Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 2400-2405
Received: 10-07-2017
Accepted: 11-08-2017

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Pharmacognostic evaluation of *Daucus carota* Linn. Leaf (Apiaceae)

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Abstract
The macroscopic, organoleptic features, microscopical and chemo-microscopical characters as well as physico-chemical parameters of the *D. carota* leaf were evaluated to establish some pharmacognostic standards for its further investigation. The plant material was prepared and evaluated according to standard methods of assessing crude drugs. Findings from the study shows the leaf has alternate covering base, tri-pinnate leaf arrangement, parallel venations, finely divided uniform division, acute leaf shape and also serrated leaf blade. The leaf width ranges between 0.30-0.50 cm, stem height 39.00-42.00 cm, leaf size 1.50-2.80 cm and has a fine hairy surfaces, greenish colour, mint odour and pleasant taste. The microscopical features revealed wavy epidermal cells and several diacytic stomata on lower and upper surfaces. The chemo-microscopy revealed the presence of starch, tannins, lignin, cellulose, starch grains, aleurones, mucilage, suberin, cutins and calcium oxalate crystal was not seen. The quantitative leaf microscopy of the lower epidermal layer revealed stomata number is 14.80±0.97, stomata index 31.22±3.09% and palisade ratio is 6.20±0.37, while the upper epidermal layer revealed stomata number is 10.60±0.87, stomata index is 32.55±1.25%, vein islet number is 12.80±1.98 and veinlet termination is 31.800±2.33. The physico-chemical parameters determined showed that the moisture content (5.56%), alcohol extractive value (15.60%), water extractive value (21.80%), total ash (11.60%), acid insoluble ash (1.00%) and water soluble ash (5.67%). These findings will serve as baseline towards establishing standards for *D. carota* leaf identity, purity and quality which can lead to its further pharmaceutical utilizations and proper differentiation from similar species.

Keywords: *Daucus carota*; Pharmacognostic evaluation; Microscopy; Physicochemical constant

Introduction
Daucus carota (Linn.) is one among the natural products from plants that are produced and obtained principally in nature. It belongs to the family of Apiaceae and it is commonly known as carrot (Pimenov and Leonov, 2004) [24]. It is native to Western or near East Asia, Southwest Asia, Tropical Africa, Australia and North and South America and also found in the Mediterranean region (Reed, 1976) [26] and it is a rich source of vitamin A, B, C and can serve as antioxidants that can inhibit or reduced cardio vascular diseases, cancer or prevent xerophthalmia among children and prevent other inflammatory diseases (Bao and Chang 1994; Davir *et al.*, 2016) [6]. Many species in the family are used ethno medicinally for gastrointestinal treatment, cardiovascular ailments, abortificient, stimulants and sedatives. All the parts have been used traditionally such as aphrodisiac, diuretic, antidiabetic, muscle and back pain treatment (Barnes, 1998). The infusions of the leaves have been used to counter cystitis and kidney stone formation, and to diminish stones that have already formed. Also the hot water extract of the leaf is taken orally as a uterine stimulant during parturition, abortifacient, emmenagogue, aphrodisiac and the dried seeds are used as a powerful abortifacient (Ross, 2005; Barnes, 2007) [28, 5]. Some prominent members of Apiaceae family include *Apium graveolens* (Celery), *Anethum graveolens* (Dill), *Anthriscus cerefolium* (Chervil), *Angelica* spp. (Angelica),*Carum carvi* (Caraway), *Coriandrum sativum* (Coriander) (Amirhossein and Mehrdad 2010) [4]. Generally, some of the species may possess antimicrobial activity and could also be used as flavorings for alcoholic beverages (Heywood *et al*., 2008) [18]. Apiaceae family are known to possess antimicrobial, antioxidants, anti-inflammatory, used in treating infections and respiratory ailments (Davir *et al.*, 2016) and could also be used in treating other ailments including cancer and have hepatoprotective activities. Nevertheless, Carrot is called *Karas* among the Hausa, *Karoti* or *Atoka* in Yoruba and *Karotu* in Igbo. The leaf is usually thrown away after harvesting or after eating the edible root part and results to wastes. The aerial part of *D. carota* is used as components of livestock feed by Hausa and Fulani of Northern Nigeria with little or no information on the safety of the *D. carota* aerial part on animals and humans.

