

# Nutritive Evaluation, Mineral Composition and Phytochemical Analysis of Leaf Protein Concentrates of *Daucus carota*

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## ABSTRACT

Fresh green leaves of *Daucus Carota* was harvested and processed with a view to analyse its photochemical constituents and nutritional potentials. Proximate analysis, mineral analysis and phytochemical analysis was determining using standard analytical techniques. The nutrient composition of the leaf protein concentrate revealed: Moisture content; (8.69±0.03)g/100g, crude protein; (18.38±0.08)g/100g, crude fibre; (14.81±0.02)g/100g, crude fat; (5.69±0.23)g/100g, Ash; (19.69±0.02g/100g), and NFE; (32.74±0.43)g/100g. The mineral analysis of the sample shows the concentrations of the elements in the following descending order of magnitude: Ca > P > Mg > Na > Fe > K > Zn > Cu > Mn > Se while Hg was not detected in the sample. The photochemical constituents also revealed: Tanin: (3.66±0.16)mg/100g, Saponin: (4.34±0.06)mg/100g, Alkaloid: (10.49±0.02)mg/100g, Oxalate: (6.89±0.04)mg/100g, Phytate: (14.62±0.05)mg/100g, Phenolics: (3.75±0.82)mg/100g, Flavonoids: (3.62±0.43)mg/100g. High Concentration of Calcium and Phosphorus indicates that *Daucus carota* leaf protein concentrates is fit for consumption by children, pregnant women and weaned infants for good teeth and bone formation. The constituents of *Daucus carota* leaf protein concentrates present values of significant nutritional benefits and can find application in various food additives, binders, flavonoids and vitamins. It could also be used as additives in some drugs if the active compounds present in it could be analysed to detect its suitability.

**KEYWORDS:** *Daucus carota*, Phytochemicals, Leaf Protein concentrates, Proximate analysis, Mineral composition

## INTRODUCTION

One of the special food nutrient obtained through the process of mechanical breakdown of green leaf components usually referred to as leaf fractionation is leaf concentrate. Although, very few people have heard of leaf concentrate and much less eaten leaf concentrate despite the fact that it is not a new idea or even a new technology.

The rapid development rate and accelerated growth in population in most of the countries in Africa (Nigeria not excluded) has led to serious food crises especially among the vulnerable groups such as the weaned infants, pre-school children, pregnant or nursing mothers and so on. People of this class are particularly susceptible to dietary mineral, protein and vitamin deficiencies. (Aletor and Fasuyi 1997, Coffman and Garcia 1977). This has led to Outbreak of some serious diseases such as marasmus, infant blindness, mortality, morbidity and Kwashiorkor (Aletor and Adebayo 2012). As a result of this, Nutritionists are in search for reliable protein and dietary mineral dietary sources to replace food inadequacies which arise mainly from the highlight cost of animal proteins.

*Daucus Carota* is a biennial plant of *Apiaceae* family, the root were known to contain high quality of alpha and beta carotene, and are a good source of vitamin K and vitamin B6. It is one of the important vegetables that have worldwide

distribution. Carrots were in earlier times used for medicinal purposes and gradually used as food.



Fig. 1: Picture of *Daucus carota*

Furthermore, the ever increasing food shortage due to increase in populations of most countries of the world cannot be alleviated by conventional food sources from

agriculture alone this is due to rapid acceleration demand for food production in most underdeveloped countries of the world and as a result of the requirement for additional source of protein, leaf protein concentrates should be given serious attention because leaves are abundantly available all the year round in most countries that this problem are widespread and many have high protein content with suitable additional food ingredients.

