DIETARY SUPPLEMENT CAPSULES CONTAINING BEETROOT AND CARROT POWDER

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Abstract

The term nutraceutical is a hybrid of "nutrition" and "pharmaceutical". Reportedly, it was coined in 1989 by DeFelice, Chairperson of the Foundation for Innovation in Medicine. It is applied to products that are isolated from herbal sources and serve as dietary supplements (nutrients). The idea of using phytocompounds in combination results in a dramatic synergism even at low concentrations. The beetroot is the taproot portion of the beet plant, usually known in North America as beets while the vegetable is referred to as beetroot in British English, and also known as the table beet, garden beet, red beet, dinner beet or golden beet. It is one of several cultivated varieties of beet vulgaris grown for their edible taproots and leaves called beet greens, They have been classified as the *B.vulgaris* Conditiva group.

Carrots (*Daucus carota Linn*) are a multi-nutritional and multifunctional root vegetable, rich in natural phytochemicals (bioactive compounds), which are recognized for their nutraceutical effects and health benefits in the human body. The main aim of our research was to create a sustainable nutraceutical capsule containing a combination of beetroot and carrot in dry powder form for daily use as a health supplement. Also to identify and quantify the contents available in the capsule by various analytical techniques.

Keywords: Nutraceutical, Beetroot, Carrot, TLC, UV Spectroscopy, HPLC

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INTRODUCTION

Nutraceutical is defined as any substance that is a food or part of a food and provides medical or health benefits, including the prevention and treatment of diseases. A nutraceutical is any non-toxic food extract. Supplement that has scientifically proven health benefits for either the treatment and/or prevention of diseases. Nutraceuticals can be functional food ingredients or dietary supplements from natural sources.[1-2]

The word "Carrot" was first recorded in English circa 1530 and was borrowed from the Middle French carotte, itself from the Late Latin "carōta", from the ancient Greek "karōtón". In Old English, carrots (typically white at the time) were not clearly distinguished from parsnips. Various languages still use the same word for carrot as they do for root; e.g. the Dutch wortel. The carrot is a biennial plant in the umbellifer family, Apiaceae. At first, it grows a rosette of leaves while building up the enlarged taproot.[3-6]

The beet is derived from the wild beet or sea beet (Beta maritima) which grows on the coasts of Eurasia. Ancient Greeks called the beet teutlion and used it for its leaves, both as a culinary herb and medicinally. Beetroot consists of multiple biologically active phytochemicals including betalains (e.g., betacyanins and betaxanthins), flavonoids, polyphenols, Saponins and inorganic Nitrate (NO3); it is also a rich source of diverse minerals such as potassium, sodium, phosphorous, calcium, magnesium, copper, iron, zinc and manganese.[7-10]

Table 1 Pharmacological Information [11-12]

Content	Beetroot	Carrot
Kingdom	Plantae	Plantae
Division	Magnoliophyta	Tracheophyta
Class	Magnoliopsid	Magnoliopsid
Order	Caryophyllale	Apiales
Family	Chenopodiacea	Apiaceae
Genus	Beta	Daucus
Species	vulgaris	D. Carrota

MATERIALS AND METHODS

Thin Layer Chromatography (TLC) [13-15]

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminum foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel. TLC depends on the separation principle. The separation relies on the relative affinity of compounds towards both phases. TLC is widely used by many industries and research fields, including pharmaceuticals, clinical testing, environmental toxicology, food, water and pesticide analysis, and cosmetics Retention Factor (Rf VALUE): Retardation or retention factor (Rf) value is the ratio of distance travelled by the analyte to that of the solvent front on a chromatogram.

$$RF = \frac{\text{Distance moved by Analyte}}{\text{Distance moved by Solvent}}$$

Requirements of TLC

Stationary Phase: Silica Gel.

Mobile Phase: Acetic Acid: Water: Ethanol

Steps for TLC

Prepare the Development Container

Prepare the TLC Plate

Spot on the TLC Plate

Development of TLC Plate

Visualize the Spot

Fig 1: Requirements of TLC

UV- Visible Spectroscopy [13-15]

UV-Visible Spectroscopy (or Spectrophotometry) is a quantitative technique used to measure how much a chemical substance absorbs light. Our testing aims to determine the lambda max of Betanin and Beta-carotene using UV-visible spectroscopy. A simple, accurate and selective UV-spectrophotometric method was developed for the estimation of Betanin and Beta-carotene in capsule dosage form. The study was carried out by using methanol as a solvent. UV – Visible range for this test was used from 200nm to 800nm.

