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Ultrasound assisted green extraction and characterization of Natural pigments from medicinal plants *Cucurbita maxima* and *Curcuma Longa*

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Abstract

Cucurbita maxima and Curcuma longa are reported to have important medicinal properties in traditional literature. Cucurbita maxima peel contains carotenes and Curcuma Longa rhizomes contain curcumin as natural pigments. Curcumin has numerous health benefits such as antioxidant, anti-inflammatory, anti-diabetic, immune boosting capacity, anti-cancer activities etc. Curcumin is a bioactive compound, and it has strong medicinal properties. Carotenes have different biological roles like strong antioxidant properties, helps in maintaining proper vision and good skin health, support in improving immune function, etc. Curcumin and carotenes are used as natural dye in fabrics and food materials. This study mainly focused on the extraction of natural pigments from peel of Cucurbita maxima and rhizome of Curcuma longa using an ultrasonic extraction technique. Cucurbita maxima peel contained significant amount of carotenoids. The principle-coloured component in dyestuff extracted from pumpkin peel is β-carotene. Extraction was done by using sunflower oil as green solvent. The extracted pigment was analysed by FTIR and UV Visible Spectroscopy.

Keywords: *Cucurbita maxima, Curcuma longa*, pigment, natural dye, carotenes.

Introduction

Medicinal plants contain various bioactive constituents like flavonoids, saponins, alkaloids, tannins, polyphenol etc. and these compounds are mainly useful as therapeutic agents[1].Traditional folk medicines are mainly dependent on medicinal plants and plant extracts[2,3]. *Curcuma longa* is a medicinal plant of ginger family. Rhizomes are most used part of the plant. Rhizomes contain a wide variety of bioactive compounds including different type of non-volatile curcuminoids. Curcumin was reported as the most active curcuminoids in *Curcuma longa*[4]. These active compounds present in volatile oils of the plant. Curcumin is a natural polyphenol compound. Naturally obtained polyphenol compound is known as the “wonder drug of life”[5]. *Curcuma longa*. has different beneficial pharmacological properties including anti-inflammatory, antidiabetic, anti-thrombotic, anti-diarrheal, diuretic, antirheumatic, antimicrobial, antioxidant, insecticidal, and antityrosinase effects[6]. Pumpkin has been considered as beneficial to health because it contains various biologically active components. Pumpkin fruits contain different important bioactive compounds, and they have various biological activities. The fruits of pumpkin are a good source of carotenoids.

Carotenes are generally a group of common and important plant pigments. Carotenes are mainly found in various plant parts like colourful fruits, vegetables, etc. β-carotene is the most common form of carotenoids. Caroteneshas various biological functions, and they have different key health benefit roles. β-carotene is considered as a pro vitamin A or precursor to vitamin A. Carotenoids produces vibrant orange or red colours in various plant parts. Carotenoids are frequently used as natural food colourants in various food products like confectionery, sauces, snacks, etc. Those are considered as safer food colourants and having different health benefit effect. Carotenoids are traditionally used as natural dye in textile fabrics. Plant extracts are used for various health benefit effects from historical age[7].

Pumpkin (*Cucurbita maxima*) is one type of annual herbaceous plant. Pumpkin contains different bioactive compounds[8]. Pumpkin peels contain various health benefit valuable nutrients. Various compounds are present in pumpkin peel which can act as natural colourants. Pigments of Pumpkin peel contain different types of carotenoids like α-carotene, xanthine, β-carotene, etc. β-caroteneproduces orange red colour in pumpkins[9]. Pigments isolated from pumpkin peel may be used as natural food colorants in wide varieties of food products. Pumpkin peel extracts are used in cosmetic products especially in skincare products.

Curcuma longa is a medicinal plant of ginger family. Rhizomes contain a wide variety of bioactive compounds including different type of non-volatile curcuminoids. Curcumin is the most active curcuminoids in *Curcuma longa*[10]. These active compounds present in volatile oils of the plant. Curcumin is one kind of natural polyphenol compound. Naturally obtained polyphenol compound is known as the “wonder drug of life”[5]. *Curcuma longa L.* has different beneficial pharmacological properties including anti-inflammatory, antidiabetic, anti-thrombotic, anti-diarrheal, diuretic, antirheumatic, antimicrobial, antioxidant, and insecticidaleffects[6].