*Daucus carota* is a biennial plant that belongs to Apiaceae or Umbelliferae family and grows up to 0.6 m (2ft) by 0.3 m (1ft in) at a medium rate. It is mostly grown in the northern part of Nigeria by Hausa tribe (most especially around major cities in Jos, Plateau State). The plant usually flower between June to August, and the seeds ripen around August to September. Suitable for: light (sandy), medium (loamy) and heavy (clay) soils and prefers well-drained soil. The wild carrot is an aromatic herb that has diuretic power, soothes the digestive tract and stimulates the uterus. It is wonderful cleansing medicine, that supports the liver, stimulates the flow of urine and enhances the removal of waste by the kidneys. Carrot leaves contain significant amounts of porphyrins, which stimulate the pituitary gland and lead to the release of increased levels of sex hormones. A warm water infusion of the flowers of *Daucus carota* has also been used in the treatment of diabetes (Mahammad, S. B., Tripathi, S. S. and Karunakar H. 2017).

Numerous research investigations about the economic importance, medicinal potential and nutritional benefit derivable from *Daucus carota* are found in the literature such as the abundance of beta-carotene with higher tendency of lowering the risk of cancer and leukemia, (Zaini, R., Clench, M.R. and Maitre, C.L. 2011), Dias, J.S. (2012) and Dias, J.S. (2012), presence of good proportion of antioxidant significant amount of various minerals, ability to lower blood pressure, diabetics control (Megan 2017). But, the leaf protein concentrates of this plant has not been given prominent attention. It is therefore, the objectives of this paper to evaluate the dietary constituents and mineral composition of *Daucus Carota* leaf protein concentrates.

## MATERIALS AND METHODS

### Sample Preparation:

Fresh green leaves of the *Daucus carota* were obtained from a *Riyom* carrot garden situated very close to National Muzeum in Jos Plateau state. The leaves were washed with distilled water and pulped by passing it through the locally produced *mincer*. The pulp was collected and strained through a cotton cloth followed by screw press. The green juice obtained from straining the pulp through the cotton cloth, was heated between 85°C – 90°C by steam injection, which resulted in coagulation of all the protein present within the pulp. The Coagulum was then centrifuge from the rest of the solution, pressed, pulverized and air dried for further chemical analysis.

### Proximate Analysis

Proximate analysis of *Daucus Carota* leaf protein concentrate was carried out as follows:

#### Moisture content determination:

Two grammes (2g) of the fresh sample of *Daucus carota* leaf concentrates was placed in the crucible and heated at 105°C until a constant weight was attained. The moisture content

was calculated as loss in weight of the original sample and expressed as percentage moisture content (FAO, 1980).

#### Determination of crude protein:

Kjeldahl method was used for the determination of crude protein with slight modification 1.0g of *Daucus carota* leaf concentrates was digested with 2.5ml of concentrated sulphuric acid in the presence of Kjeldahl catalyst. The nitrogen from the protein in the sample was converted to ammonium sulphate that reacted with 2ml of 2.5% Brucine reagent, 2.5ml of 98% sulphuric acid to give a coloured derivative and the absorbance read at 470nm. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein (AOAC 1990).

#### Estimation of crude fat:

This estimation crude fat was performed using the Soxhlet extraction method. 5g of *Daucus carota* leaf concentrates were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser. 150ml of n – Hexane was used to extract the lipid (AOAC 1990).

#### Determination of crude fibre:

The estimation was done using the method of AOAC (1990), 10g of the *Daucus carota* leaf concentrates and 220ml of 1.25% H<sub>2</sub>SO<sub>4</sub> were heated for 25min and filtered with a Buchner funnel. The residue was washed with distilled water until it was acid free. 220ml of 1.25% NaOH was used to boil the residue 30min, it was filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCl and twice with ethanol. Finally it was rinsed with petroleum ether three times. The residue was put in a crucible and dried at 105°C in an oven overnight. After cooling in desiccators, it was ignited in a muffle furnace at 550°C for 90 minutes to obtain the weight of the ash.

#### Determination of ash content:

The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited 5g of the pulverized *Daucus carota* leaf concentrates samples was placed in a crucible and ignited in a muffle furnace at 550°C for 6 hours. It was then cooled in desiccators and weighed at room temperature to get the weight of the ash.

#### Determination of Nitrogen free extractive:

The Nitrogen free extractive content was determined by subtracting the summed up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100 (Otitoju, 2009).