The needs for its pharmacognostic studies will ensure its proper identity, quality and purity for its further utilization. These evaluations involve quality control assessment, establishing pharmacognostic standards in guiding towards its further utilizations as well as prevention of adulteration of natural drugs (Ghani, 1990) [16] Since plants as a natural product have been the main sources of drug, food, clothing, shelter, vegetables, herbal beverages, cosmetics, insect repellant, herbicides, animal food and health care management in developing countries including Nigeria (Etkins, 1993 and Riewpaiboon, 2004) [13, 27]. The aim and objectives of this study is to establish some pharmacognostic of *Daucus carota L.* leaf and aerial part and also provide useful scientific evidences that could guide further studies.

Materials and Methodology

Light microscope – Rating: 85-265V (Fisher Scientific UK), Bleaching agents (Sodium hypochlorite), glass slides, cover slips, camera lucida, razor blade, analytical weighing scale (Sartorius ED-2245), stage micrometer and ocular lens (Graticles LTD, Ton Bridge, Kent, England), Oven (Light water, Surrey GU-185-TA-UK), crucibles, metal scrappers, water bath, desiccators, forceps, syringes and needles, razor blades, ruler, measuring cylinder, capillary tubes, photographic camera, funnels, beakers, aluminum foils, conical flask, and other sourced equipment and instruments. All chemicals and solvent were of analytical grades purchased from Sigma Aldrich Chemical Company and Merck (From Lagos, Nigeria distributors). The instruments were also well calibrated before use.

Plant Identification, Collection and Preparation: *D. carota* aerial part was collected from Samaru market in Sabon Gari Local Government Area, Zaria, Kaduna State - Nigeria. It was identified and authenticated by Mallam Namadi Sanusi, a Taxonomist in the Herbarium unit of the Department of Botany, Ahmadu Bello University Zaria, Nigeria. The collected plant was cross referenced and the voucher specimen (no. 12034) was obtained. The plant material was collected in large quantities, air dried under shade, pulverized and stored in an air tight container for further use. The fresh leaf of the plant was used for the microscopic studies.

Macroscopic and Organoleptic features

Various characteristics features of the fresh leaves of *D. carota* such as type of leaf base, characters of lamina (vennation, margin, apex, base, surface and texture), taste, odour and appearance were observed.

Microscopic studies of *D. carota* leaf

The transverse section across the midrib of the *D. carota* fresh leaf was prepared, cleared, mounted in dilute glycerol and observed under the microscope (X 400). The upper and lower epidermal layer was observed under the microscope and the diagnostic features were observed and documented as described by Evans, (2009) [14] and WHO, (2011).

Quantitative leaf microscopy

Some numerical constants of the leaves of *D. carota* namely: stomatal number and index, palisade ratio, vein islet and veinlet termination number was carried out as reported by Evans (2009) [14].

Determination of Physicochemical Parameters

The physico-chemical parameters such as the moisture content (loss on drying), total ash, water soluble ash, acid insoluble ash values, water and ethanol extractives of the *D. carota* aerial parts were determined as described in World Health Organization guidelines on method of assessing crude herbal drugs (WHO, 2011).

Chemo-microscopic Examination

The histo-chemical detection of cell walls and cell contents of the *D. carota* aerial part were carried out following the methods outlined as described in WHO guidelines on quality control methods for medicinal plant materials (WHO, 2011).

Fluorescence analysis

The *D. carota* aerial parts powdered were screened for fluorescence characteristic using chemical and non-chemical treatment. The colour observations in day light and under ultra-violet light between 254 nm and 365 nm was observed as reported by Kokashivj *et al.*, (1958); Shivani *et al.*, (2013) [30].

