The Biochromatics of Plants: Extraction, Characterization, and Application of Pigments from Various Plants

Mrunali Patel[1], Sneeha Veerakumar[2], Tejesh Reddy Chirathanamettu[3], P. Mala[4], Jignesh Mevada[5]

[1]*Department of Biotechnology, U.V. Patel College of Engineering, Ganpat University, Mehsana, Gujarat, India*
[2]*School of Sciences, P P Savani University, Kosamba, Surat, Gujarat, India*
[3]*Department of Biotechnology, U.V. Patel College of Engineering, Ganpat University, Mehsana, Gujarat, India*
[4]*Department of Biotechnology, Periyar Maniammai Institute of Science and Technology, Vallam, Thanjavur*
[5]*Department of Mechatronics engineering, U.V. Patel College of Engineering, Ganpat University, Mehsana, Gujarat, India*

Corresponding Author
Jignesh Mevada: Department of Mechatronics engineering, U.V. Patel College of Engineering, Ganpat University, Mehsana, Gujarat, India
Email: Jignesh.mevada@ganpatuniversity.ac.in

KEYWORDS

Natural colour, plant pigment, Phytoconstituents, Stability testing, Antibacterial and Antioxidant activity

ABSTRACT

Pigments are chemical compounds that absorb light in the wavelength range of the visible region. Synthetic pigments in effluents pose a serious environmental threat due to their non-biodegradable nature, prompting a shift towards exploring eco-friendly alternatives. The focus has shifted towards the diverse array of natural pigments for applications in food, pharmaceuticals, and textiles, prioritizing human health preservation. The stability of pigments is influenced by various factors such as pH, light exposure, temperature, and complexation with other substances. This study aimed to develop a stable, eco-friendly biocolour using extracts from *Rubus fruticosus* L., *Bixa orellana* L., *Adhatoda vasica* L., and *Beta vulgaris* L. Phytochemical analysis revealed the presence of key constituents like phenols, flavonoids, alkaloids, and tannins. Purification through flash column chromatography and thin-layer chromatography determined pigment separation, with UV spectrophotometry used for quantification based on Lambert beer law. HPTLC fingerprinting identified carotenoids, anthocyanins, betalains, and chlorophylls. FTIR analysis determined functional groups, while LCMS provided information on molecular aspects. Encapsulation, co-pigmentation, and entrapment methods were employed to enhance stability. Antioxidant and antibacterial activities were assessed, and heavy metal content determined using atomic absorption spectroscopy. Extracted pigments found applications in food, textiles, tablet coating, cosmetics, and candles. The methanol extract of *Bixa orellana* L. demonstrated superior stability across various applications. This study advocates for the promising use of natural pigments, promoting consumer health and facilitating the production of eco-friendly biocolours devoid of synthetic additives.

INTRODUCTION

Plant pigments are exclusive compounds found in plants absorbing specific light wavelengths, play crucial roles in regulating plant processes like photosynthesis, growth, and development. These pigments, categorized as biochromes, contribute to plant coloration and have potential therapeutic applications in diseases. Natural pigments, derived from plant, fruit, or vegetable sources, are generally considered safe for human consumption. They offer vibrant colors without synthetic chemicals, aligning with consumer preferences for clean and natural products. Besides being safe, natural pigments also provide health benefits, exhibiting antioxidant, anti-inflammatory, and immune-regulatory properties. Plant pigments, including chlorophylls, anthocyanins, carotenoids, and betalains, contribute to the array of colors observed in plants.(Gahlawat, 2019)

Synthetic pigments, widely used in various industries for their stability and vibrant colors, raise environmental and health concerns. Some contain heavy metals like lead, mercury, and chromium, posing potential toxicity risks. The persistence of synthetic pigments in the environment contributes to long-term pollution in water bodies and soil. The environmental impact and potential health hazards associated with synthetic pigments emphasize the need for eco-friendly and non-toxic alternatives.(Rodríguez-Mena *et al*., 2023)

Encapsulation technology and copigmentation are methods employed to enhance the stability of natural pigments. Encapsulation involves protecting pigment molecules with a shell to prevent degradation from light, heat, and oxygen. Copigmentation materials, such as polymers and phenolic mixtures, form complexes with natural pigments, improving stability and color retention.(Chung *et al*., 2017)

Entrapment of natural pigments creates a protective environment, shielding them from environmental factors like light, heat, oxygen, and moisture. This prevents color fading and chemical breakdown, ensuring the stability of natural pigments.(Salimeh *et al*., 2019)

The exploration of plant pigments and the development of methods to enhance their stability provide valuable insights into plant biology, support sustainable practices, and cater to consumer preferences for natural and safe products.(Mohammad Azmin *et al*., 2022) The ongoing shift towards natural pigments reflects a broader trend towards eco-friendly and health-conscious choices in various industries.(Fernández-López *et al*., 2020)

MATERIAL AND METHODS

Collection of selected plants

Various parts of the plant were chosen for research analysis, including leaves, seeds, and roots from four selected plants. The first plant, '*Rubus fruticosus* L.' was collected from the Bavla Taluka of Ahmedabad district on January 24, 2022. The second plant, named '*Bixa orellana* L.', was collected from the Serenity botanical garden of Ahmedabad District on January 16, 2022. The third plant, named '*Adhatoda vasica* L.', was collected from the Sanand Taluka of Ahmedabad District on January 8, 2022. The fourth plant named '*Beta vulgaris* L.' was collected from the Serenitybotanical garden of Ahmedabad District on January 16, 2022. Authentication is vital for establishing the botanical identity of a plant species prior to its collection from the wild. 'Dr. Hitesh Solanki' from Gujarat University authenticated the collected plants.

Processing of plant material

Washing is crucial for removing undesired particles from plant materials. Collected specimens underwent soaking in tap water, and any foreign material was removed. Healthy leaves, seeds, and roots were rinsed 2-3 times, sometimes under cold tap water. Excess water was eliminated before drying. Drying, essential for further processing, occurred by spreading materials on cotton cloth, minimizing 'photooxidative changes.' Drying duration ranged from 7-15 days.(Salminen, 2003) For grinding and storage, plant powders were ground for homogeneity, stored at a lower temperature for future studies. Drying impacts sensory quality, bioactive component stability, and activity.(Alsaud & Farid, 2020)

Figure 1: Processing of plant material

Physicochemical properties of plant powder

Physicochemical properties specified by "The Unani Pharmacopoeia of India" were investigated, including moisture content, odor, taste, colour, water-soluble ash value, acid insoluble ash value, total ash value, and extraction yield.(Forage *et al*., 2009) Foreign matter was assessed by isolating and weighing particles. Moisture determination involved drying plant material for 5 hours at 105°C. Ash values were determined post-moisture assessment. Extractive values were obtained by macerating powdered plant material in various solvents, filtering, and calculating % w/w of extractive value.(Mushtaq *et al*., 2014)

Extraction by maceration method

Plant materials (coarse or powdered) were soaked in a stoppered bottle with a solvent throughout the maceration process, which required constant stirring for a minimum of six days. (Odey M O *et al*., 2012)

Figure 2: Extraction process by maceration method

Thermo-labile components were extracted from 30 gram of plant powder using Methanol, Acetone, Chloroform, Petroleum ether, Dichloromethane, Ethyl acetate, Ethanol, Aqueous methanol, Aqueous ethanol, and Aqueous acetone (1:10 W/V). After six days, filtrates were obtained using Whatman No. 1 filter paper, followed by solvent evaporation and water bath concentration to dryness. The resulting extracts were stored at 4°C for further analysis. The % extractive yield was computed using the formula shown below,

$$(\%)\ \text{Extractive yield (w/w)} = \frac{(\text{weight of dried extract})}{(\text{weight of dried plant material})} \times 100$$

Phytochemical screening of plant extracts

For alkaloid testing, a 0.5g extract was mixed with 1% aqueous HCl, heated, and filtered. Wagner's Test revealed a positive result with a reddish-brown precipitate. Hager's Test confirmed alkaloids with a yellow precipitate. Molisch's and Fehling's tests detected carbohydrates and sugars, respectively. Total phenol was verified by a dark green color, tannin by a bulky white precipitate, flavonoid by yellow fluorescence, saponin by foam development, fixed oil by an oil stain, protein by blue color with ninhydrin, and phytosterol by a distinctive color change.(Poongothai, 2019 , Doss *et al*., 2009)

Purification of pigment by flash column chromatography

Crude plant extracts were purified via flash column chromatography using a Sabarscientific column. The 20 mm × 24 mm column, 450 mm in length, employed compressed air through a Filter-Regulator (FR) unit, regulated to 1.2 bar. A flow control valve managed air velocity, and colored fractions were collected for further analysis.(Oliveira-júnior *et al.*, 2020)

Separation of pigment by thin layer chromatography

Purified pigments underwent Thin Layer Chromatography (TLC) using silica-coated aluminum sheets. The TLC plates were activated in a preheated oven and employed specific solvent systems for each plant species: methanol: water (90:10) for *Beta vulgaris* L., hexane: acetone: trichloromethane (60:20:20) for *Bixa orellana* L. and *Adhatoda vasica* L., and chloroform: methanol: formic acid (85:10:5) for *Rubus fruticosus* L. The separation was visualized by capillary action, and Rf values were determined for comparison with standard pigment values.(Quach *et al*., 2004)