#### Analysis of Mineral Content:

Five grams (5g) of the sample was ashed on a muffle furnace at 550°C for 12 hours, the resulting ash was cooled in desiccators. The ash was dissolved in 2ml of concentrated HCL and few drops of concentrated HNO<sub>3</sub> were added, the resulting solution was evaporated almost to dryness in water bath. The content was diluted to the mark level in 100ml volumetric flask with distilled water. Bulk scientific Atomic Absorption Spectrophotometer was used to determine each metal reported for the sample after the appropriate dilutions were made for each element.

### Phytochemical Analysis

Determination of Tannin content: Tannin contents of the Sample were determined using method described by Price and Butter 1977. 5g of plant samples is added 30 min. after filtration, the solution is further transferred to a 30ml flask and water was added to 50ml. 5ml aliquots are finally transferred to vials, 1 ml, 1%  $K_3Fe(CN)_6$  and 1 ml 1%.  $FeCl_3$  are added and water is added to make 10ml volume. After 5 min, the solutions are measured spectrophotometrically at 720nm. The actual tannin concentrations are calculated on the basis of the absorbance values obtained for the standard solution in range 5-25mg /10ml.

### Determination of Saponin content

The method described by Obadori and Chuko 2001 was used for determination of saponin. 10g of powdered samples is located into 150ml of 20% aqueous ethanol. The samples are located with continuous stirring at 55°C for 4 hours. The mixture was filtered and the residue is extracted with another 200ml of 20% ethanol. The combined extracts were concentrated to 40ml over water bath at about 90°C. The concentrate is extracted with 200ml of diethyl ether. The aqueous layer is recovered while the ether layer is discarded. The purification process is repeated; 50ml of n-butanol is added. The combined n-butanol extracts are washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution is located in water bath. After evaporation the sample is dried in the oven to a constant weight and the saponin content is calculated as percentage.

### Determination of Alkaloid

The total alkaloid contents of the samples were determined by method described by Manjunath et al., 2012. The sample extract was dissolved in 2N HCl and then filtered. 1ml of this solution was transferred to separating funnel and washed with 10ml chloroform. The pH of phosphate buffer solution was adjusted to neutral with 0.1N NaOH. 1ml of this solution was transferred to a separating funnel and then 5ml of phosphate buffer and the complex formed was fractionated with chloroform by vigorous shaking. The fractions were collected in 10ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470nm.

### Determination of Flavonoid content

5g of *Daucus carota* leaf concentrate was weighed into 100ml plastic bottle and extracted repeatedly with 100ml of 80% aqueous ethanol at room temperature. It was then filtered with Whatman filter paper into 100ml flask. This filtrate was transferred into a crucible dish and evaporated to dryness over a water bath. This was further dried in an oven at 60°C for 30 minutes and later cooled in desiccators. Both the crucible and the content were weighed and recorded (Ukpabi et al., 2013).

### Determination of Total Phenolic Content

The fat free sample was boiled with 50ml of ether for 15 minutes. 5ml of the extract was pipette into a 50ml flask and 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of conc. alcohol were also added. This sample was left to react for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 500nm. A blank sample for each extract was used for background subtraction. A standard phenol was prepared as 0.005mg/l and absorbance

measured the total phenolic content was expressed as mg/100g

### Result and Discussion

**Table 1:** Proximate Composition of *Daucus Carota* leaf Protein Concentrates

Parameter	Value/g/100g
Moisture	8.69±0.03
Ash	19.69±0.02
Crude Fat	5.69±0.23
Crude Protein	18.38±0.08
Crude fiber	14.81±0.02
NFE	32.74±0.43

Table 1 present the results of proximate composition of *Daucus carota* leaf protein concentrates. The sample contained 8.69±0.03g/100g moisture content. The moisture in food determines the characteristics keeping quality; it facilitates the rate of digestion assimilation and absorption within the body. The moisture content of *Daucus Carota* leaf protein concentrate is higher than (6.69g/100g) reported for *Telfaira occidentalis* leaf protein concentrates by (Akindahunsi and Salawu 2005) but lower than 9.94 ± 0.01g /100g reported for *Thaumatococcus danielli* (Sodamide 2014). The low moisture content of the samples means that there is a concentration of solutes and decreased ability to perish ability (Fennema and Tannen Baum 1996).

