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Molecular Spectroscopic Analysis of *Daucus carota* Plant Pigment Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Various solvents (distilled water, methanol, ethanol, acetone and chloroform) extracts of *Daucus carota* were scanned with UV – Visible spectrophotometer (Thermo - spectronic) and Perkin – Elmer FT – IR model (Spectrum BX) infra – red spectrometer. All the extracts though with different pH showed typical orange colour with more polar solvent (H₂O) giving the lighter hue. The UV – Visible spectra analyses of the extracts revealed a good absorption between 202 – 452 nm and λ_{max} range of 272 – 340 nm. The IR spectrometry revealed various functional groups such as alcohol, arenes, alkanes and particularly, the conjugated dienes. These functional groups obviously are attributable to the structural chemistry of the *Daucus carota* plant pigment.

Keywords: Molecular spectroscopy; pigment; UV- visible; infra-red; spectra.

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1. INTRODUCTION

Plant pigments include a variety of different kinds of molecules, including porphyrins, carotenoids, anthocyanins and betalains. All biological pigments selectively absorb certain wavelength of light while reflecting others. The light that is absorbed may be used by plants to power chemical reactions while the reflected wavelengths of the light determine the colour pigment that will appear to the eye. Pigments also serve to attract pollinators. Plants pigments in fruits and vegetable are mainly chlorophyll (green), carotenoids (red, yellow, orange), and anthocyanin (blue, purple, red). The orange in carrots is due to the carotene [1].

Major chemical constituent of carrots is lycopene which has eleven conjugated double bonds that gives it, its deep red colour and are responsible for its anti-oxidant activity. Due to its non-toxicity, lycopene is used for food coloring [2]. Plants and photosynthetic bacteria naturally produce all-trans α & β carotene [3]. When exposed to light and heat, α & β carotene undergo isomerization to number of cis- isomer, which have a bent rather than linear shape.

In synergy with other nutrients, has been shown to decrease biomarker of oxidative stress and carcinogenesis [2]. In different studies, α & β carotene were even found to have inhibitory effect on cataract development [4] to several different kinds of cancer including breast cancer [5]. α & β carotene may be the most powerful carotenoids quencher of singlet oxygen [6], being 100times more efficient in test-tube studies of singlet oxygen quencher action than vitamin E, which in turn has 125 times the quenching action of glutathione (water soluble). Singlet-oxygen produced during exposure to ultra-violet light is the primary cause of skin ageing [7].

Though carotenoid is non-toxic and is commonly found in diet, but cases of excessive carotenoid intake have been reported. In a middle aged woman who had prolonged and excessive consumption of tomato juice, her skin and liver were colored orange-yellow and she had elevated levels of lycopene in her blood. Her skin colour returned to normal after three weeks on carotenoid-free diet [8]. This coloration of the skin is known as α & β carotene dermia.

In recent years, complementary and alternative medicine, together with their various practices

has become increasingly popular and even considered a highly acclaimed discipline in the western world [9]. It is noted that 40-50% of medicine are direct or synthetic copies of plants ingredient [10]. The federal government of Nigeria inaugurated the Presidential Initiative Committee (PICO) in 2006 on the development, promotion and commercialization of Nigerian herbal medicine plants. The committee's blue print is yet to be implemented despite the global resurgence of medicinal plants. This Study have, therefore intends to elucidate the pigment chemistry of *Daucus carota* commonly consumed in Nigeria. It is hoped that this could provide some basis for its utilization in food, health and related industries.

2. EXPERIMENTAL

2.1 Sample Collection and Preparation

2 kg. of fresh sample of carrot (*Daucus carota*) was purchased from vegetable market at Airport Road, Benin city, Oredo local government area of Edo state. The vegetable was identified by comparison with Herbarium Reference Material at Department of Botany, Faculty of Life Science, University of Benin. The sample was prepared for extraction by carefully cleaning and slicing them into small portions and thoroughly crushed with mortar and pestle. The crushed vegetable was used as starting material for the extraction.

2.2 Chemical Extraction

Cold extraction and maceration was used by taking 800 g of the crushed vegetable (carrot), mixed with 1200 cm³ each of the various analar grade solvents (methanol, ethanol, acetone and chloroform) and distilled water in 250 cm³ conical flask. The flask was carefully swirled for 15 minutes after which the mixture was filtered with Whatman filter paper No.1 (11 cm). All the pigment extracts were preserved by keeping them in refrigerator temperature for physical and chemical evaluation studies [11].

2.3 UV-visible Spectra

The UV-visible spectra of solvents (methanol, ethanol, acetone and chloroform and distilled water) extracts of carrot sample in a rectangular cuvette were scanned respectively, using spectrophotometer (Thermo-Spectronic, England). The extracts were first sufficiently

diluted with relevant solvents before introducing into cuvette and then scanned [12]. UV-visible spectroscopy involves the examination of the electronic transition associated with absorption in the UV (180-390 nm) and visible (390-700 nm) regions of the electromagnetic spectrum [13].

2.4 Infra-Red Spectra (IR)

Two equal sizes of flat IR transparent circular cells of 2 cm in diameter were thoroughly cleaned with solvent. Two drops of the sample extracts were added to one of the cells with glass rod and then carefully covered with the other cell. The covered cell was then inserted into the Perkin Elmer FT - IR model – Spectrum BX. The spectra were finally printed out for interpretation [14].

The pH meter was standardized by using buffers of pH 4 and pH 10. The pH of the extract were determined by using pH meter(Hanna Instrument model H196107), also the pH was altered by adding several concentration (0.05 M,0.5 M & 0.8 M) of hydrochloric acid (HCl) and (0.05 M,0.5 M & 0.8 M) sodium hydroxide (NaOH). The pH values were finally measured using the pH meter.