Characterization of plant pigments

UV visible spectrometry

Purified pigments were analyzed using a Cystronic double-beam spectrophotometer in the 200-800 nm range. After mixing with an organic solvent, samples were filtered or centrifuged. Absorbance at the absorption maxima was measured, and the Lambert-Beer law equation used absorbance values to quantify pigment concentration. Methanol served as a blank. The total concentration of pigment was calculated by using the formula below:
Concentration= $(A \times MW \times DF \times 1000)/(\epsilon \times L)$

HPTLC fingerprinting

Following Wagner et al.'s (1996) protocol, 25mg of plant pigments were dissolved in 0.5ml methanol and centrifuged. Test solutions were injected onto silica gel TLC plates using CAMAG Linomat 5. Plates were developed using specific mobile phases, dried, and images were captured under visible, UV 254 nm, and UV 366 nm. WinCATS software, version 1.3.4, facilitated peak analysis.(Srivastava *et al*., 2021)

Fourier transform infrared spectroscopy

FTIR, using a Bruker alpha model, is a potent tool for identifying chemical bonds in plant pigments. Powdered plant samples, made into KBr pellets or thin films, were analyzed under specific conditions. A diamond ATR top plate facilitated analysis of 2 µl plant pigments. Spectra, obtained with 32 scans and 64 repetitions, identified functional groups, with triplicate analyses and plain KBr pellets as blanks.(Westfall *et al*., 2020)

Liquid chromatography mass spectrometry

LCMS separated the sample via liquid chromatography, converting it into ions in an ion source. The mass analyzer sorted ions by mass-to-charge ratio, creating a mass spectrum. Detected compounds revealed elemental isotopes and aided in elucidating chemical structures, potentially explaining pigment stability.(García-de Blas *et al*., 2011)

To enhance pigment stability

Various approaches were examined to improve the stability of pigments, including encapsulation with maltodextrin and gum arabic solution and copigmentation using ascorbic acid and citric acid. Physicochemical factors such as pH, light exposure, temperature, and complexation with other compounds can influence pigment stability, impacting product quality and consumer acceptance.(Ngamwonglumlert *et al*., 2017)

For encapsulation, a 30% maltodextrin solution and a 30% gum arabic solution were prepared separately. A 1:1 ratio of maltodextrin to gum arabic solution was also created. Microencapsulation involved emulsifying purified pigments in the maltodextrin: gum arabic solution using an ultrasonicator, followed by drying. Various parameters, such as moisture content, bulk density, hygroscopicity, solubility, redispersion time, and microencapsulation yield, were analyzed to assess pigment activity.(Kang *et al*., 2019)

Additionally, copigmentation was achieved by adding 2% ascorbic acid and citric acid to pigment samples, subjecting them to heat treatment, and assessing color stability.(Dimitrić Marković *et al*., 2005) The ionic gelation method with sodium alginate was employed to entrap pigments. The beads' size, moisture content, color stability, and entrapment efficiency were evaluated over time. These techniques provide insights into strategies for maintaining and enhancing the stability of pigments in various applications.(Tavlasoglu *et al*., 2022)

Antibacterial and Antioxidant activity of pigments

The antibacterial activity was examined through the agar well diffusion method, involving triplicate experiments for each treatment. Plant pigments were allowed to diffuse into agar plates seeded with test organisms, including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*. After 24 hours of incubation, the zone of

inhibition was measured, with final pigment concentrations ranging from 2 to 10 mg/ml. Negative controls involved plates with the DMSO reagent, while positive controls utilized chloramphenicol antibiotic solution.(Zia *et al.,* 2021)

For antioxidant activity assessment, the DPPH free radical scavenging assay was employed. Pigment concentrations of 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL were prepared, and a DPPH solution was created at a concentration of 0.001% in ethanol. Ascorbic acid served as the positive control. The resulting mixtures were incubated, and absorbance was measured at 517 nm. The DPPH scavenging effect was calculated using a formula, and results were reported as IC50 values and DPPH scavenging activity percentage.(Çekiç & Özgen, 2010)

Applications of plant pigments

Plant pigments used in diverse applications, including textiles, tablet coatings, food products, lip balm, and candles. In textile dyeing, plant pigments extracted in methanol were used to color fibers and fabrics. Fastness properties such as washing, rubbing, and light fastness were assessed to gauge color stability.(Brudzyńska *et al*., 2021) Tablet coating in pharmaceuticals involved the development of a 3D-printed machine for coating tablets with plant pigment solutions. The process included spraying the coating solution onto tablets in a rotating drum, followed by air drying for color stability.(Luzardo-Ocampo *et al*., 2021)

In the food industry, plant pigments were incorporated into ice cream formulations for color enhancement, differentiation, and flavor identification. Organoleptic testing, including color and appearance evaluations, was conducted on ice cream products. Lip balm formulation included the addition of plant pigments to create a visually appealing product. Various tests, including color stability, melting point determination, spreadability, and skin irritation, were performed to assess the lip balm's quality and suitability for safe application.(Kokil *et al*., 2015)

Plant pigments were integrated into candle-making by adding them to melted wax, enhancing the aesthetic appeal of candles. Quality assessments involved preference testing, burn time determination, and appearance evaluations, ensuring the candles met consumer expectations.

RESULTS AND DISCUSSION

The research focuses on four selected plants—*Rubus fruticosus* L., *Bixa orellana* L., *Adhatoda vasica* L., and *Beta vulgaris* L.—gathered from the Ahmedabad region of Gujarat in 2022. The botanical authentication was performed by Dr. Hitesh Solanki, a professor at the Department of Botany, Bioinformatics, and Climate Change Impact Management, University School of Sciences, Gujarat University.

The plant material endured treatment to create a dried, homogeneous plant powder devoid of extraneous particles. This involved washing, drying, and grinding, with the resulting powders stored at lower temperatures for future use.

The physicochemical properties of the plant powders were analyzed, covering parameters such as odor, taste, color, moisture content, total ash value, acid insoluble ash value, water-soluble ash value, and extraction yield. The moisture content of all four plants was below 6%, as determined by the loss-on-drying method. Total ash values for three plants—*Rubus fruticosus* L., *Bixa orellana* L., and *Beta vulgaris* L.—were below 10%, while *Adhatoda vasica*

L. exceeded this threshold with 15.05 ± 0.6%. Acid insoluble ash and water-soluble ash values varied across the plants.

The physical characteristics of plant extracts were examined using different solvent systems, and the extraction yield of each extract was determined. The extracts exhibited diverse colors, with varying extraction yields. For example, the extraction yield for *Bixa orellana* L. was highest in methanol (27.80 ± 0.3%) and lowest in dichloromethane (13.47 ± 0.2%).

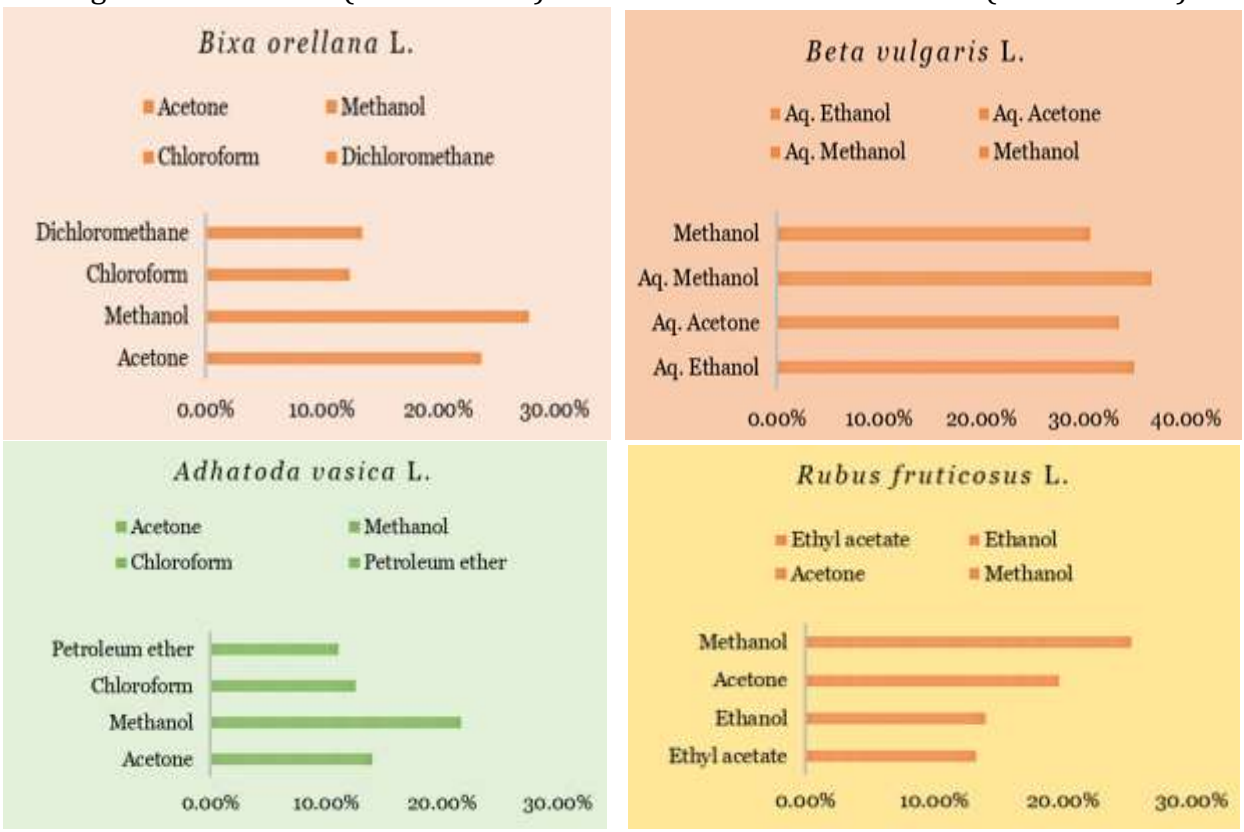


Figure 3: Extraction yield of plant extract

The phytochemical screening of the plant extracts involved the analysis of compounds such as alkaloids, flavonoids, terpenoids, tannins, saponins, and other secondary metabolites.