Food industries generate huge amount of waste materials. Plant based natural dyes require large amounts of raw materials which may ultimately cause depletion of natural resources. This problem can be solved to some extent by utilizing the waste materials from agricultural and food wastes. The proper management and utilization of bio-wastes is very much important. By-products produced in the food processing industries may be considered as valuable sources of natural dyes[11].Various techniques are used and investigated for the extraction of natural dyes from plant-based sources. Waste pumpkin peel may be rich source of sustainable natural dye.

Curcumin is the main pigment of turmeric[12]. Curcumin is mainly oil soluble pigment. It is the main active compound of turmeric. Curcumin has strong anti-inflammatory effects. It can inhibit various biomolecules which main role in inflammation. Curcumin has the ability to suppress pain and swelling in arthritis, inflammation in the digestive tract[15]. Curcumin is one kind of polyphenolic compound isolated from *Curcuma longa* rhizomes. Demethoxycurcumin, cyclocurcumin, and bisdemethoxycurcuminare identified in Turmeric[13]. Curcumin isused as therapeutic agents in various types of illnesses[15]. Curcumin can be used as anti-proliferative, anti-angiogenic agent, and chemopreventive agent. Curcumin has curative effect in cardiovascular diseases, parkinson's disease, atherosclerosis, colon cancer, inflammatory bowel disease, etc[16,17]. The present study was designed to extract the natural pigments from peel of fruit of pumpkin (*Cucurbita maxima*) and turmeric *(Curcuma Longa)* rhizome through ultrasound assisted green extraction using sunflower oil as a green solvent.

Materials and Methods

Raw Materials and reagents: Pumpkin (*Cucurbita maxima*) and turmeric (*Curcuma Longa*) were collected from the local village area of Nalbari district, Assam, located in between 26°N and 27°N latitude and 91°E and 91°47′ E longitude. Good quality sunflower oil was collected from the local market of Guwahati, Assam, India.

Preparation of samples of Plant materials: The fresh ripe fruit of pumpkin (*Cucurbita maxima*) peel and turmeric (*Curcuma Longa*) rhizome were collected. The sample was washed thoroughly with clean water for removing all unwanted contaminants from the surfaces of plant materials. The peels of pumpkin were separated from the fruits and those peels were utilized as raw material for the investigation. The raw materials were processed as small pieces and thoroughly dried in open air under shade at ambient temperature. The raw material was powdered and stored in closed airtight sample bottle until further investigation.

Ultrasound-Assisted Extraction (UAE): Dry powdered pumpkin peels and rhizome of turmeric were used as samples. In a 250-ml beaker, 12g dry powdered samples were transferred, and 120 mL sunflower oil was added at material to liquor ratio 1:10. The sonication was done at temperature 40°C inside the sonicator. In the ultrasound assisted extraction, the sonication periods were 90 minutes. The crude extract of natural dye was then filtered. After the extraction the mixture was centrifuged for 3500 rpm for 30 mins to separate the pigment and the oil sample.

Preparation of test solution for the analysis in UV Visible Spectroscopy: 1 mg of the extracted pigments were weighed and transferred into 100 mL volumetric flask. Methanol was used as a solvent for analysis. Methanol was added up to the mark and clear solution was prepared and then the resulting solutions were used for UV Visible spectral analysis.

Analysis in UV-Vis spectrophotometer: Extracted natural dye solution was scanned in UV spectrophotometer in the range of 200-800nm. Methanol was used as a blank in this analysis. Wavelength corresponding to maximum absorbance (λ_{max}) for each sample was recorded.

Identification of functional groups through FT-IR spectroscopy: The functional groups of the extracted pigments were identified and characterized by using FT-IR spectral analysis. The scan range in the FT-IR was within 500 to 4000 cm^{-1}.