2.5 Hue

Hues were visually assessed in conjugation with the λ maximum values obtained from UV– visible spectra from various spectra.

3. RESULTS AND DISCUSSION

3.1 Effect of Varying the Types of Solvent

The colours of the extracted pigments obtained using various solvents is presented in Table 1.

It would be observed that the colour of the carrot extract using various solvents was basically orange. In other words, the use of the more polar solvents (H₂O, MeOH, C₂H₅OH, CH₃OCH₃) and non-polar solvent (CHCl₃) did not affect the

colouration of the carrot extract. However, it is obvious in Table 1, that the hue of the orange colour was lighter when the extraction was done with distilled water. Of the more polar solvents used in the extraction, water would be considered the most polar. Thus, the most polar solvents gave the least hue of colour. The inference, is that all the solvents extracted the same type of pigment from carrots (orange – colour pigment) but the most polar solvent (H₂O) gave a lighter hue of orange, which would tantamount to a less intensive extraction of orange pigments. This can be ascribed to differences in the affinities of the orange pigment in the carrot to the various types of solvents used in these studies [11] reported a similar phenomenon with meat extracts in which various λ max values were observed with meat extracts when different solvents were used in the extraction.

Table 1. Colours of extracts of carrot using different solvents

Extraction solvent	Colours of extracts	λ max.
Distilled water	Light orange	272
Methanol	Orange	268
ethanol	Orange (Deeper hue)	294
Acetone	Orange	292
chloroform	Orange	286

The orange colouration of the extracts observed in this study, is probable due to primarily, vitamin A (retinol) which is known to be present significantly in carrots. Carrot is the major source of vitamin A [15]. The largely hydrocarbon and therefore hydrophobic nature of vitamin A would make it more soluble in less polar solvents.

3.2 UV Visible Spectral Analysis of Extracts

These spectral studies were done with a view to gaining more insight into the physical attributes of the pigments.

Table 2. UV – visible spectral analysis of carrot extracts

Extracting solvent and sample	Region of absorption (nm)	λ max. (nm)
Carrot/ distilled water	202 – 388 nm	272 nm (0.253A)
Carrot / methanol	208 – 448 nm	294 nm (0.426A)
Carrot / Ethanol	204 – 452 nm	268 nm (0.410A)
Carrot / Acetone	204 – 422 nm	340 nm (6.000A)
Carrot / Chloroform	206 – 368 nm	286 nm (0.463A)

From Table 2, the carrot extracts showed absorption from about 200 nm to 450 nm; absorption in both uv and visible regions of electromagnetic spectrum. Ultraviolet – visible (UV - visible) spectroscopy involves the examination of the electronic transitions associated with absorption in the UV (180 -390 nm) and visible (390 – 780 nm) regions of the electromagnetic spectrum [13]. The energies associated with these regions are capable of promoting the outer electrons of a molecule from one electronic energy level to a higher level. The part of the molecule containing the electrons involved in electronic transition responsible for the observed absorption is called the chromophore.

The types of transitions that results UV – visible absorption consists of excitation of an electron from the highest occupied molecular orbital (usually of non – bonding p or bonding π orbital) to the next lowest unoccupied molecular orbital (an anti – bonding π^* or σ^* molecular orbitals) [16].

Generally, the study revealed that the various solvent extracts gives different λ max. (Water extract, 272 nm; methanol extract, 294 nm; ethanol extract, 268 nm; acetone extract 340 nm and chloroform extract 280 nm). UV – visible spectra are sensitive to differences in solvent, pH and conjugation [16]. Many of the benzene – based pigments such as anthocyanins, flavonoids etc. [17] could show this uv absorption because of the conjugation in their structures.

3.3 Infra – Red Spectroscopy (IR)

The IR spectroscopy was carried out on the extracts in an attempt to gain further insight into their physico – chemical properties. Infra-red spectroscopy is a technique based on the vibrations within a molecule [18-21]. In this study, various functional groups are inferred in the ethanol extracts in accordance with the use of spectroscopy. Conjugation lowers the bond order because of contributions of the resonance forms with lower bond orders.

Among the IR bands inferred includes 3080 cm^{-1} of C – H stretch, coupled with C = C stretch with IR band of 1633 cm^{-1} , strong band around 3350 cm^{-1} which shows presence of hydroxyl group and 1200 cm^{-1} which is due to C – O stretch of alcohol. The IR band of 1623 cm^{-1} is lower due to effect of conjugation which reduces the adsorption frequencies of the conjugated groups.

A sharp band at 3600 cm^{-1} tend to suggest, there are free O – H stretch. Bonded O – H stretch appears at lower frequency than free O – H stretch. Finally, a strong band at 3000 cm^{-1} was seen, which is typical of C – H stretching of the aromatics.

4. CONCLUSION

Though *Daucus carota* pigments extracted with various solvents showed typical orange color, the most polar solvent (H_2O) gave a lighter hue of orange; indicating less intensive extraction of orange pigment. Similarly, the UV. visible spectral analysis of the extracts gave λ_{max} range (272 nm-340 nm) with the more polar solvent (H_2O) recording the least λ_{max} (272 nm or 10253Å). Various functional groups are inferred in the extracts in accordance with the use of IR spectroscopy in functional group detection and analysis. The infra-red region ($4000\text{-}650\text{ cm}^{-1}$) is of prime importance for the study of organic compounds. Possibility of two different compounds having the same IR spectrum is exceedingly small. Thus IR could be used for identification of organic molecule. In this study, various functional groups like OH, CO, C-H and effect of conjugation on the absorption bands were inferred.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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