For *Bixa orellana* L. extracts, alkaloids were present in varying amounts, total phenol concentrations were moderate, and tannins were observed. Flavonoids were more concentrated in methanol and acetone extracts. Saponins were present at low to moderate levels in all extracts.

Rubus fruticosus L. extracts showed the presence of alkaloids, total phenols, tannins, and flavonoids. Methanol extracts exhibited a solid positive result for alkaloids and total phenols. Ethanol and ethyl acetate extracts showed positive results for alkaloids and tannins.

Beta vulgaris L. extracts had detectable alkaloids, higher concentrations of total phenols in aqueous ethanol, and moderate levels of tannins. Flavonoids were more concentrated in aqueous ethanol and aqueous methanol extracts.

Adhatoda vasica L. extracts displayed alkaloids, total phenols, tannins, carbohydrates, saponins, and fixed oil. Flavonoids were detected in acetone and methanol extracts.

Extraction yields varied based on the solvent and plant material. For *Bixa orellana* L., the methanol extract yielded the highest (27.80 ± 0.3%), while dichloromethane yielded the

lowest (13.47 ± 0.2%). *Beta vulgaris* L. had the highest extraction yield in aqueous ethanol (35.02 ± 0.2%) and the lowest in methanol (30.58 ± 0.2%). *Adhatoda vasica* L. showed the highest extraction yield in methanol (21.65 ± 0.3%) and the lowest in petroleum ether (11.09 ± 0.4%). *Rubus fruticosus* L. had the highest yield in methanol (25.33 ± 0.6%) and the lowest in ethyl acetate (13.20 ± 0.3%).

Purification and Separation of pigments

The study delves into the purification and separation of pigments obtained from four different plants—*Adhatoda vasica* L., *Bixa orellana* L., *Beta vulgaris* L., and *Rubus fruticosus* L. The purification process utilized flash column chromatography, employing a hexane:acetone (2:1) solvent system for *Adhatoda vasica* L. The eluted fractions were collected for further analysis, revealing that acetone, chloroform, and methanol extracts underwent effective purification, while the petroleum ether extract exhibited comparatively lower purification efficiency.

Similarly, flash column chromatography was applied to *Bixa orellana* L., where chloroform, acetone, and methanol extracts demonstrated favorable purification results. However, dichloromethane extract showed impurities, indicating the presence of substances not part of the desired compounds.

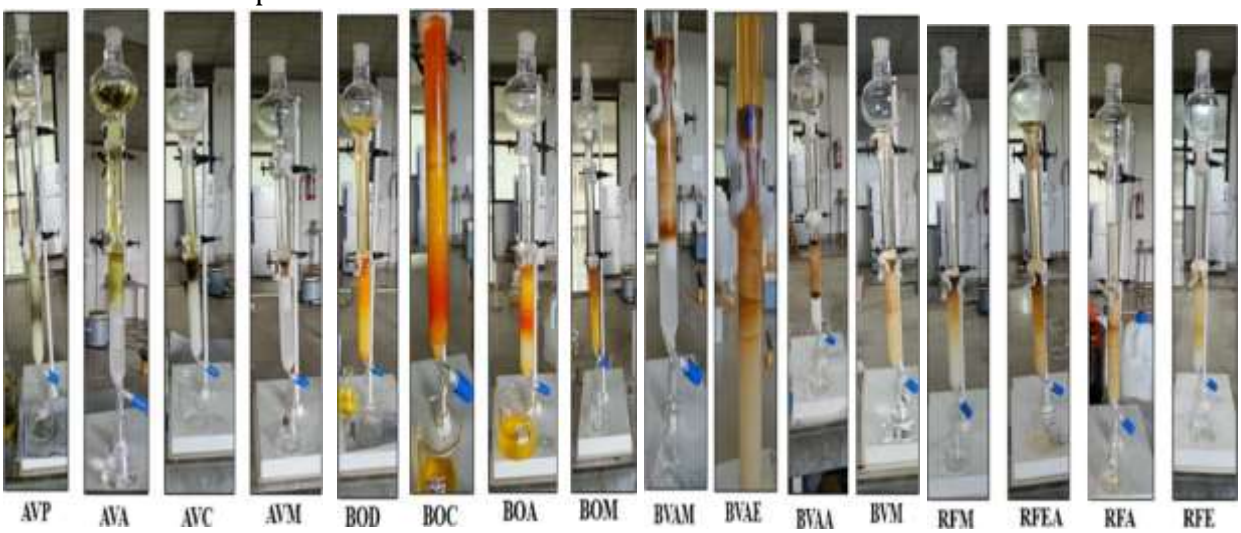


Figure 4: Purification of crude extract

For *Beta vulgaris* L., aqueous methanol, aqueous ethanol, and aqueous acetone extracts exhibited superior purification compared to pure methanol extract. The use of methanol and water as the solvent system proved effective in removing impurities and isolating desired compounds.

Rubus fruticosus L. purification results varied, with ethanol, acetone, and methanol extracts showing a high degree of efficiency, while ethyl acetate exhibited lower purification levels. The outcomes underscore the importance of optimizing parameters for effective purification.

After purification, the pigmented extracts were subjected to thin-layer chromatography (TLC) for separation analysis. Sharp, well-defined spots on the TLC plate suggested purity, while additional spots or smearing indicated impurities or degradation.

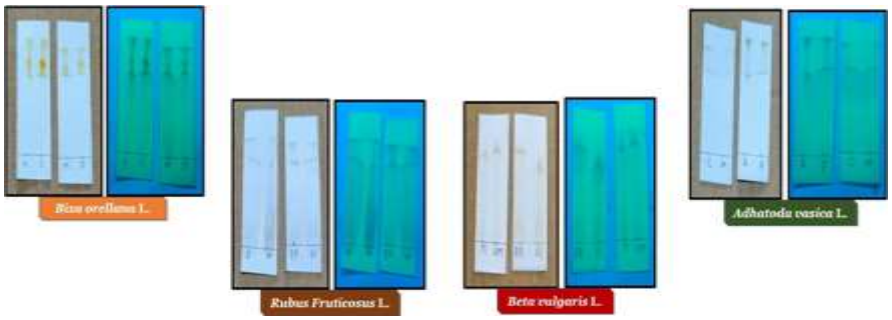


Figure 5: TLC developed plate

The R*f* values ranged from 0.55 to 0.80 for carotenoid pigments in *Bixa orellana* L., 0.62 to 0.80 for anthocyanin pigments in *Rubus fruticosus* L., 0.55 to 0.69 for betalain pigments in Beta vulgaris L., and 0.61 to 0.89 for chlorophyll pigments in *Adhatoda vasica* L. These results offer valuable information about the mobility of pigments and the effectiveness of the purification process in isolating specific compounds.

Characterization of Pigments

UV-Visible Spectroscopy Analysis

In the methanol extract of *Bixa orellana* L., UV-visible spectroscopy analysis showed a peak absorbance at 409 nm, indicating the presence of a pigment with maximum absorption at this wavelength. The concentration of carotenoid in different extracts of *Bixa orellana* L. varied, with Chloroform extract having the highest concentration at 26.58 mg/ml. In the analysis of *Rubus fruticosus* L., distinct peaks in absorbance were observed at different wavelengths for various extracts. The concentrations of anthocyanin ranged from 61.66 mg/ml to 73.69 mg/ml, with the Methanol extract having the highest concentration.

For *Adhatoda vasica* L., UV-visible spectroscopy analysis revealed a distinctive peak in the absorption spectrum for various extracts. The concentrations of carotenoids varied among the extracts, with the Methanol extract having the highest concentration at 45.30 mg/ml.

The UV-visible spectroscopy analysis of *Beta vulgaris* L. showed distinct peaks at specific wavelengths for different extracts. Betalain concentrations varied among extracts, with 50% Aqueous acetone extract having the highest concentration at 32.08 mg/ml.

HPTLC Fingerprinting

Carotenoid Profile (*Bixa orellana* L.)