Requirements

Apparatus - Volumetric flask, Syringe filter.
Chemicals and materials - Beetroot Extract, Carrot Extract, Methanol.

Instruments – UV- Visible Spectrometer, Sonicator.
Instrumentation

The development of the method was performed on a UV Spectrophotometer, made by Shimadzu and model UV-1900. A lab solution was applied for data collection and processing.

Procedure:

For Beet root extract (Betanin)

Dissolved 75mg of Beetroot extract containing Betanin as a main component in 100 ml methanol in a 100 ml volumetric flask with sonication. Filter the solution through a 0.45 µ syringe filter. Scan the resulting solution in UV-visible spectrophotometry from 200nm to 800nm using methanol as a blank.

For Carrot root extract (Beta-Carotene)

Dissolved 75mg of Carrot root extract containing Beta-Carotene as a main component in 100 ml methanol in a 100 ml volumetric flask with sonication. Filter the solution through a 0.45 µ syringe filter. Scan the resulting solution in UV-visible spectrophotometry from 200nm to 800nm using methanol as a blank.

High-Performance Liquid Chromatography (HPLC) [13-15]

High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. HPLC is a type of liquid chromatography. It has high resolution and separating capacity. It is important for the determination of volatile and non-volatile compounds as well as qualitative and quantitative analysis. A Simple, accurate and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of Betanin and Beta-carotene in the capsule dosage form. Different mobile phase and stationary phase compositions were tried to optimise the chromatographic conditions.

Instrumentation

The method was developed on an HPLC system Alliance ® Waters model 2695 with the Empower 3 software version applied for data collection and processing. Detectors 2498 PDA detector. pH meter (lab India pico +), Analytical balance (Radwag) AS82/220.x2.

Chromatographic Condition

Column	:	Inertsil ODS-3V C18, 250 x 4.6mm, 5 µ
Column Temperature	:	30°C
Detector	:	535 nm for Betanin and 447nm for Beta-Carotene
Flow rate	:	1.0 mL/min
Injection Volume	:	20 µL
Run time	:	15 minutes
Diluent	:	Methanol

Procedure

Preparation of Mobile phase

Preparation of Buffer Solution

Weigh and transfer about 2.2 g of 1-Octane sulphonic acid sodium salt monohydrate and 0.3 g of Sodium acetate into 1000 mL of Milli-Q water, mix and adjust the pH to 3.8 with glacial acetic acid. Filter through 0.45 µ membrane filter.

Preparation of Mobile Phase

Prepare a degassed mixture of buffer solution and acetonitrile in the ratio of 70:30 v/v.

Preparation of Standard Solution

Weigh accurately about 75.20 mg of Betanin (Beetroot Extract) and 75.10 mg of Beta-Carotene (Carrot root Extract) and transfer them into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate to dissolve. Cool to room temperature and dilute to volume with diluent and mix well.

Preparation of Sample Solution:

Transfer the contents of NLT 10 capsules into a mortar and pestle and crush them into fine powder. Weigh and transfer a sample equivalent to 75 mg of Betanin into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate to dissolve. Cool to room temperature and dilute to volume with diluent and mix well. Filter the solution through a 0.45 µ syringe filter and inject it into the HPLC system.

Formulation of Capsules[16]

Formula for Plant Extract capsules

Extract 1:- 75mg
Extract 2:- 75mg
Microcrystalline cellulose (PH 102):- 100mg
Lactose Monohydrate: - 100mg
Sodium starch glycolate: - 20mg
Talc: - 3mg
Magnesium stearate: - 2mg

Use capsule size"0" having a capacity minimum of 300mg and a maximum of 430mg.

Add plant extract + microcrystalline cellulose + lactose monohydrates + sodium starch glycolate + talc, mix well and pass through sieve no.60#. Add magnesium stearate and mix for 5 minutes. Fill the powder in capsules size "0".

Results and Discussion

Thin Layer Chromatography (TLC)

The standard retardation values of phyto-components are specified by researchers in various books. Using that data the following results were obtained.