Preliminary phytochemical screening: A qualitative analysis was done to identify the phytochemical constituents, or secondary metabolites present in dye extracts by the preliminary phytochemical screening. Preliminary phytochemical screenings of plant extracts of peel of *Cucurbita maxima* and rhizome of *Curcuma Longa* were done using standard chemical tests.

Results and Discussion

Result of phytochemical screening: The result of phytochemical tests is presented in the Table-1.

Table-1: Result of phytochemical tests.

Name of the Phytochemicals	Extracted dye from *Curcuma Longa* rhizome	Extracted dye from *Cucurbita maxima* peel
Phenols	+	+
Phytosterols	-	-
Terpenoids	+	+
Glycosides	-	-
Saponins	-	-

Flavonoids	+	+
Tannins	+	-
Alkaloids	+	+
Quinones	-	-

FT-IR spectral analysis: FT-IR spectral analysis is very much helpful for the identification and characterization of various functional groups present in the compounds. Different functional groups such as carboxylic acid, ester compounds, amines, aromatic compounds, hydrocarbons, aryl disulfides, ether groups, amide compounds, etc. can be characterized by their respective absorption bands in IR spectroscopy. In the present investigation, the natural dyestuffs extracted from pumpkin peel and turmeric was subjected to FT-IR analysis. The results of FT-IR analysis were represented in Table-5, 6 and 7. The detected peaks, corresponding bond stretching or bending, corresponding functional groups for the peaks are summarized in the tables.

A peak was observed at 1001 cm^{-1}, which is corresponding to the C-H bending of the aromatic C-H bond. FT-IR analysis of natural dye isolated from *Curcuma longa* rhizome indicated the presence of 2921cm^{-1} (C-H stretching), 3264 cm^{-1} (aliphatic O-H stretching), 1634 cm^{-1} (ketone C=O stretching), 1505 cm^{-1} (C=C stretching) and 1281 cm^{-1} (O-C stretching) as represented in Table-2.

FTIR spectra of extracted pigment from turmeric (*Curcuma Longa*) rhizome showed similarity in band stretching pattern of pure standard curcumin. It indicated that extracted pigment is mainly curcumin or curcumin derivative.

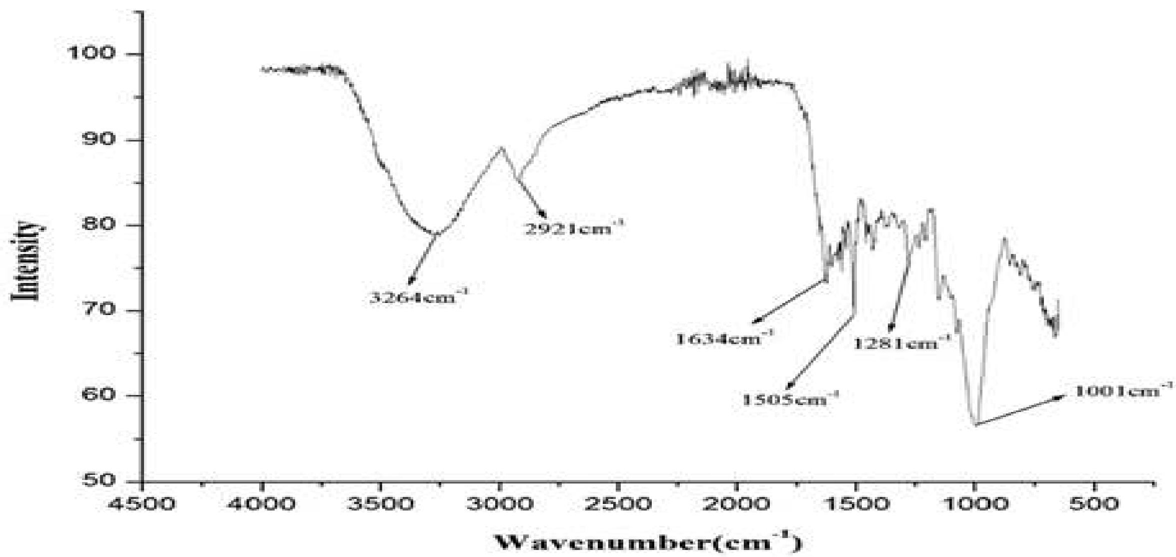

Figure-1: FT-IR spectrum representing potential bands in the pigment extracted from *Curcuma Longa* rhizome.