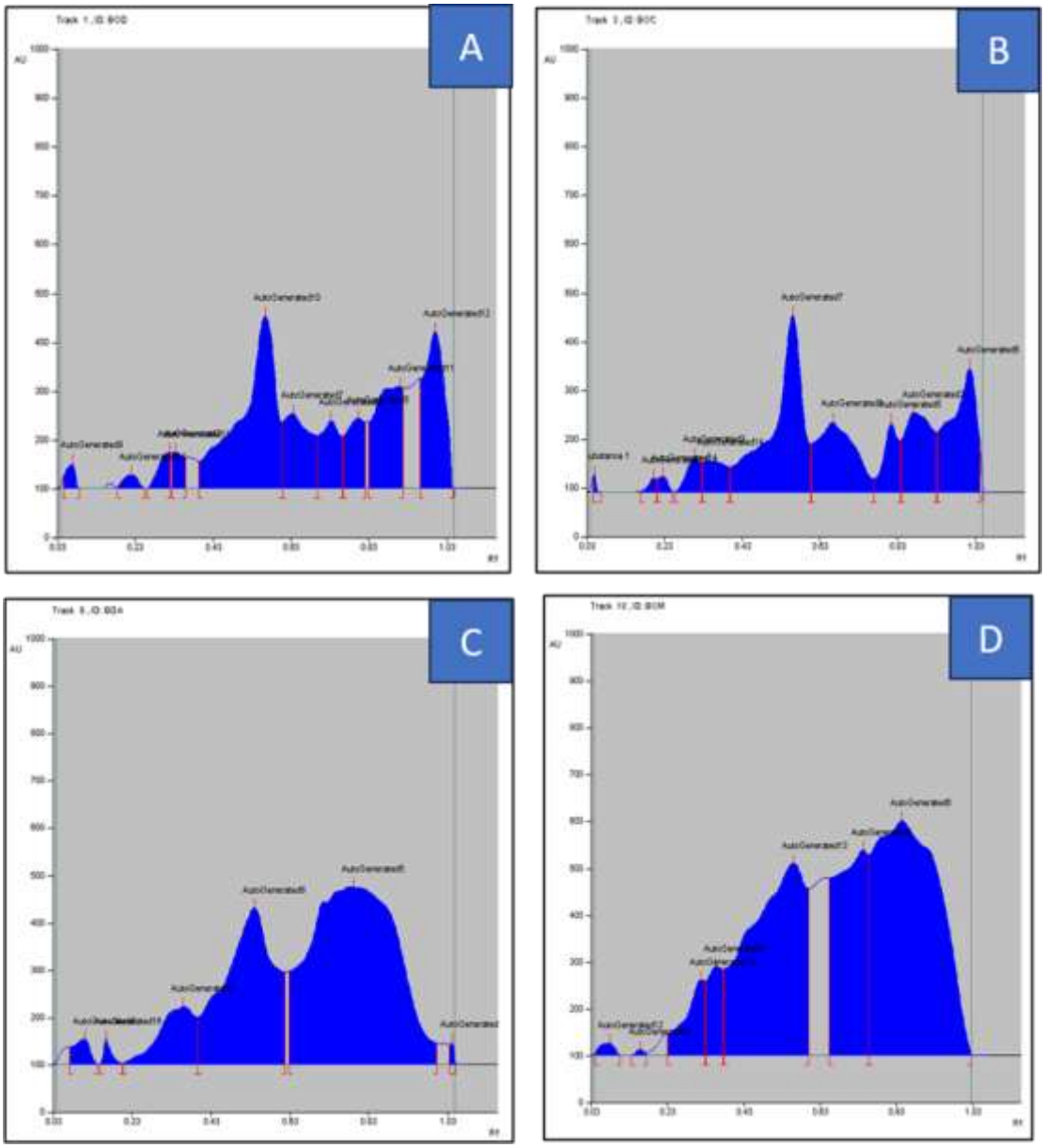


Figure 6: Peak densitogram (254 nm) of different extracts for carotenoid compound A:BOD,B:BOC,C:BOA,D:BOM

HPTLC fingerprinting for carotenoid compounds in *Bixa orellana* L. extracts revealed distinct bands with Rf values corresponding to different carotenoids. The extracts showed the presence of bixin, norbixin, zeaxanthin, lutein, and astaxanthin.

Anthocyanin Profile (*Rubus fruticosus* L.)

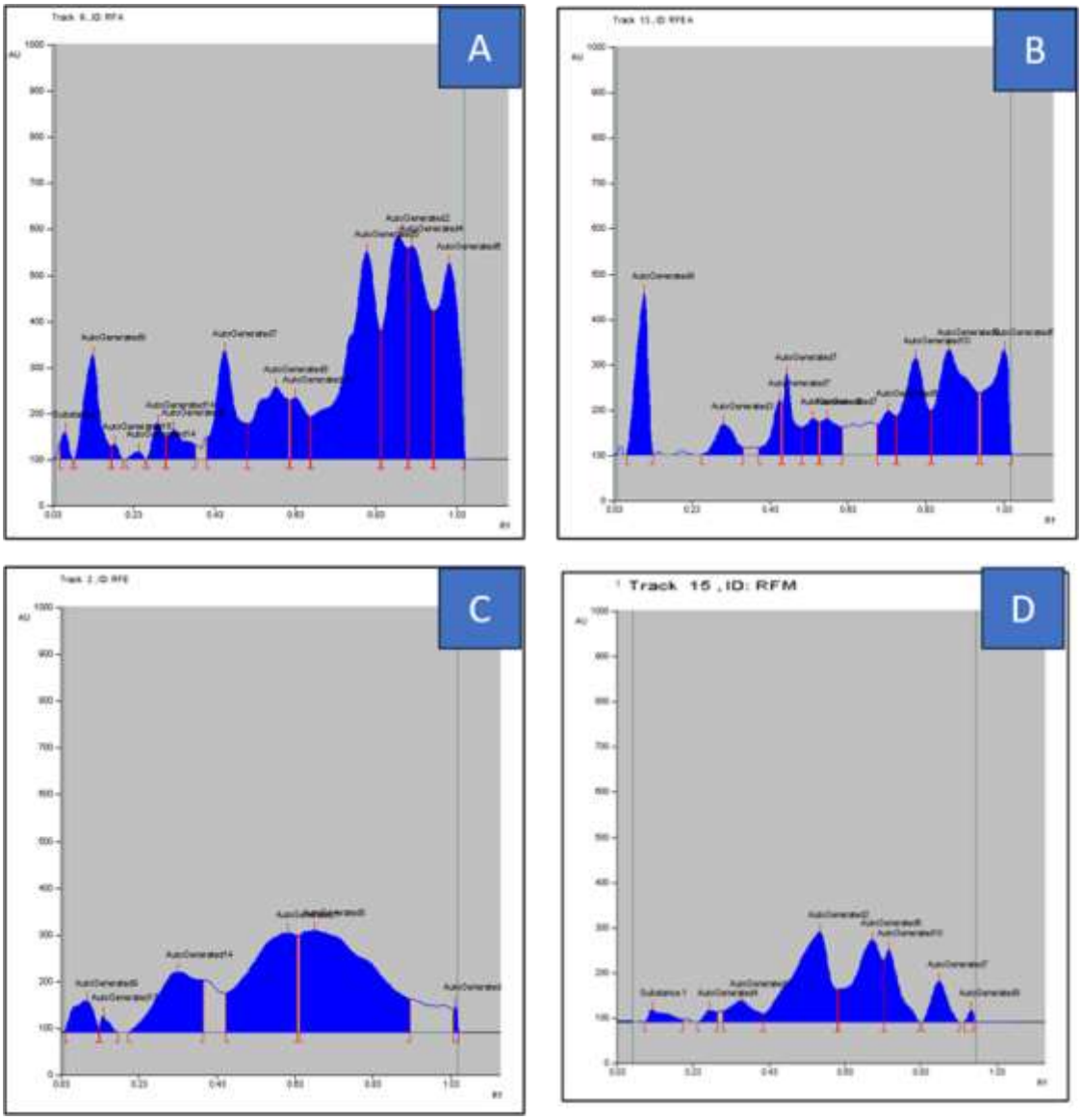


Figure 7: Peak densitogram (254 nm) of different extracts for anthocyanin compound A: RFA, B: RFEA, C: RFE, D: RFM

HPTLC fingerprinting for anthocyanin compounds in *Rubus fruticosus* L. extracts revealed bands corresponding to delphinidin-3-O-galactoside, cyanidin-3-O-glucoside, malvidin-3-O-galactoside, peonidin-3-glucoside, and pelargonidin-3-O-glucoside.

Chlorophyll Profile (*Adhatoda vasica* L.)