Ash content of *Daucus Carota* leaf protein concentrates is 19.69±0.02g/100g the value is higher than 11.60g/100g and 11.37g /100g reported for two varieties of *Ipomea batatas* leaf sample (Hard 1996). High ash content implied higher availability of mineral in food. The value of ash content in *Daucus Carota* agreed with those reported in literature for some common green leafy vegetables. (Saidu and Adunbarin 1998).

Crude fat content of *Daucus carota* leaf protein concentrates is 5.69±0.23g/100g. The values is lower than 6.80± 0.1g/100g and 6.81±0.49g/100g reported for leafy vegetables of *Solanum Microcarpon* and *Cochorus Olitorus* respectively (Adanlowo and Dairo 2006). Fat determines the proportion dietary energy available in food, it increase the palatability due to capability of absorbing and retaining flavours. Any foods capable of providing 1-2% of fat furnish man with sufficient caloric energy. (Davidson et al., 1975).

Crude protein content of *Daucus carota* leaf protein concentrate is 18.38±0.08, the value is lower than 24.85g/100g reported for sweet potato leaf protein concentrates (Akindaunsi and Salawu 2005). It is also lower than 52.07±0.20g/100g reported for *Thaumatococcus Danielli* leaf protein concentrates (Sodamide 2014). The value is lower than crude protein content reported for lentil, cowpea and pigeon pea which are highly recommended as substitute for animal protein (Kay, 1979).

Crude fibre content of *Daucus Carota* leaf protein concentrates is 14.81±0.02g/100g. Significant proportion of Fibre in food reduces the risk of cardiovascular disease, coronary heart disorder, obesity and gastro intestinal disorder. Crude fibre concentration of this sample is a true prediction of the concentration of mineral element in the sample. The value is lower than 28.60g/400g reported for *Amaranthus cruentus* (Oguntona 1988).

Nitrogen free extract of *Daucus Carota* leaf protein concentrate ( $32.74 \pm 0.43$ ) is higher than  $23.58 \pm 3.64/100g$  and  $1.12 \pm 0.43g/100g$  reported for the leaf protein concentrates of *Vernonia amygdalina* and *Thaumatococcus danielli* leaf protein concentrates respectively (Sodamade 2013 and 2014). The carbohydrates content shows that this

sample may not be suitable for those who want to cut down on carbohydrate intake and for obese who need less carbohydrate in their diet. This is because of excess glucose as the end product of digestion of carbohydrates. (Nelson and Cox 2000).

**Table 2:** Concentration of Mineral elements in *Daucus Carota* leaf protein concentrates (mg/100g).

Mineral	Na	K	Ca	P	Mg	Fe	Cu	Mn	Zn	Se	Hg
Concentration mg/100g	23.50 $\pm 0.22$	9.72 $\pm 0.34$	49.30 $\pm 0.87$	40.10 $\pm 0.15$	29.90 $\pm 0.07$	16.25 $\pm 0.65$	4.40 $\pm 0.43$	0.60 $\pm 0.16$	7.80 $\pm 0.27$	0.20 $\pm 0.65$	ND

Table 2 shows the various concentrations of minerals present in *Daucus carota* leaf protein concentrates. The concentration of sodium in the leaf protein concentrates of *Daucus carota* is  $23.50mg/100g$ . The value is lower than  $500mg$  value of adults recommended daily allowance (NRC 1989) this shows that *Daucus carota* leaf protein concentrate can contribute 4.7% of the recommended daily allowance implicating that the sample is good for hypertensive patient but significant quantity of the sample must be eaten if the recommended daily allowance has to be met by eating the sample alone. Sodium is an important source of electrolyte within the body but too much of sodium in combination with chloride could lead to increase blood pressure.