Table-2: FT-IR peak values and functional groups of pigment extracted from *Curcuma longa* rhizome.

Wave number in cm^{-1}	Bond stretching or bending	Corresponding functionality
2921 cm^{-1}	C-H stretching	aromatic C-H
1001 cm^{-1}	C-H bending	aromatic C-H
3264 cm^{-1}	O-H stretching	O-H stretching
1634 cm^{-1}	C=O stretching	ketone C=O stretching for conjugated enone
1505 cm^{-1}	C=C stretching	C=C stretching for conjugated enone and aromatic rings
1281 cm^{-1}	O-C stretching	O-C stretching of ester linkage

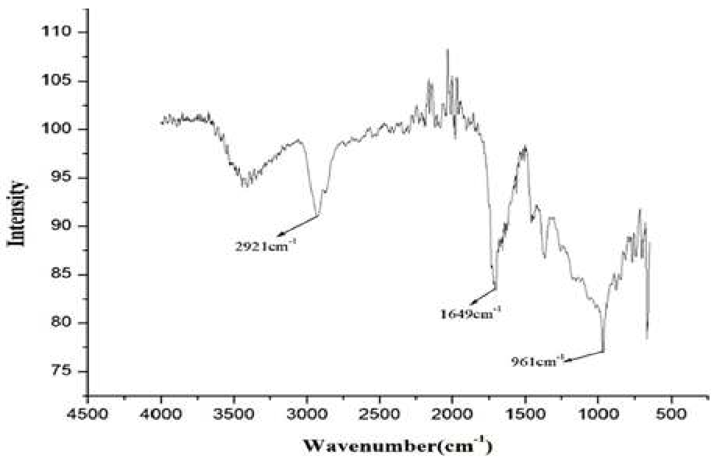

Figure-2: FT-IR spectra representing potential bands in the pigment extracted from *Cucurbita maxima* peel.

Table-3: FT-IR peak values and functional groups of pigment extracted from *Cucurbita maxima* peel.

Wave number in cm^{-1}	Bond stretching or bending	Corresponding functionality
2921cm^{-1}	C-H stretching	aromatic C-H
961 cm^{-1}	C-H bending	aromatic C-H
1649 cm^{-1}	C=C stretching	C=C stretching of the conjugated double bond.

A peak was observed at 1649 cm^{-1} which is corresponding to the C=C stretching of the conjugated double bond. FT-IR analysis of natural dye isolated from pumpkin peel indicated the presence of 2921cm^{-1} (C-H stretching), 961 cm^{-1} (C-H bending) and 1649 cm^{-1} (C=C stretching) as represented in Table-3.

Thus, IR data of the sample were compared with the IR signals of β carotene standard. The IR spectrum of the natural dye extract exhibited transmission peaks at 2921, 961 and 1649 cm^{-1} in agreement with IR peaks exhibited by the pure β carotene standard. Peaks from IR spectra has indicated that the extracted dye stuff from pumpkin peel is carotenoid and mainly it is β-carotene in a trans configuration.

UV-Vis spectrophotometric analysis: Extracted natural dye solutions were scanned in UV spectrophotometer. UV Visible spectra of the extracted natural dye was recorded in methanol as shown in Figure-3 and 4. Natural dye extracted from *Curcuma Longa* rhizome in methanolic solution showed a broad characteristic UV-visible absorption within a range of 300-500 nm. Wavelength corresponding to maximum absorption band of pigments extracted from turmeric in methanol was observed at wavelength around 424 nm as shown in Figure-3. This λ_{max} value is also characteristic value of standard pure curcumin. UV Visible spectra of natural dye extracted from pumpkin peel showed the maximum absorption at around 445 nm as shown in Figure-4. It is observed that the characteristic maximum absorbance of the standard β carotene solution at around 450 nm. This result indicated that extracted natural dye from *Cucurbita maxima* peel contained significant amount of β carotene. The research work revealed that the peel of Pumpkin is a rich and profitable source of β carotene.