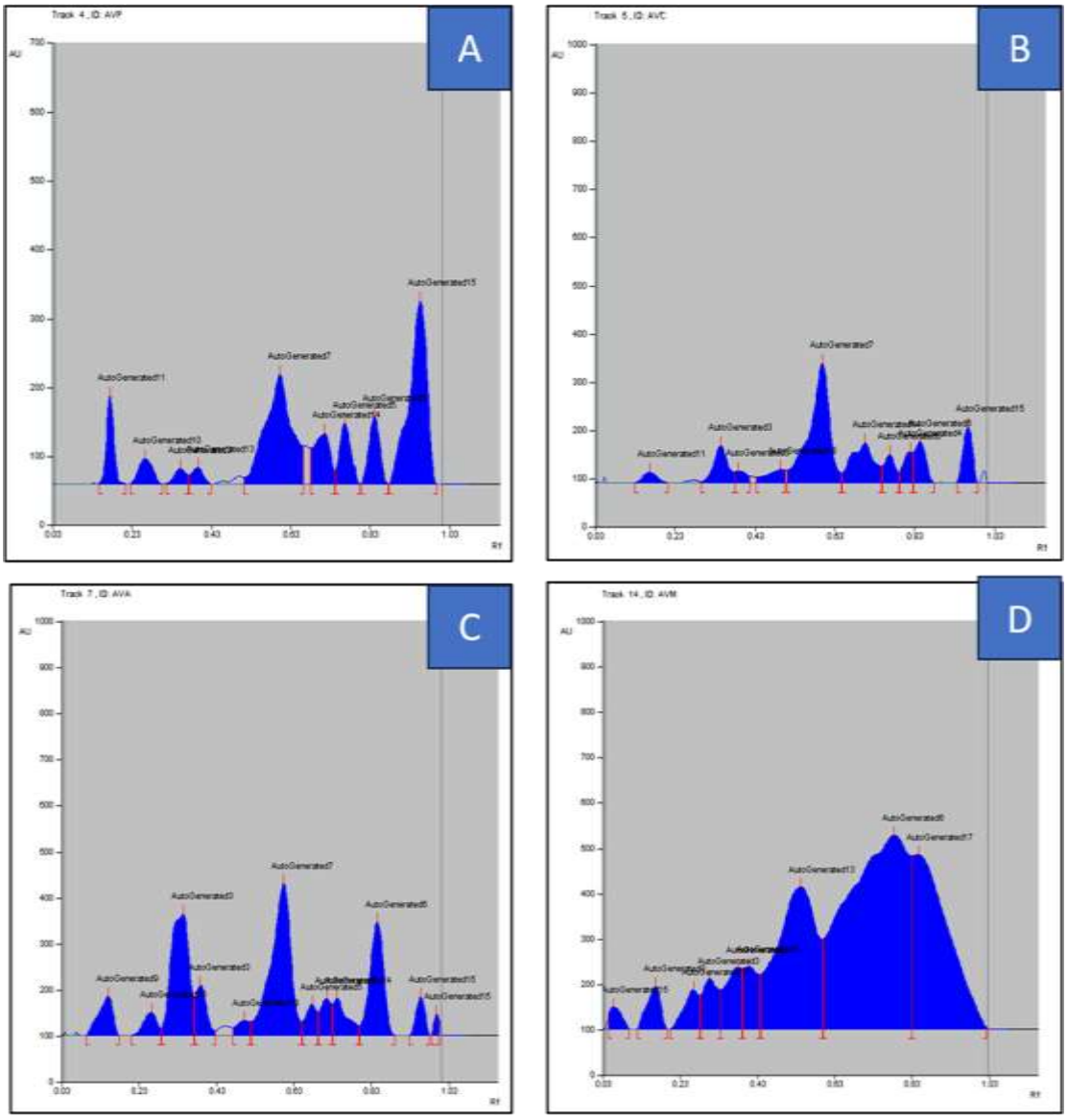


Figure 8: Peak densitogram (254 nm) of different extracts for chlorophyll compound A: AVP, B: AVC, C: AVA, D: AVM

HPTLC fingerprinting for chlorophyll compounds in *Adhatoda vasica* L. extracts showed bands corresponding to Chlorophyll C, Chlorophyll C1, Chlorophyll C2, and Chlorophyll C3.

Betalain Profile (*Beta vulgaris* L.)

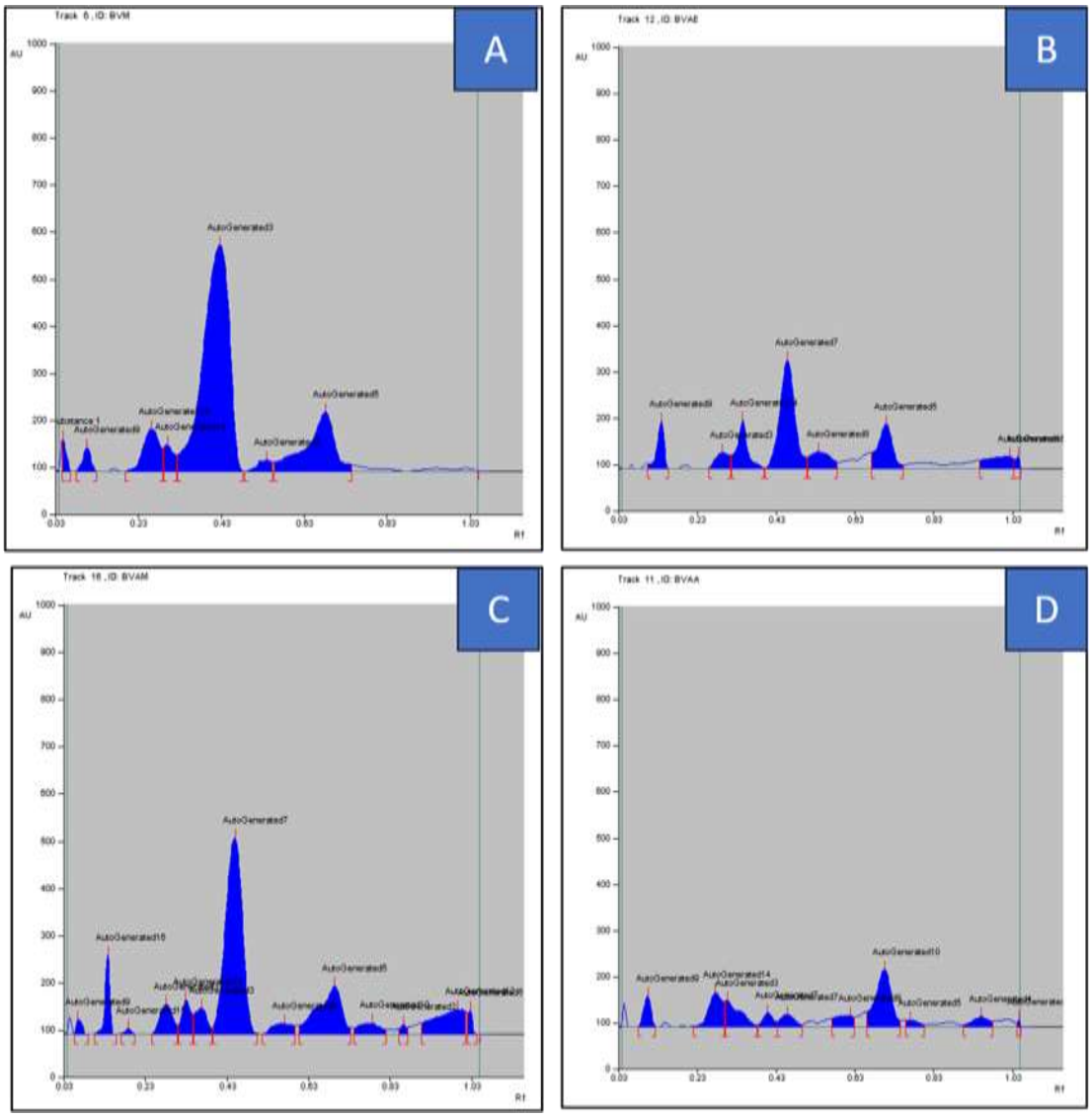


Figure 9: Peak densitogram (254 nm) of different extracts for betalain compound A: BVM, B: BVAE, C: BVAM, D: BVAA

HPTLC fingerprinting for betalain compounds in *Beta vulgaris* L. extracts revealed bands corresponding to basic betanin, isobutane, and Vulgaxanthin I.

The HPTLC fingerprinting allowed for the visualization and identification of different pigmented compounds in the plant extracts, providing a detailed profile of carotenoids, anthocyanins, chlorophyll, and betalains.

Fourier transform infrared spectroscopy

The FTIR spectra of plant pigments underwent a thorough analysis to identify characteristic absorption bands, indicating the presence of functional groups. Carotenoids exhibit a linear polyene structure with isoprene units forming the backbone, and variations in hydroxyl and carbonyl groups. Specific functional groups in different plant extracts were highlighted, such as alkane and ester groups in *Bixa orellana* L.

Sr. No.	Plant extract	Functional Group	Frequency (cm^{-1})
1	BOD	C-H alkane (stretching)	2919 (3000-2840)
		C=O stretching (ester)	1741 (1750-1735)
		C=C bending (alkene)	695 (730-665)
2	BOM	C-H alkane (stretching)	2920, 2856 (3000-2840)
		C=O stretching (ester)	1740 (1750-1735)
		C=C bending (alkene)	696 (730-665)
3	BOA	C-H alkane (stretching)	2922, 2855 (3000-2840)
		C=O stretching (ester)	1736 (1750-1735)
		C=C bending (alkane)	730 (730-665)
4	BOC	C-H alkane (stretching)	2919, 2855 (3000-2840)
		C=O stretching (ester)	1737 (1750-1735)
		C=C bending (alkene)	695 (730-665)

Table 1: FTIR peak assignment of *Bixa orellana* L.

The analysis also explored anthocyanin structures in the Methanol extract of *Rubus fruticosus* L., offering valuable insights into plant pigment composition.

Sr. No.	Plant extract	Functional Group	Frequency (cm^{-1})
1	RFM	O-H Alcohol group (stretching)	3659 (3700-3584)
		C-H Alkane group (stretching)	2991, 2911 (3000-2840)
		O-H bending (carboxylic acid)	1434 (1440-1395)
		C-O-C	1042 (1050 to 1250)
2	RFA	O-H Alcohol group (stretching)	3650 (3700-3584)
		C-H Alkane group (stretching)	2987 (3000-2840)
		C=C alkene group (stretching)	1578 (1600 to 1670)
		O-H bending (carboxylic acid)	1426 (1440-1395)
		C-O-C	1043 (1050 to

			1250)
3	RFE	C-H alkyne (stretching)	3307 (3333-3267)
		C-H alkane (stretching)	2913 (3000-2840)
		O-H bending (carboxylic acid)	1404 (1440-1395)
		C-O-C	1011 (1050 to 1250)
4	RFEA	O-H Alcohol group (stretching)	3656 (3700-3584)
		C-H alkane (stretching)	2987, 2910 (3000-2840)
		COOH (carboxylic acid)	1425 (1440-1395)
		C-O stretching (aeromatic ester)	1244 (1310-1250)
		C-O-C	1044 (1050 to 1250)

Table 2: FTIR peak assignment of *Rubus fruticosus* L.