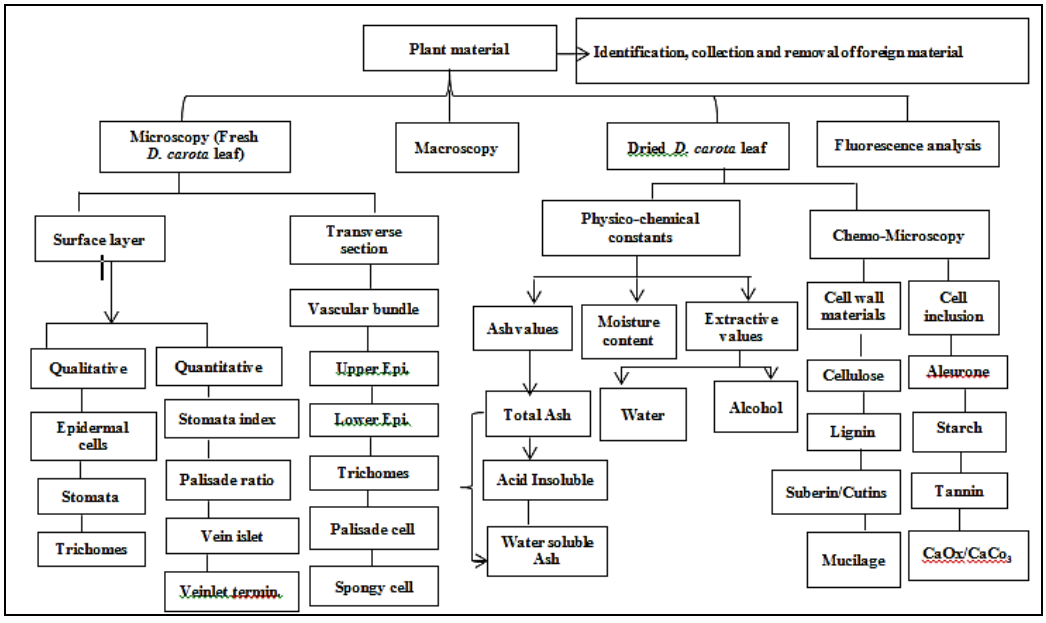

Fig 1: Schematic methodology of some pharmacognostic evaluation of *D. carota* leaf.

Results and Discussion

Plate I: *D. carota* aerial part in its natural habitat

Plate II: Powdered D. carota aerial part

Table 1: Organoleptic and Macroscopic features of *D. carota* aerial part

Organoleptic/Macroscopic features	Physical characteristics
Colour	Green leafy
Odour	Mint/pleasant
Taste	Flavour
Appearance/texture	Smooth hairy surface along the stem Yellowish during spoilage
Leaf size	2.52 ± 0.20 cm
Leaf width (cm)	0.44 ± 0.04 cm
Aerial part height (cm)	39.00 ± 1.34 cm
Leaf shape	Acute/ Serrated leaf blade
Apex	Acute apex
Vennation	Parallel vennation
Leaf arrangement	Tri-pinnate

Microscopic features of *D. carota* fresh Leaf

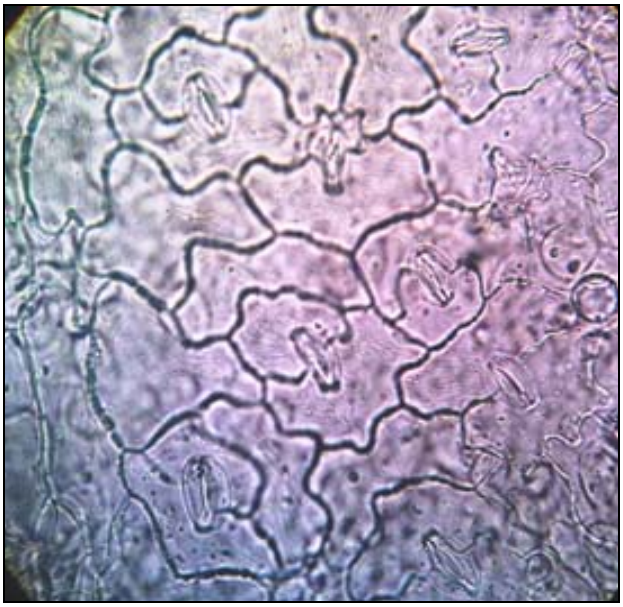

Plate III: The Lower Epidermal surface) of *carota* Leaf (Mag.: X 400)