Potassium concentration of the leaf protein concentrate of *Daucus Carota* is  $9.72mg/100g$ . The concentration of potassium in this sample is lower than  $100g$  reported for *astragalina* leaves (Gafar et al., 2011). Potassium concentration value in this sample is lower than  $90.3 \pm 0.42mg/100g$  reported for *Thaumatococcus danieli* by (Sodamade, 2014). The recommended daily allowance of potassium is  $2000mg$  for adult (NRC, 1989). High amount of potassium in the body was reported to increase iron utilization (Adeyeye, 2002), beneficial to people to control herpes and patient that suffer from excessive loss of potassium through the body fluid (Arinathan et al., 2003). *Daucus Carota* leaf protein concentrate is not viable to function in this capacity due to significantly lower quantity of potassium.

Calcium concentration of *Daucus Carota* leaf protein concentrate is  $49.30mg/100g$  while phosphorus concentration is  $40.10mg/100g$ . Calcium and phosphorus are mostly required by children, pregnant and lactating women for proper bone and teeth development. The values of calcium and phosphorus concentration in *Daucus Carota* leaf protein concentrate cannot furnish man with the recommended daily allowance requirement of calcium and phosphorus. However, it is important to stress that care should be taken in the choice of calcium richer foods to prevent kidney stones. Because, approximately 85% of kidney stones issues are reported to originate predominantly from calcium compounds. Kidney stones can also be contacted through high level of oxalate and uric acids, low levels of citrate and inadequate amount of selenium (kidney stones overviewed 2008).

*Daucus Carota* leaf protein concentrates contain  $29.90mg/100g$  of magnesium concentration. The value is lower than  $350mg$  recommended as daily requirement of Adult magnesium and  $170mg$  concentration of daily dietary recommend allowance for children. Magnesium is very important in calcium metabolism in bones and also involved

in prevention of circulatory disease of the heart.  $58.5g$  of the *Daucus Carota* leaf protein concentrate have to be eaten by adult to meet recommended daily allowance of magnesium.

The concentration of Iron in *Daucus Carota* leaf protein concentrate is  $16.25mg/100g$ . The value is lower than  $28.97 \pm 0.04mg/100g$  reported for *astragalina* leaves (Gafar et al., 2011). The concentration of iron in *Daucus Carota* is higher than the recommended dietary allowance of iron in adult male and children ( $10mg$ ) and  $15mg$  per day of an adult female (NRC 1989). Iron is required for the formation of haemoglobin in man and animals. Deficiency of iron in the body leads to anaemia. (Adeyeye and Fagbohon, 2005) and too much of iron in food could lead to increase in the blood pressure level.

*Daucus Carota* leaf protein concentrate contain copper concentration of  $4.40mg/100g$  the concentration is higher than  $3mg$  per day recommended for adult and  $2mg$  per day recommended as daily allowance for children (NRC 1989). Copper plays vital role in the biological transfer of electron and production of enzymes in the body. Zinc concentration in carrot leaf protein concentrates is  $7.80mg/100g$ . Zinc plays a major role in metabolic activity and food assimilation within the body it is also required for proper functioning of reproductive system. The proportion of zinc in *Daucus Carota* leaf protein concentrate is higher that reported by Gafar et al., 2011 for *astragalina* leaf.

Manganese concentration is  $0.60mg/100g$  the value is lower compared to  $2.50 \pm 0.88mg/100g$  reported for *Thaumatococcus danielli* leaf protein concentrates by Sodamade (2014). Manganese assist in the regulation of blood sugar level, it is involve in the cell production, energy and reinforce the immune system (Deborah 2008).

Mercury was not detected in the sample but selenium is present in the concentration of  $0.2mg/100g$ . Though the concentration is very small too much of selenium, mercury, cadmium and lead in food is not good because it could lead to metal poisoning.

