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Phytochemicals Investigation, Chromatographic Evaluation and Antioxidant Activity Assay on the Root Extract of Carrot (*Daucus carota*)

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Abstract: **Daucus carota roots were chosen for this study in order to examine both their phytochemicals and antioxidant activity (free radical scavenging activity). Ethanol was used to extract the plant's roots' powder sample. n-hexane, chloroform, ethyl acetate, and methanol were used to macerate the crude ethanol extract, which was then screened for alkaloids, flavonoids, saponins, tannins, and water-soluble phenols. Thin Layer Chromatography (TLC) analysis was performed on the crude extract as well as on the other fractions. For the optimum separation of the phytochemicals present in the fractions, solvent systems were designed. Chloroform:Ethyl Acetate was chosen as the solvent system for TLC of the n-hexane, methanol, and ethanol fractions (1:1). 100% of chloroform was used for the chloroform fraction. The phytochemical analysis established the presence of water-soluble phenols, saponins, and flavonoids. The results of the TLC study have shown different spots. According to the results of the antioxidant activity test, the ethanol fraction has a higher level of antioxidant activity than the other fractions.**

Keywords: **Daucus carota, Phytochemicals, Antioxidant activity, Thin layer chromatography (TLC).**

1. INTRODUCTION

The most significant crop in the Apiaceae family is the carrot (Daucus carota). It is a root vegetable that is available worldwide (Dias, 2014). Carrots come in a variety of colors, but their predominant ones are purple, orange, red, and yellow, which are caused by anthocyanins, β-carotenes, lycopene, and lutein (Raman et al, 2019). Many carrot tissues share a similar chemical make-up, although each one's phenolic content varies and falls from the peel to the interior (xylem) (Sheila et al., 2017). China, Russia, and the United States are the top 3 carrot producers worldwide, accounting for about 50% of the global carrot output (Arscott and Tanumihardjo, 2010). The Ancient Egyptians employed Daucus carota as a stimulant, carminative, diuretic, anthelmintic, and in a decoction for infantile diarrhea (Al-Snafi, 2019). Apart from being used traditionally in salads and the production of curries in India, carrot roots could be processed commercially into products like juice, dry powder, canned goods, preserved goods, and candies that are nutrient-dense (Singha and Srivastava, 2021). In addition to being a significant source of provitamin A, which accounts for 17% of all vitamin A consumed, carrots are also a good source of carotenes. Carotenes have been shown to have antioxidant properties (Zhang and Hamauzu, 2004). The maximum antioxidant content was found in purple-yellow carrots, followed by purple-orange carrots; the other carrots did not significantly vary (Ting et al., 2009). Carotenoids, polyacetylenes, phenolic compounds, and ascorbic acid are the key four types of phytochemicals that make carrots nutritious (Ahmad et al, 2019). Polyacetylenes are found in carrot roots.

Previously thought to be toxicants because they are strong skin sensitizers and irritants and are neurotoxic at high concentrations, they are now thought to be beneficial substances (Muharrem and Zahide, 2018). Additionally, carrots are a fantastic source of calcium pectate, a remarkable pectin fiber with cholesterol-lowering qualities (Ayo and Gidado, 2017).

2. MATERIALS AND METHODS

2.1 Plant material

Fresh roots of *Daucus carota* were bought in Kurna Area, Fagge Local Government, Kano State. The plant was identified and authenticated by Dr. Yusif Nuhu of the botany department at Bayero University, Kano, with herbarium accession number (BUKHAN 630). The roots were washed, dried under shade, and made into a fine powder (Adoum, 2016).

2.2 Extraction and fractionation

The dried powder of *Daucus carota* (200 g) was percolated with ethanol (1000 ml) at room temperature for two weeks. The percolate was decanted, filtrated, and concentrated using a rotary evaporator machine at 40°C to obtain the ethanol crude extract, which is labeled as ethanol extract. The ethanol extract obtained was then weighed and kept for use. The ethanol extract was macerated with solvents of different polarities: n-Hexane, chloroform, ethyl acetate, and methanol. The fractions were labeled as: F1, F2, F3, and F4, respectively. The percentage yield was calculated (Adoum *et al*, 1997).

2.3 Phytochemical screening

The crude extract and other fractions of *Daucus carota* were screened for phytochemical constituents such as alkaloids, flavonoids, saponins, tannins, and water-soluble phenols using the technique described by De *et al*, 2010.