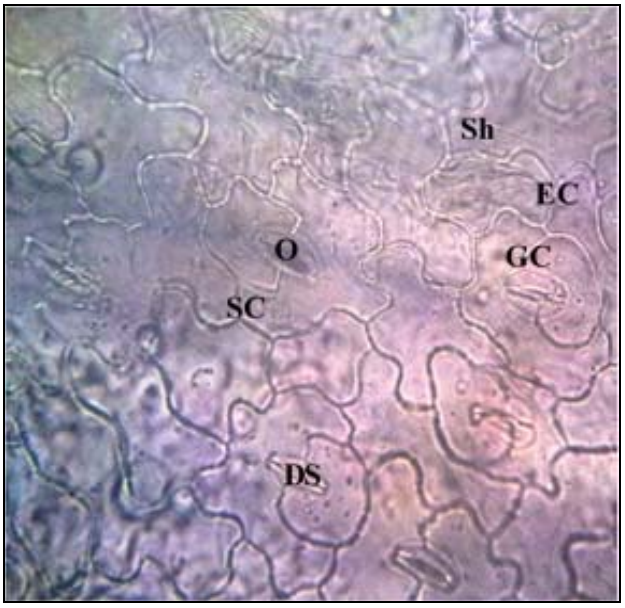

Plate IV: The Upper Epidermal surface of *D. D.* Leaf (Mag. X 400)

Key: EC = Epidermal cell; DS = Diacytic stoma; SC = Subsidiary cell; O = Osteole; GC = Guard cell; SH = Sheath covering.

Table 2: Quantitative microscopic of *D. carota* leaf

Quantitative standards	Upper surface	Lower surface
Epidermal cells	21.80 ± 0.97	31.40 ± 1.86
Stomata number	10.60 ± 0.87	14.80 ± 0.97
Stomata index %	32.55 ± 1.25	31.22 ± 3.09
Palisade ratio	4.00 ± 0.05	6.20 ± 0.37
Vein islet number	12.80 ± 1.98	12.80 ± 1.98
Vein termination number	31.80 ± 2.33	31.80 ± 2.33

*Average values of five determination

Table 3: Chemo-microscopical Features of *D. carota* Powdered Leaf

Constituents	Detecting reagents	Observation	Inference
Starch	N/50 iodine	Blue-black colour on grains within the cell.	Present
Lignin	Phloroglucinol	Red-pink colour on the walls of lignified cell.	Present
Tannins	5% $FeCl_3$	Greenish-black colour in some parenchyma cells.	Present
Mucilage	Ruthemium red	Dilated fragments	Present
Calcium oxalate	HCl	Dissolution of shining crystals on the anatomical sections of the Leaf.	Absent
Calcium carbonate	HCl	Effervescence in the cell.	Present
Cellulose	Chlor-Zinc- Iodine	Blue coloration of the cell wall	Present
Suberin	Sudan red	Orange red colour on cell wall.	Present
Aleurone grains	Iodine in ethanol	Yellowish brown	Present

Table 4: Some physico-chemical parameters of powdered *D. carota* aerial part

Parameters	Values (% w/w) ± SD
Moisture content	5.57 ± 0.51
Total ash value	11.60 ± 1.02
Acid Insoluble ash	1.00 ± 0.90
Water Soluble ash	5.67 ± 0.76
Ethanol Extractives	15.60 ± 0.55
Water Extractives	21.80 ± 1.10