Liquid chromatography mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS) plays a crucial role in identifying and characterizing plant pigments. Peaks in the LC-MS chromatogram represent distinct pigments, with their retention times serving as unique identifiers. Molecular weight determination through mass spectra analysis offers insights into chemical composition and potential structures.

Pigments	Molecular mass of compounds	Fragment 1	Fragment 2
BOM	564.82 (Canthaxanthin)	427.30	290.20
BOA	394.5 (Bixin)	349.22	283.17
RFE	463.4 (Peonidin-3-glucoside)	286.08	269.08
RFM	484.8 (Cyanidin-3-glucoside)	357.12	288.06
AVA	652.9 (Chlorophyll-C3)	569.18, 515.14	341.05
AVM	588.7 Chlorophyll-C)	529.22	412.17
BVAM, BVAE	550.47 (Betanin)	371.09	326.09

Table 3: Identified compound by LCMS

Fragmentation patterns aid in understanding structural characteristics. Comparison with databases validates compound identification. UV-Visible spectroscopy, HPLC, and FT-IR findings are corroborated by LC-MS. HPTLC chromatograms reveal bands matching LC-MS-identified R*f* values. Molecular masses for pigments like Canthaxanthin and Bixin, along with specific fragmentation patterns, confirm their presence in various plant extracts.

Enhancement of plant pigment stability

Ensuring the stability of plant pigments is crucial for preserving their color intensity and quality across various applications. One effective strategy involves encapsulating pigments using a solution of maltodextrin and gum arabic. This encapsulation forms a protective barrier around individual pigment particles, safeguarding them from oxygen, moisture, and light-induced degradation. This process prevents issues such as oxidation, moisture absorption, and photodegradation, thereby preserving the color and stability of plant pigments. Additionally, encapsulation prevents the agglomeration of pigment particles, ensuring even distribution and improved solubility in various media. The encapsulated pigments exhibit controlled and sustained color release, enhancing their longevity.

The encapsulated pigments underwent thorough testing, including assessments of moisture content, bulk density, hygroscopicity, solubility, redispersion time, and microencapsulation yield. Variations in moisture retention, bulk density, and hygroscopicity were observed, reflecting the efficiency of encapsulating materials. Controlled and low moisture content was maintained to ensure stability and prevent microbial growth.

Plant pigments	Moisture content (%)	Bulk density (g/cm3)	Hygroscopicity (g/100g)	Solubility (%)	Redispersion time (sec)	Micro encapsulation yield
BOM	4.95±0.03	0.41±0.02	37±0.20	84±0.50	33±0.50	73±0.50
BOC	5.01±0.06	0.44±0.05	45±0.50	83±0.58	39±0.58	70±0.58
BOD	4.99±0.07	0.47±0.06	49±0.60	80±0.60	41±0.60	71±0.60
BOA	4.98±0.04	0.42±0.02	40±0.30	80±0.45	42±0.45	72±0.45
BVAM	4.45±0.04	0.46±0.03	34±0.30	91±0.38	34±0.38	79±0.38
BVAE	4.43±0.05	0.44±0.03	38±0.34	95±0.47	30±0.47	74±0.47
BVAA	4.48±0.08	0.49±0.07	41±0.40	88±0.68	38±0.68	71±0.68
BVM	4.46±0.10	0.51±0.08	46±0.49	84±0.99	44±0.99	73±0.99
AVA	3.97±0.05	0.42±0.02	38±0.20	91±0.35	32±0.35	68±0.35
AVM	3.95±0.03	0.44±0.03	41±0.32	89±0.37	35±0.37	70±0.37
AVC	3.99±0.07	0.48±0.07	46±0.71	81±0.60	41±0.60	62±0.60
AVP	4.00±0.09	0.54±0.09	54±0.92	79±0.96	43±0.96	60±0.96
RFE	5.57±0.05	0.44±0.04	33±0.30	90±0.30	33±0.30	70±0.30
RFM	5.59±0.07	0.46±0.06	40±0.45	93±0.45	30±0.45	73±0.45
RFA	5.61±0.09	0.49±0.09	49±0.58	88±0.58	38±0.58	70±0.58
RFEA	5.64±0.10	0.55±0.10	55±0.10	87±0.10	37±0.10	69±0.10

Table 4: The moisture content, bulk density, hygroscopicity, solubility, redispersion time and microencapsulated yield of encapsulated pigment

Another approach to enhance pigment stability involves copigmentation using ascorbic acid and citric acid. These compounds form complexes with pigments, stabilizing them and intensifying their color. The study demonstrated the varying color retention abilities of

different pigments over time, influenced by the copigmentation process.

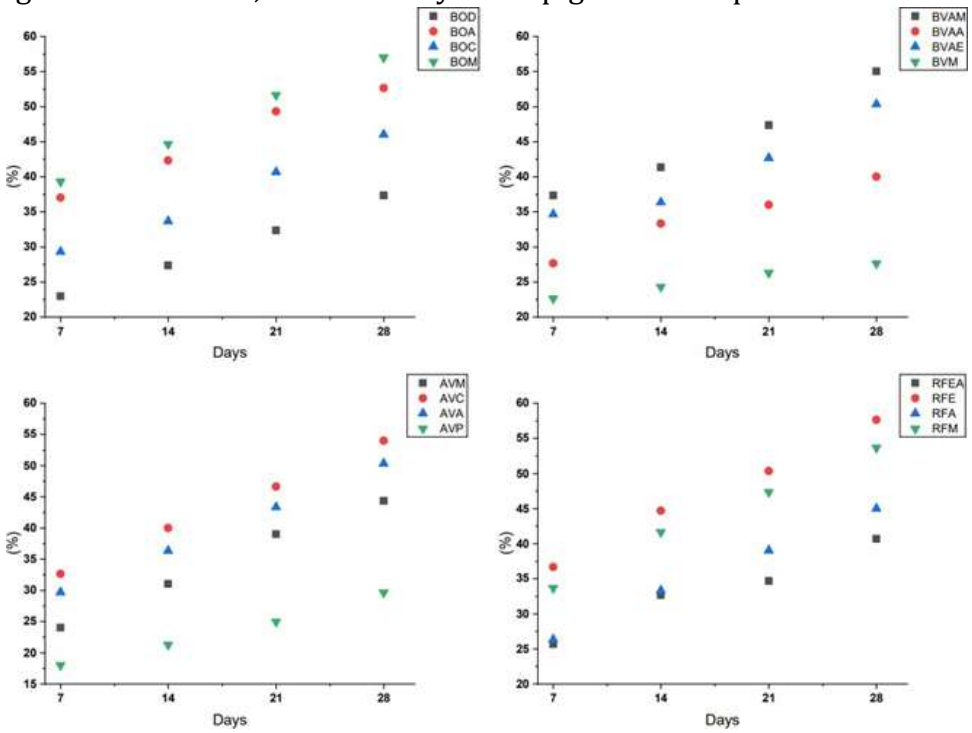
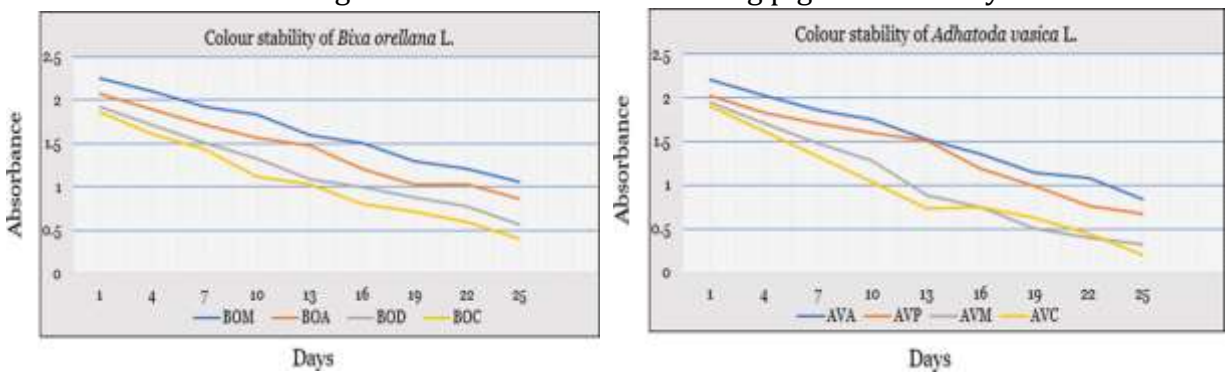


Figure 10: Colour retention of copigmented extract

The entrapment of pigments through the ionic gelation method provides precise control over release and protection. The gel matrix formed offers stability, controlled release, and protection against external factors, ensuring prolonged color preservation. The study includes detailed characterizations of the beads, evaluating size, moisture content, and entrapment efficiency for various plant pigments. Additionally, the color stability of pigment-containing beads was assessed over different storage durations, demonstrating the effectiveness of the ionic gelation method in maintaining pigment stability.



