Table 4(g)* show the details of the results obtained from the antibacterial activity test of the n-hexane, ethyl acetate and ethanol fractions of carrot leaves extract. The results deduced that all the three fractions, has activity on bacterial strain with zone of inhibition ranging between 2-10 mm.

***Table 4(a)**. Antibacterial activity of n-Hexane fraction of carrot leaves.*

Concentration (mg/ml)	Zone of inhibition		
	S-aureus	E-coli	Shigella
0.25	8.00	0.00	0.00
0.50	6.00	2.00	0.00
1.00	4.00	2.00	2.00
2.00	4.00	3.00	4.00

***Table 4(b)**. Antibacterial activity of ethyl acetate fraction of carrot leaves.*

Concentration (mg/ml)	Zone of inhibition		
	S-aureus	E-coli	Shigella
0.25	2.00	2.00	0.00
0.50	5.00	5.00	2.00
1.00	8.00	4.00	2.00
2.00	10.00	3.00	6.00

***Table 4(c)**. Antibacterial activity of ethanol fraction of carrot leaves.*

Concentration (mg/ml)	Zone of inhibition		
	S-aureus	E-coli	Shigella
0.25	7.00	0.00	0.00
0.50	5.00	2.00	0.00
1.00	6.00	2.00	6.00
2.00	8.00	3.00	5.00

***Table 4(d)**. Antibacterial activity of standard antibiotic (Amoxicillin).*

Antibiotic concentration	Zone of inhibition (mm)		
	S-aureus	E-coli	Shigella
Amoxicillin (125 mg/ml)	12.00	10.00	10.00

***Table 4(e)**. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of n-hexane fraction of carrot leaves.*

Test organism	MIC (mg/ml)	MBC (mg/ml)
S.aureus	0.25	2.00
E.coli	1.00	0.25
Shigella	0.25	0.20

Table 4(f). *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethyl acetate fraction of carrot leaves.*

Test organism	MIC (mg/ml)	MBC (mg/ml)
S.aureus	2.00	0.25
E.coli	0.50	1.00
Shigella	0.25	0.50

Table 4(g). *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol fraction of carrot leaves.*

Test organism	MIC (mg/ml)	MBC (mg/ml)
S.aureus	0.50	0.25
E.coli	1.00	0.25
Shigella	2.00	0.25

The dried leaves of Daucus carota were successively extracted by cold maceration based on solvent polarity using n-hexane, ethyl acetate and ethanol respectively. The percentage moisture content obtained are 67.00% presented in (*Table 1*) with mass of 3.16 g, 090 g, 0.40 g and the percentages yield of 6.32%, 2.895, and 1.35% respectively (*Table 2*). The investigated phytoactive constituents includes alkaloids, flavonoids, saponins, and steroid. And also the results obtained from the qualitative analysis of extracts showed that all the fractions contained flavonoid, alkaloids, saponin and steroids as presented in (*Table 3*). The result also revealed that tanins, cardiac glycoside and anthraquinone were not detected in all the three fractions. The result obtained in this studies for alkaloids and flavonoids agrees with the reports of Sule et al. (2011), as well as Usman and Osuji (2007) in their respective studies. Although, it was reported that the aqueous and methanol extract contains tannins which recorded negative reaction in the present study. This however may be due to the solvent used for the extraction or the plant part extracted. Daucus carota plant has been found to contain natural antibacterial agents that are effective against common bacteria. Three different bacteria isolates that were used in this research work; Echerichia coli, Staphyloccus aureus and Shigella. The susceptibility test of all the extract of the plant tested showed varying degree of antibacterial activities against the test bacteria species, as shown in *Tables 4(a)* to *Table 4(c).* The results of the sensitivity test showed that the ethyl acetate extract had wider zones of inhibition than the other two extracts. However, E-coli shigella and S. aureus showed zones of inhibition in n-haxane, ethyl acetate and ethanol respectively. Generally, all the extract displayed better sensitivity against S. aureus with zone of inhibition ranging from 2- 10 mm while the zones of inhibition for E. coli and Shigella species ranges from 2-8 mm respectively. Although, ethyl acetate extract showed more antibacterial activity to the test organisms. It should also be also noted that technique employed for the antibacterial studies of the extracts is agar well diffusion method, using 6 mm cork borer. This means that any recorded inhibitory diameter values that equal 6 mm showed no inhibition at all. The results when compared with Amoxicillin (Standard Antibiotic-*Table 4(d),* the zone of inhibition produced by the antibiotic against the test organisms was found to be appreciable in relation to those activities produced by the organism under study. However, according to Usman and Osuji (2007), diameter of zones of inhibition ≥ 10 mm are considered active. The minimum inhibitory concentration results presented in (*Table 4(e)* to *Table 4(g)* showed that the lowest concentration at which the various extract is 0.25 mg/mL, the finding of this research work showed that the all the extracts showed better minimum inhibitory concentration

and minimum bactericidal concentration activities. In view of the above results, all the extracts showed considerable activity against S. aureus, a gram positive bacterium known to play a significant role in invasive skin diseases and also on E-coli (gram-ve) and Shigella (gram-ve) (Usman and Osuji, 2007).

Conclusion

Based on the proportion of the yield of the plant extract for the selected solvents, ethyl acetate extract produced more of the bioactive compounds than those of ethanol and ethyl acetate combined. The qualitative phytochemical screening of the n-hexane, ethyl acetate and ethanol extracts of the Daucas carota leaves used in this research showed the presence of bioactive constituents such as alkaloids, flavonoids, saponins and steroids. Some of the phytochemical constituents present in this plant have been reported for their medicinal purposes. The various extracts of the leaves of Daucas carota used in this study also displayed antimicrobial activity against E. coli, S. aureus and Shigella at varying concentrations as seen from the measured zones of inhibition. The therapeutic effects of these plants can justifiably be attributed to, among others, the possession of bioactive compounds such as flavonoids, alkaloids, steroids present therein. The results of this research work therefore have established that Daucus carota leaves have bioactive compounds and possess antibacterial properties.

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Conflict of interest

The authors declare that there is no conflict of interest involve in this research study.

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