Values (% w/w) are means ±SD (Standard deviation) of five determinations

Table 5: Fluorescence analysis of *D. carota* Aerial part

Reagents/solvent Used	Daylight		Short Wavelength (254 nm)	Long Wavelength (365 nm)
	Immediately	After one hour		
Conc. Hcl	Green	Green	Deep green	Dark green
Dil. Hcl	Brown	Brown	Brown	Black
Dilute NaOH	Light green	Greenish yellow	Deep green	Dark green
H_2SO_4	Dark green	Greenish black	No fluorescence	Green
n-Hexane	Yellowish green	Yellowish Green	Light green	Green
NH_4OH	Yellow	Greenish yellow	Deep green	Dark green
Water	Green	Green	No fluorescence	No fluorescence
Ethyl acetate	Green	Green	Light green	Brown
Nitric acid	Deep brown	Deep brown	Brown	Deep brown
Methanol	Blue green	Greenish black	Light green	Green
Picric acid	Light green	Light green	Light green	Dark green
Acetic acid	Green	Green	Dark green	Dark green
Iodine	Green	Green	Green	Green
Magnesium chloride	Green	Green	Green	Dark green
Ferric chloride	Blue-black	Blue-black	Dark green	Black
Ferrous sulphate	Blue-black	Black	Black	Black
Benzene	Green	Green	Green	Dark green

The results above (Table 1-5), established some pharmacognostic standards in *D. carota* aerial parts that could ensure its quality, safety and purity as a crude drug. The macroscopic and organoleptic features of the *D. carota* leaf (Table 1) shows greenish colouration, smooth hairy surface but not spiking and may become yellowish colour during spoilage. The leaf has flavour taste and pleasant odour especially when fresh and could be characteristic odour in the plant genus. The average leaf size was 2.52±0.20 cm with the width of 0.44±0.04 cm and the aerial height could grow to the average of 39.00±1.34 cm to harvesting. The leaf has parallel vennation, mainly tri-pinnate leaf arrangement and acute leaf shape. All these morphological features will serve as a tool for its proper identification from similar plant species. These results are in lined with Reed, (1976) [26]; (Heywood et al., 2008) [18]; Alina *et al*., (2009) [3] and Wikipedia, (2015) [34] who reported similar morphological features in the plant genus.

Microscopically, the stomata have diacytic type stoma with numerous numbers on lower epidermal surface and frequent number on the upper epidermal surface. The presence of stomata may increase it photosynthetic potential (Woodward, 1990). The epidermal layer was wavy epidermal type with lateral covering sheath, possession of osteole, subsidiary and guard cells which make it an organized crude drug and revealed its potentials for transpiration and water propensity (plate III-IV). This results was in lined with Ghani, (1990) [16]; WHO, (2011); Santhan (2014) [29] who reported that some diagnostic features in some medicinal plants species which could guide and preventing adulterations (Kunle *et al*., 2012) [23]. Alina *et al*., (2009) [3] reported similar microscopic features on *Anethum graveolens* (Dill.) in the Apiaceae family and also reported diacytic stoma as the stomata. This could be an important diagnostic feature in the Apiaceae family. But Santhan, (2014) [29] reported paracytic stomata type in *Centella asiatica* in the Apiaceae family, these differences could be attributed to different geographical distributions and wide evolutionary taxonomical relationship in the family. Also, the transverse section of the leaf showed the adaxial layer which is the upper epidermal, abaxial layer which is the lower epidermal, hypodermis, vascular bundles principally xylem and phloem, trichomes, palisade and spongy parenchyma which are common features of higher and vascular plants as also reported by Dutta, (2000) [10] and Gupta, (2003). However, the transverse section across the midrib showed dorsi-ventrally leaves pattern which is a

common feature among dicot plants (Dutta, 2003) [11].

The quantitative leaf microscopy showed the average stomata number of 14.80±0.97 on the lower surface and 31.40±1.86 on the upper surface while stomata index on the lower surface is 31.22±3.09 and 21.80±0.97 on the upper surface that could serve as baseline for establishing standards The palisade ratio is 6.20±0.37, vein islet number 12.80±1.98 and vein termination number 31.80±2.33 could be used as numerical and quantitative standards of the *D. carota* leaf (Table 2). These numerical standards could be useful as baseline for standardization and for further evaluation. However, there could be variation base on geographical distribution (Brain and Tuner, 1975) [7] when carried out in another geographical zone.