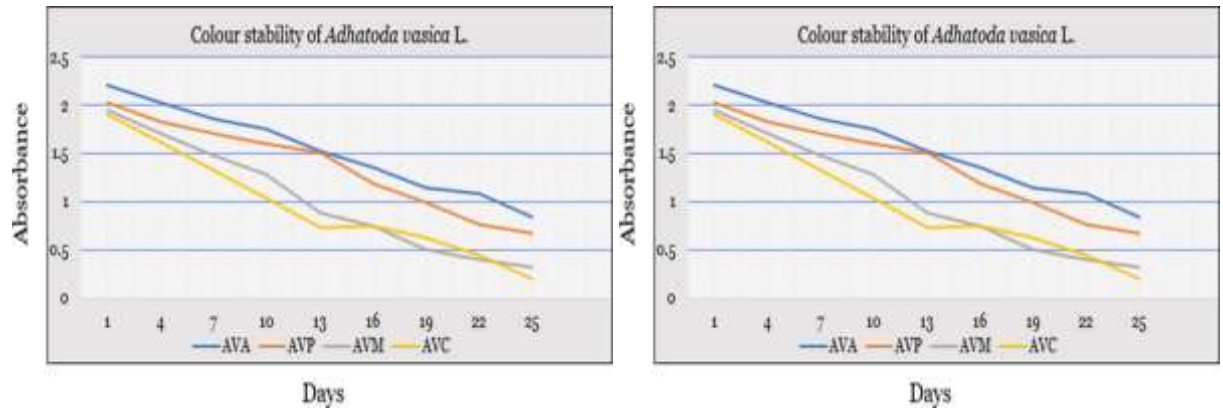


Figure 11: Colour stability of pigments in different storage duration

Antibacterial and Antioxidant activity of plant pigment

The investigation into plant pigments' antibacterial and antioxidant properties underscores their potential in disease prevention and treatment. Antibacterial effects can inhibit harmful bacteria growth, crucial for reducing infection risks. Additionally, antioxidant activity safeguards cells from oxidative damage linked to chronic diseases. As a response to rising antibiotic resistance concerns, plant pigments offer safer, sustainable solutions. Their antibacterial prowess finds applications in food preservation, enhancing safety, and reducing waste.

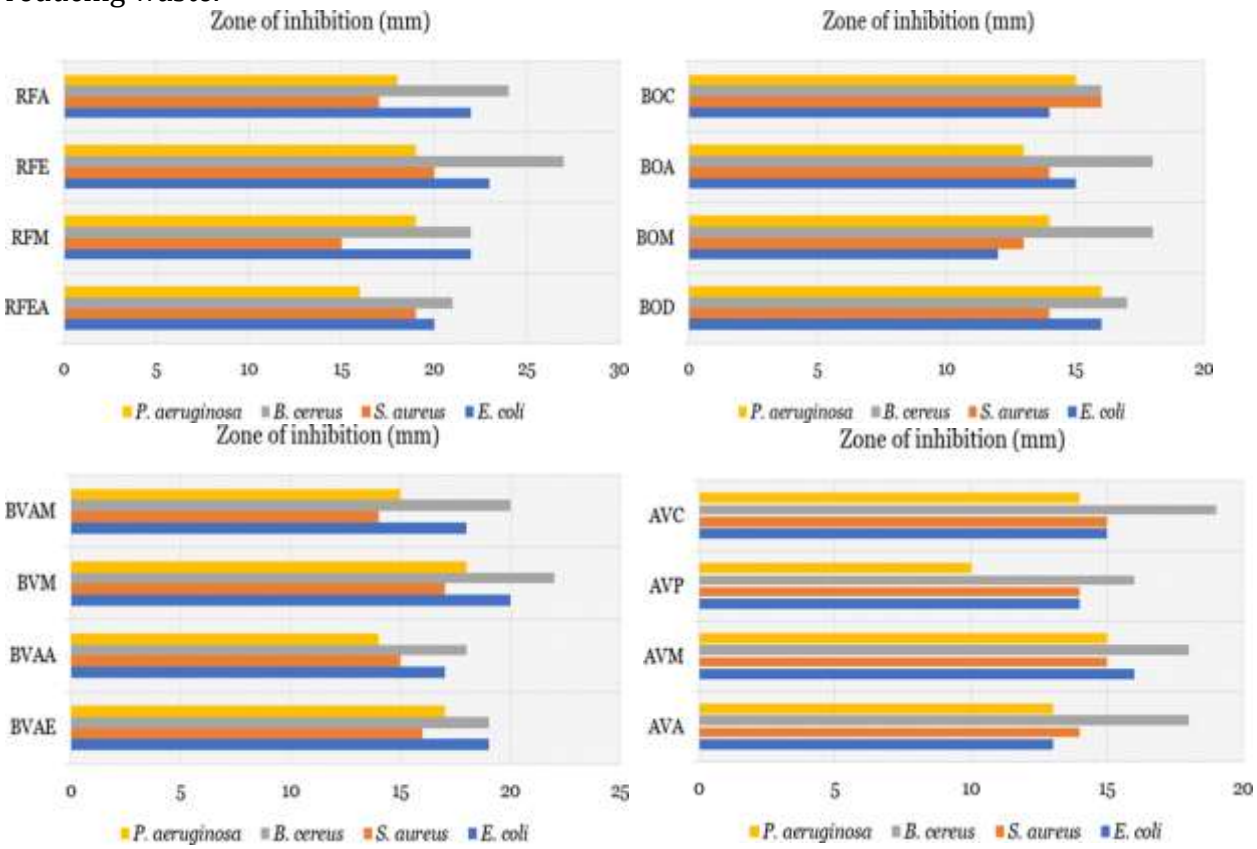


Figure 12: Effectiveness of Pigments on *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa and Escherichia coli*

The study employed the agar well diffusion method to assess antibacterial activity. Results for *Rubus fruticosus* L. and *Bixa orellana* L. extracts displayed concentration-dependent increases in inhibition zones against various pathogens. *Rubus fruticosus* L. ethanol extract, particularly at 10 mg concentration, exhibited notable effectiveness against Staphylococcus aureus.

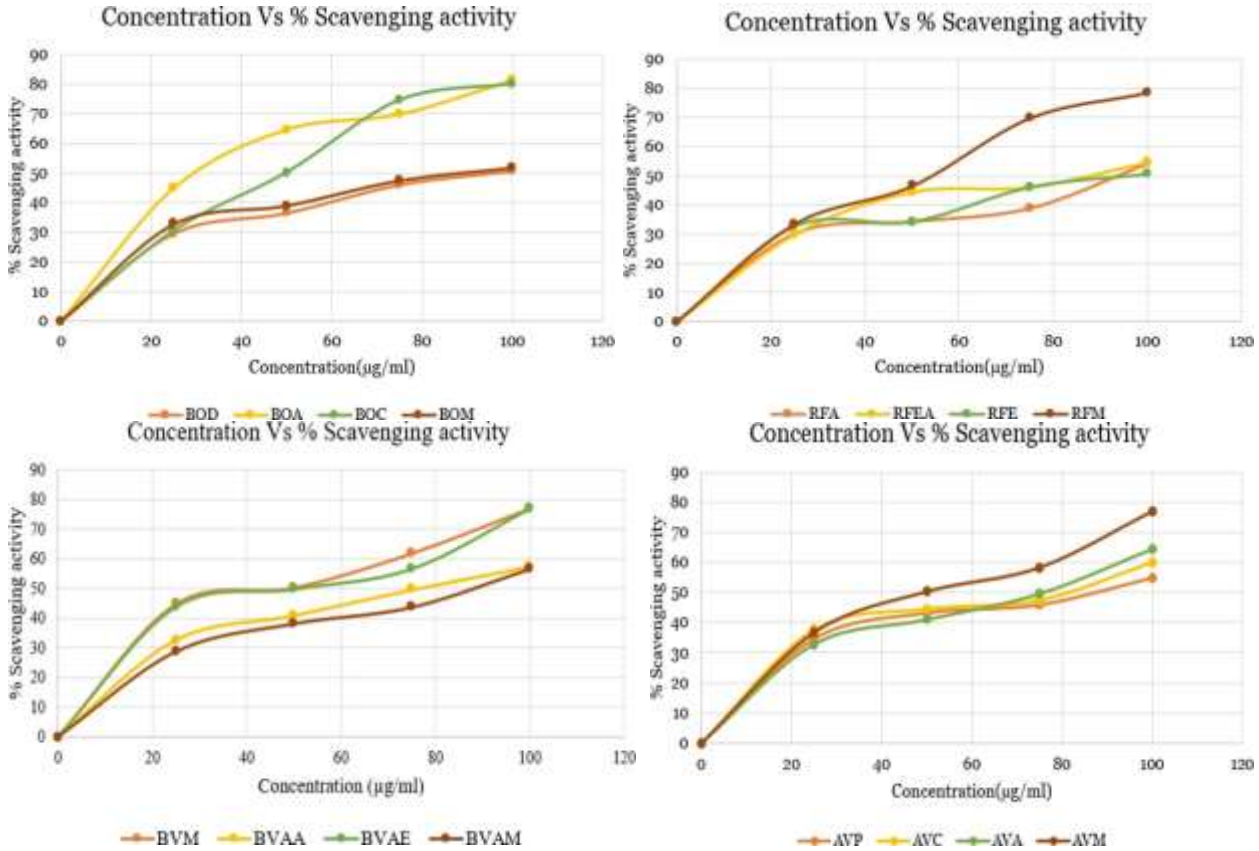


Figure 13: Antioxidant activity of pigment

Antioxidant activity, evaluated through DPPH scavenging, revealed concentration-dependent efficacy for *Bixa orellana* L., *Rubus fruticosus* L., *Beta vulgaris* L., and *Adhatoda vasica* L. Extracts, especially from *Beta vulgaris* L. and *Rubus fruticosus* L., demonstrated potent antioxidant capabilities. The IC50 values indicated concentration levels needed for 50% antioxidant activity inhibition.