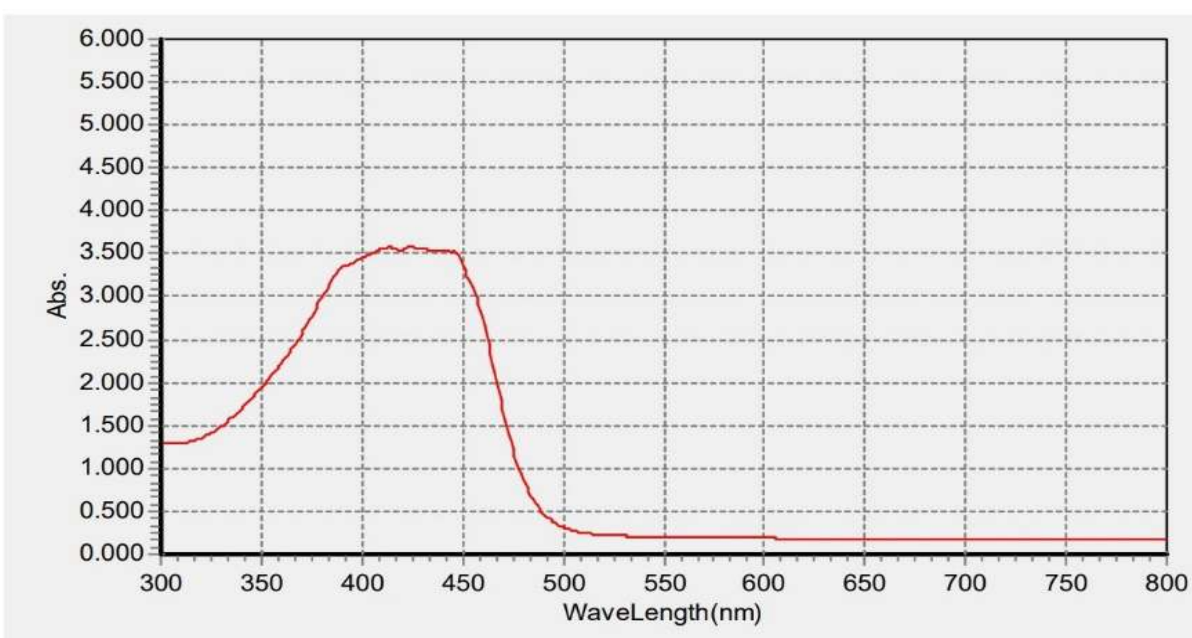

Figure-3: UV -Vis spectra of pigment extracted from *Curcuma Longa* rhizome.

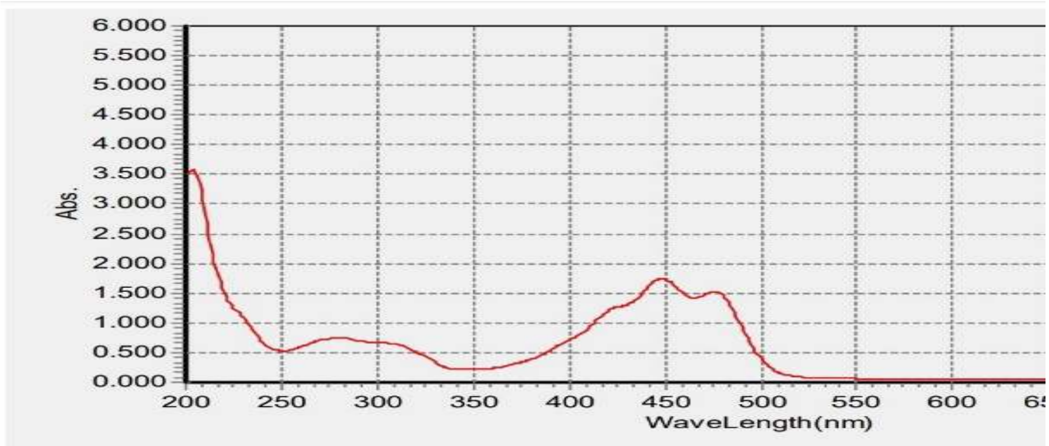

Figure-4: UV-Visspectra of pigment extracted from *Cucurbita maxima*peel.