The powdered chemo-microscopical features in the aerial part revealed some cell inclusion and cell contents (Table 3) using detecting reagents revealed the presence of cellulose, mucilage, starch, lignin, tannins, suberins, calcium carbonate and aleurones grains which could be attributed to its potentials as pharmaceutical agents. However, calcium oxalate crystal was not seen, this may be due to the fact that the plant aerial part may be weak, have no mechanical support and could have no protection from herbivores (Albert, 1901) [2] which could make the aerial part susceptible to diseases and other predators.

Furthermore, the physico-chemical parameters of the *D. carota* aerial part were assessed for moisture content, total ash, acid insoluble ash, water soluble ash, ethanol extractive and water extractives (Table 5) which may serve as a reference standards in assessing quality and purity of *D. carota* as a crude drug (WHO, 2011). The moisture content of 5.57±0.51 % may discourage the growth of bacteria, yeast, mould and fungi and could be stored for long period of time without spoilage. However, the average percentage of moisture content in crude drug should be within 12-14 % (BHP, 1990; EP, 2011; WHO, 2011) and the value obtained was within the permissible limits. This percentage of moisture content was also similar to the work of Fazal and Singlaash, (2012) [15] who reported similar moisture content in *Apium graveolens* (Linn.). The total ash value (11.6%) obtained could be attributed to organic material like carbonate, oxalate and silicate and presence of other impurities in *D. carota* aerial part. The high value of total ash percentage could be used as criteria to judge the purity of drug (Prasad *et al*., 2012) and load of contaminations that could be directly or indirectly deposited on the aerial part. The acid insoluble ash of 1.00 ± 0.90 % indicated some levels of contamination with earthy material, sand and other impurities in the crude drug however, the result is within the permissible standard in evaluation of crude drug (WHO, 2011). The water soluble ash of 5.67±0.76 % could be used to estimate the amount of inorganic compound present in the crude drugs. This results was in agreement with BHP, (1990); EP, (2011); WHO, (2011); (Kaneria and Chanda, 2011) and (Sumitra, 2014) that reported the permissible limits and the necessities for physicochemical evaluation of crude drugs. This study found water extractive value (21.80%) to be the highest and alcohol extractive (15.60%) respectively. The high water extractive values probably revealed that water extract have the ability to extract more phytoconstituents than alcohol extract based on their polarity scale. This is in agreement with the work of Ajazuddin and Shailendra, (2010) [1] who reported that having high water extractive value is a better solvent of extraction than ethanol. Nevertheless, water is a universal solvent and its use mostly as solvent by traditional healers and individual in crude drug preparation. Alcohol extraction is still given preference in terms of choice of solvent when it comes to medicinal plant researches due to the fact it does not accommodate or increase the microorganism growth and could easily be evaporated. These results are similar to the findings of Fazal and Singlaash, (2012) [15]; Kumar *et al.,* (2012). The choice of solvent in a research involving plants depend on many factors among which include the diversity of different phytochemicals to be extracted and also what is intended to carried out with the extract (Kokate *et al.,* 2009; Tiwari and Mishra, 2010) [20, 33].

Similarly, the fluorescence analysis revealed the nature of the aerial plant under daylight and UV- visible at short (254 nm) and also under long wave length (365 nm) using different solvents and chemicals (Table 5). The results showed visible fluorescence region ranging from green to dark green. Brown fluorescence was also observed when the aerial part was mixed with dilute HCL while there was no fluorescence when *D. carota* aerial part was mixed with water under short and long wave length. Similar finding was also reported by Kokashivj *et al.,* (1958) and Shivani *et al.,* (2013) [30]. This could also be used as guide in pharmacognostic standardization (Sumitra, 2014) and could formed basis of its utilization as crude drug and also prevent adulteration.

Conclusion

The pharmacognostic evaluation of *D. carota* leaf provided both qualitative and quantitative standards that will serve as baseline in ascertaining the identity, quality and purity and also guide its further pharmaceutical utilizations.

Acknowledgement

The authors are grateful to Mal. Kabiru Mohammed and Mal. Kamilu of Department of Pharmacognosy and Drug Development, ABU, Zaria for their laboratory assistance during the study.

Declaration of conflict of interest: The authors declare no conflict of interest. The authors alone are responsible for the content of the paper.

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