2.4 Thin-layer chromatography (TLC)

Thin-layer chromatography was carried out on the crude extract and other fractions of *Daucus carota* root on an aluminum plate (5×4) coated with silica gel. The solvent system selected for TLC of n-hexane, methanol, and ethanol fractions was chloroform:ethyl acetate (1:1). Chloroform (100%) was used for the chloroform fraction. The solvent mixture was poured into a 100 ml beaker which was used as the developing chamber, the samples were spotted 0.5 cm at the baseline of the TLC plate using a capillary tube, and the plate was placed inside the developing chamber containing the solvent system, and covered, the solvent was allowed to move with the spots to the front line of the TLC plate (De *et al*, 2010).

2.5 Antioxidant Activity Assay using 2,2- Diphenyl -1- picrylhydrazyl (DPPH)

According to the procedure outlined by Sharma and Bhart (2009), the antioxidant activity of the Daucus carota root extract against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed. The final concentrations of each stock solution sample (10 mg/ml) were 1000, 500, 250, 125, 62.5, and 31.25 µg/ml. The sample solution (100µL) was then mixed with a total of (200µL) DPPH solution before being incubated at room temperature for 30 minutes in the dark. At 517 nm, the mixes' absorbance was measured. A positive control was ascorbic acid. Higher free radical scavenging activity is indicated by a reaction mixture with a lower absorbance, and vice versa. Inhibition of DPPH radical in percent (%I) was calculated.

3. RESULTS AND DISCUSSION

Table I showed the weight, % yield, and appearance of the fractions obtained. The preliminary phytochemical analysis of the Daucus carota root extracts in (Table II), showed the various phytochemicals present in the different extracts. The n-Hexane fraction contains flavonoids and saponins. The chloroform fraction also contains flavonoids and saponins. The methanol fraction contains flavonoids, saponins, and water-soluble phenols. The crude extract (ethanol extract) contains flavonoids, saponins, and water-soluble phenols. Table III, showed the solvent system selected for the TLC of n-Hexane, Chloroform, Methanol, and Ethanol fractions. The solvent system for the n-Hexane fraction was chloroform: ethyl acetate(1:1), which resulted in five (5) different spots with Rf value of 0.225, 0.35, 0.45, 0.5, and 0.6. The solvent system for chloroform fraction was 100% chloroform which resulted in five (5) different spots with Rf values of 0.24, 0.375, 0.575, 0.65, and 0.9. The solvent system for methanol was chloroform:ethyl acetate(1:1) which resulted in three (3) different spots with Rf values of 0.325, 0.675, and 0.925. The solvent system for Ethanol extract was chloroform:ethyl acetate(1:1) which resulted in six (6) different spots with Rf values of 0.225, 0.275, 0.45, 0.75, 0.8, and 0.95. Different spots with different Rf values were identified from the TLC result. The antioxidant assay result shown various percentage inhibition and the

free radicals scavenging activity was found to be concentration dependent. The greatest antioxidant activity was demonstrated by the 1000 µg/ml and among them largest percentage was found on the crude extract (93.1469%), then methanol fraction (83.76792%), n-Hexane (79.7877%) and chloroform fraction (61.4873%) at their greatest concentration (1000 µg/ml) which is lower than the free radicals scavenging activity of the control i.e Ascorbic acid at the dosage.

Table I: Weight, % yield, and appearance of Daucus carota Fractions

Fractions	Weight (g)	% yield	Appearance
F1	1.744	14.444	Sticky brown
F2	0.146	1.209	Yellow
F3	0	0	-
F4	8.886	73.596	Sticky brown
F5	24.178	12.089	Sticky dark orange

Keys: Key: F1: n-Hexane Fraction, F2: Chloroform Fraction, F3: Ethyl acetate, F4: Methanol Fraction, F5: Crude extract

Table II: Phytochemicals screening of the samples

Extract	Alkaloids	Flavonoids	Saponins	Tannins	Water-soluble phenols
n- Hexane	_	+	+	_	_
Chloroform	_	+	+	_	_
Methanol	_	+	+	_	+
Ethanol	_	+	+	_	+

Key: +: Presence -: Absence

Table III: TLC profile of *Daucus carota* fractions

Key: R_f = Retention factor.

La Plate No.	Sample	No. of spots	Solvent system(s)	R_f values
1	n-Hexane Fraction	5	chloroform:ethyl acetate(1:1)	0.225, 0.35, 0.45, 0.5, 0.6.
	Chloroform Fraction	5	Chloroform (100%)	0.24, 0.375, 0.575, 0.65, 0.9.
4	Methanol Fraction	3	chloroform:ethyl acetate(1:1)	0.325, 0.675, 0.925.
	Ethanol Fraction	6	chloroform:ethyl acetate (1:1)	0.225, 0.275, 0.45, 0.75, 0.8, 0.95

Table IV: Result for Antioxidant Activity Assay using DPPH.