Discussion: Bioactive compounds present in the medicinal plants have numerous health benefit effects[18]. Pumpkin by-products are considered as valuable source of different health-promoting compounds, including zeaxanthin, antioxidants, Lutein, dietary fibres, polyphenols, carotenoids, etc. Various parts of pumpkin plants including pulp, seeds, roots, flowers etc are utilized in common diets throughout the world and in pharmaceutical; food and cosmetics industries[19]. Pigment can be extracted from pumpkin peel more effectively by using sunflower oil than organic solvents. Sunflower oil belongs to bio-based solvent category. It is considered as a green solvent. It is biodegradable, renewable, non-volatile and non-toxic. Sunflower oil is not evaporated easily due to their non-volatile nature. It is less harmful and safe to the surrounding environment and biodegradable. Carotenoids are lipophilic compounds.

Systematic studies of medicinal plants are essential to explore their beneficial effect[20]. Curcumin extracted from turmeric is effectively used as a natural dye in food as well as textile industries. It produces bright yellow colour in food materials. Curcumin can prevent the bacterial growth on fabric due to their natural antimicrobial properties. Curcumin is a natural food colorant. It imparts yellow hue in various food items. Curcumin is a popular natural food colorant. Curcumin has different important health benefits. Curcumin has certain limitation such as low water solubility; degradation in the exposure of light, etc. The yield of pigments is influenced by the amplitude of the applied ultrasound radiation, time of extraction, and material to solvent ratio. With reducing ultrasound amplitude level for the same extraction period, the yield of natural dye decreased. Natural dye isolated from turmeric powder are used to dye fabrics in different dyeing conditions. Carotenoids are natural pigments, and it can produce various shades like red, yellow, orange red, etc.

Colour is an important component of food products. Consumers generally prefer a food colour having proper safety. Many synthetic food colours are reported to have toxic and carcinogenic effects[21]. The usage of synthetic dyes causes health hazards to human. Majority of food colourants used in food items are synthetic and those are derived through synthetic processes. Although these synthetic dyes possess better stability, more verities of hue, their regular consumption can cause different adverse and serious health effects[22]. Nowadays the important of natural dye are gradually increasing in food industries. The increasing demand can be fulfilled by proper research on naturally derived dyes and pigments so that new effective natural dyes can be explored. The most common types of plant-based pigments are carotenoids, chlorophylls, anthocyanins and betalains. Both carotenoids and curcumin are very much important plant-based pigments which have lots of health benefit effects. Carotenoids have different biological roles such as cancer prevention, skin protection, immune boosting, maintaining normal eye health, etc. Carotenoids can protect the retina of eyes from oxidative damage. Curcumins are found to have potent anti-inflammatory activities. Curcumin can improve digestive health by reducing the unwanted inflammation in the gut[23,24].

Conclusion

The effectiveness of sunflower oil as a solvent for the extraction of pigments from the *Cucurbitamaxima* (pumpkin) peel and *Curcuma Longa* (turmeric) rhizome was investigated in the study. *Cucurbita maxima* peel was found to be a suitable raw material to isolate carotenoid based natural dye. FTIR spectra of extracted natural dyes from pumpkin peel showed similarities in band stretching pattern with standard β-carotene and the naturel dyes extracted from turmeric showed similarities with standard curcumin. Sunflower oil is used as a green solvent for the extraction of natural dyes from pumpkin peel and turmeric. Sunflower oil is found to be a suitable and effective solvent for β-carotene and turmeric extraction. There is a scope of increasing research on sunflower oil to be developed as a better green solvent which can effectively replace other hazardous solvents in industrial processes. Further research works are essential to optimize the isolation methods of natural dyes by testing extractions under different conditions and changing the parameters of extractions.

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