Betanin: Standard RF value for Betanin is from 0.5 to 0.7.

Total run – 5.5
Spot 1- 3.8
Spot 2 – 2.9
Rf value for (spot 1) = 3.8 / 5.5 = 0.7
Therefore, the RF value of spot 1 is 0.7.
Rf value for (spot 2) = 2.9 / 5.5 = 0.5
Therefore, the RF value of spot 2 is 0.5.
Hence, the Rf value of the Betanin component matches with the standard values i.e. 0.5

Beta-Carotene: Standard RF value for Carotene is from 0.7 to 0.9.

Total run – 6.7
Spot 1- 6.2
Rf value for (spot 1) = 6.2 / 6.7 = 0.92
Therefore, the RF value of spot 1 is 0.92.
Hence, the Rf value of Beta-Carotene matches with the standard values and it is 0.9.

UV- Visible Spectroscopy

The results obtained by UV Spectroscopy are given below

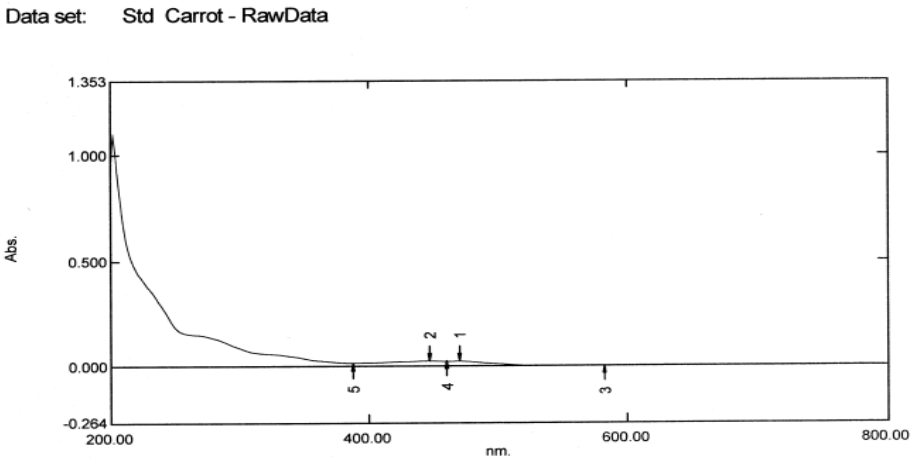

Fig 2: UV-Visible Spectrum for Beta-Carotene

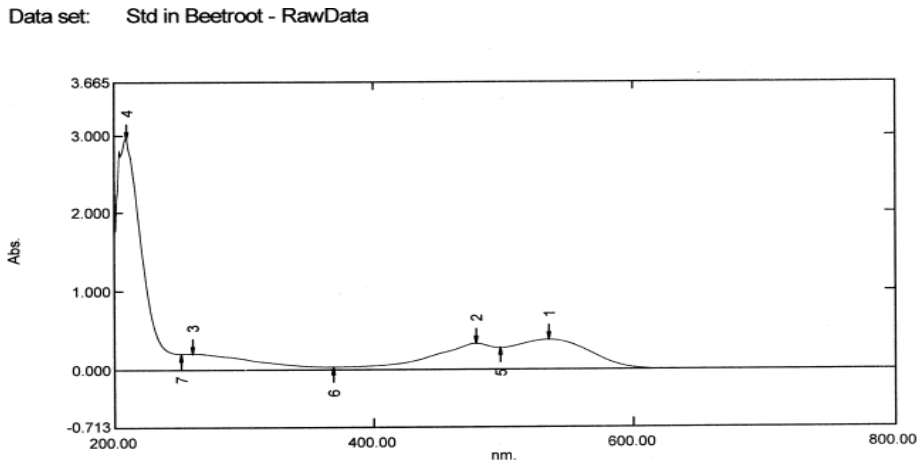

Fig 3: UV-Visible Spectrum for Betanin

Observation

The maximum absorbance for Betanin was found at 535 nm.

The maximum absorbance for Beta-Carotene was found at 447 nm.