**Table 3: Phytochemical Analysis of *Daucus carota***

Plant Constituents	W/W of phytochemical mg/100g
Tannin	$3.66 \pm 0.16$
Saponin	$4.34 \pm 0.06$
Alkaloids	$10.49 \pm 0.02$
Oxalate	$6.89 \pm 0.04$
Phytate	$14.62 \pm 0.05$
Total Phenolics	$3.75 \pm 0.82$
Flavonoids	$3.62 \pm 0.43$
Terpenes	$11.07 \pm 0.23$

The concentration of tannin in leaf concentrate of *Daucus carota* is  $3.66 \pm 0.16 \text{ mg/100g}$  one of the secondary metabolites that protect human body against infectious diseases is tannin. It exerts many physiological effects such as accelerating blood clotting; it lowers blood pressure and decrease the concentration of fat soluble materials on the blood serum (Chinedu and Friday 2015).

Saponin concentration in *Daucus Carota* leaf protein concentrate is  $4.34 \pm 0.06 \text{ mg/100g}$ . The proportion is lower than  $59.11 \text{ mg/100g}$  reported for Gas chromatographic analysis of *Gongronema Latifolium* Benth leaf (Chinedu and Friday 2015). One of the low molecular weight substances present in food is saponin. It is secondary metabolites containing either a pentacyclic terpenoid or tetracyclic steroidal which has diverse range of properties, some of which are known to be determined while some are beneficial to human health (Haralampidis et al., 2002). saponins are helpful in lowering blood cholesterol level while high saponin in diet can cause dental caries inhibition and platelet aggregation. It is also an antidote against acute lead poisoning. The proportion of saponin on this leaf concentrates cannot cause any risk of health hazard, because the amount of saponin present in it is low.

The concentration of Alkaloids in *Daucus carota* is  $10.49 \pm 0.02 \text{ mg/100g}$ . the value is higher than  $1.16 \pm 0.09$  and  $0.99 \pm 0.0$  reported for two species of *Solanum melongena* respectively by Agoreyo et al., 2012. Alkaloids are chemical compounds that contain mostly basic nitrogen atoms which occur naturally. It contains some substrates that are helpful in prevention of malaria and antipyretic in nature. The appreciable amount of alkaloid in this sample makes it suitable in for some drug application.

Oxalate concentration is  $6.89 \pm 0.04 \text{ mg/100g}$  oxalate concentration is lower than values reported for two varieties of *Solanum melongena* ( $41.72 \pm 0.6$  and  $23.97 \pm 0.5$  respectively) by Agoreyo et al., 2012.

Too much of phytate and oxalate in food are dangerous because they can bind most of the macro minerals in food such as calcium, magnesium, iron and zinc making them unavailable the level of oxalate in this leaf protein concentrate is not high rendering it safe for consumption. Phytate concentration is  $14.62 \pm 0.05 \text{ mg/100g}$ . it is also lower than that reported for the leaf of two varieties of *Solanum melongena*. Total phenolics concentration is  $3.75 \pm 0.82$  phenols are derivatives of phenylalanine and it is often controlled by dirigent proteins. Group of phenols are very important in food because they have shown anti inflammatory and anti oxidant activity in basic research models of human diseases (Korkina et al., 2011).

Flavonoids concentration is  $3.62 \pm 0.43 \text{ mg/100g}$  flavonoids are known to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility. It is also reported to inhibit varieties of enzymes like hydrolases, phosphatase and lipase (Cook and Samman 1996). Terpenes concentration of *Daucus carota* leaf protein concentrates is  $11.07 \pm 0.23 \text{ mg/100g}$ , the value fall in range with values reports for total terpenes in *Gongronema latifolium* (Chinedu and Friday 2015). Terpenes have been identified as high-valued chemicals in food, cosmetics, pharmaceuticals and technological industries (Timmappa et al., 2014).

## Conclusion

Carrot leaf protein constituent present values of significant nutritional benefit and can find application in various food additives, binders, flavonoids and vitamins. It could also be used as additives in some drugs if the active compounds present in it could be analysed to detect its suitability.

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