Concentration (µg/ml)	(I%) CRUDE	(I%) F1	(I%) F2	(I%) F4	Ascorbic Acid
1000	93.1469	79.7877	61.4873	83.76792	95.85259
500	74.2767	73.3276	33.7824	83.63525	94.88449
250	58.8611	58.8764	27.2338	79.36419	94.76347
125	51.8906	56.7281	26.7881	72.24065	94.36744
62.5	48.339	53.5643	21.124	67.56646	93.91639
31.25	46.0989	48.0839	19.4877	63.59137	93.85039

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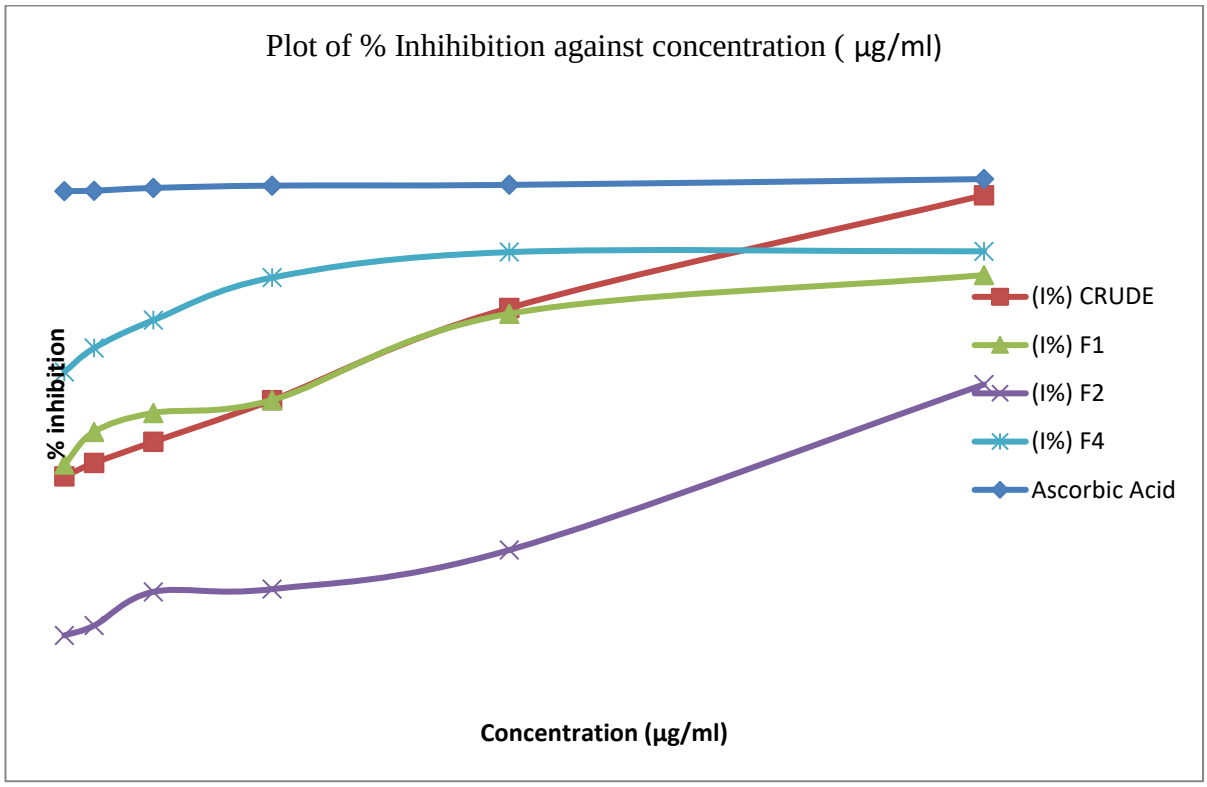

Figure 1: Showing the free radicals scavenging activity of the different fractions compared with the Ascorbic acid.

4. CONCLUSION

In conclusion, the result demonstrated that ethanolic extract and the other fractions of Daucus Carota root confirmed the presence of phytochemicals such as Flavonoids, Saponins, and Water-soluble phenols. The TLC analysis of n-Hexane fraction, chloroform, methanol, and ethanol extract has shown different spots. From the result of the Antioxidant potential Assay in Table IV, the ethanol extract has the highest % Inhibition followed by the methanol fraction, n-Hexane fraction, and then the chloroform fraction.

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