High-Performance Liquid Chromatography

Typical chromatographs for Betanin and Beta-Carotene are as follows

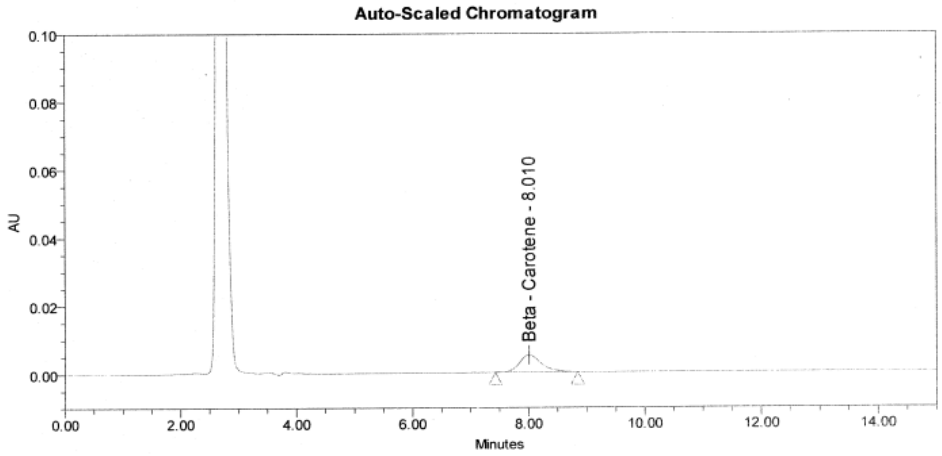

Fig 4: HPLC Chromatograph for Beta-Carotene

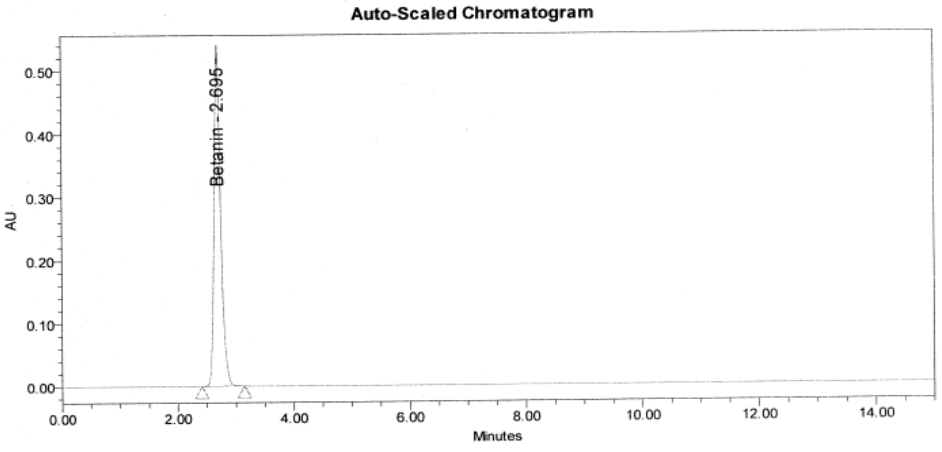

Fig 5: HPLC Chromatograph for Betanin

The formula for calculation of the percentage of content:

$$\%\text{Content Present in sample} = \frac{AT \times Wstd \times Dspl \times Avg\ Wt. \times Potency}{As \times Wspl \times Dstd \times L.C. \times 100} \times 100$$

Where,

AT = Peak Area of Sample
As = Average peak Area of Standard
Wstd = Weight of standard in mg
Wspl = Weight of sample in mg
Dstd = Dilution factor for standard
Dspl = Dilution factor for sample
Avg Wt = Average net content of the capsule(mg)
L.C. = Label claim(mg)

%Content of Betanin=

4098451 / 4182010 x 75.20/100 x 100/375 x 100/100 x 375/75 x 100 = **98.3%**

% Content of Beta Carotene =

131018 / 132041 x 75.10/100 x 100/375 x 100/100 x 375/75 x 100 = **99.4%**

Conclusion

The results obtained from the tests performed were accurate and qualified (matched) with the standard values found earlier by various researchers. Capsules produced were successfully stored and all the procedures were performed as per the instructions mentioned in the books. Through UV Spectroscopy and HPLC analysis, it is confirmed that the high recovery of Betanin and Beta Carotene from capsules and the methods are simple and can be used for routine analysis.

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