Applications

Plant pigments have gained significant importance across industries such as textiles, pharmaceuticals, food, cosmetics, and candle-making due to their natural and sustainable characteristics. In textiles, these pigments provide vibrant and eco-friendly color options, meeting the demands of environmentally-conscious consumers. The stability of plant pigments was assessed, demonstrating good fastness properties in washing, light exposure, and rubbing for both cotton and polyester fabrics.

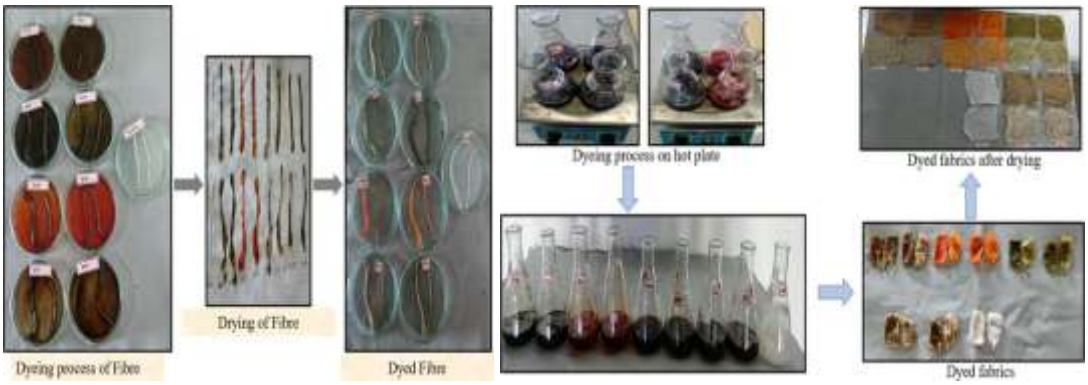


Figure 14: Dyeing process of fibres and Fabrics

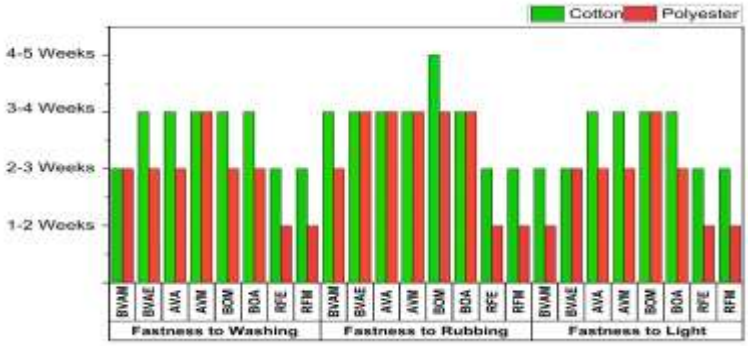


Figure 15: The fastness properties such as washing fastness, light fastness and rubbing fastness of dyed fabrics

Tablet coating benefits from visually appealing coatings, aiding in brand recognition. In tablet coating, 3D-printed machines were developed for efficient and cost-effective processes. Evaluating the color stability of coated tablets revealed varying levels of performance for different plant pigments.

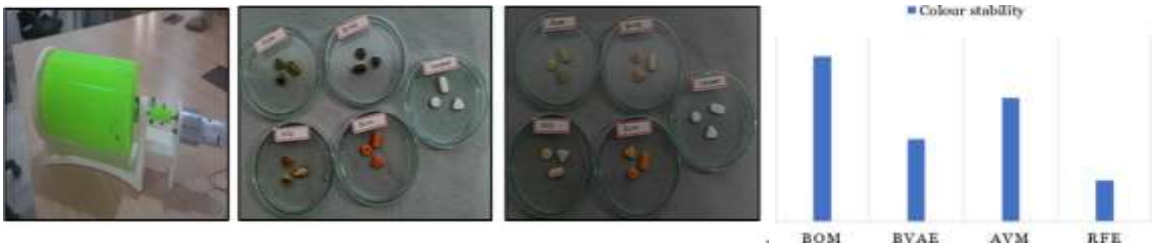


Figure 16: Coated tablet with pigments

As natural food colorants, plant pigments replace synthetic counterparts, aligning with the clean label trend in the food industry. The application of plant pigments in food products, such as vanilla ice cream and jelly, showed promising results in terms of color enhancement. However, the stability of color in ice cream diminished over time, especially when stored at higher temperatures.

Figure 17: Formulation of ice cream and jelly with extracted pigment

Lip balms can incorporate these pigments for tinted options and potential lip care benefits. In lip balm formulations, plant pigments exhibited pleasant characteristics and stability at different temperatures.

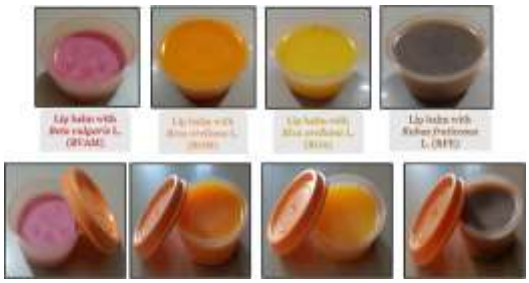


Figure 18: Formulation of lip balm from pigments

Additionally, plant pigments play a crucial role in creating colorful and environmentally-friendly candles. In candle-making, plant pigments contributed to visual appeal, with consumer preferences for natural colors and favorable aroma changes after burning.

Figure 19: Coloured candle from pigments

CONCLUSION

Synthetic pigments, once widely used for coloring various products, are facing scrutiny due to their environmental and health hazards. The present study emphasizes the emergence of natural plant pigments, such as *Bixa orellana* L., *Beta vulgaris* L., *Rubus fruticosus* L., and *Adhatoda vasica* L., as safer and cost-effective alternatives. These plant pigments, notably the Methanol extract of *Bixa orellana* L., Ethanol extract of *Rubus fruticosus* L., 50% Aqueous methanol extract of *Beta vulgaris* L., and Methanol extract of *Adhatoda vasica* L., offer antioxidant and antibacterial properties with high stability. Their versatility makes them valuable for various applications, promoting environmentally friendly and consumer-friendly formulations across industries seeking safe and eco-friendly biocolours.

REFERENCES

Alsaud, N., & Farid, M. (2020). *applied sciences Insight into the Influence of Grinding on the Extraction E ffi ciency of Selected Bioactive Compounds from Various Plant Leaves*.

Brudzyńska, P., Sionkowska, A., & Grisel, M. (2021). Plant-derived colorants for food, cosmetic and textile industries: A review. *Materials*, *14*(13), 1–18. https://doi.org/10.3390/ma14133484

Çekiç, Ç., & Özgen, M. (2010). Comparison of antioxidant capacity and phytochemical properties of wild and cultivated red raspberries (Rubus idaeus L.). *Journal of Food Composition and Analysis*, *23*(6), 540–544. https://doi.org/10.1016/j.jfca.2009.07.002

Chung, C., Rojanasasithara, T., Mutilangi, W., & McClements, D. J. (2017). Stability improvement of natural food colors: Impact of amino acid and peptide addition on anthocyanin stability in model beverages. *Food Chemistry*, *218*, 277–284. https://doi.org/10.1016/j.foodchem.2016.09.087

Dimitrić Marković, J. M., Petranović, N. A., & Baranac, J. M. (2005). The copigmentation effect of sinapic acid on malvin: a spectroscopic investigation on colour enhancement. *Journal of Photochemistry and Photobiology B: Biology*, *78*(3), 223–228. https://doi.org/10.1016/J.JPHOTOBIOL.2004.11.009

Doss, A., Sc, M., & Phil, M. (2009). *Preliminary phytochemical screening of some Indian Medicinal Plants*. *29*(2), 12–16.

Fernández-López, J. A., Fernández-Lledó, V., & Angosto, J. M. (2020). New insights into red plant pigments: More than just natural colorants. *RSC Advances*, *10*(41), 24669–24682. https://doi.org/10.1039/d0ra03514a

Forage, N., Staff, C., Grain, D., & Council, T. (2009). *Evaluation of Analytical Methods for the Determination of Moisture, Crude Protein, Crude Fat, and Crude Fiber in Distillers Dried Grains with Solubles*. 61–74.

Gahlawat, I. N. (2019). *Emerging new insights into significance and applications of Plant Pigments*. *7*(2), 29–34.

García-de Blas, E., Mateo, R., Viñuela, J., & Alonso-Álvarez, C. (2011). Identification of carotenoid pigments and their fatty acid esters in an avian integument combining HPLC-DAD and LC-MS analyses. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *879*(5–6), 341–348. https://doi.org/10.1016/j.jchromb.2010.12.019

Kang, Y. R., Lee, Y. K., Kim, Y. J., & Chang, Y. H. (2019). Characterization and storage stability of chlorophylls microencapsulated in different combination of gum Arabic and

maltodextrin. *Food Chemistry*, *272*(March 2018), 337–346. https://doi.org/10.1016/j.foodchem.2018.08.063

Kokil, S., Kadu, M., Vishwasrao, S., & Singh, S. (2015). Review on Natural Lip Balm. *International Journal of Research in Cosmetic Science*, *5*(1), 1–7. http://www.urpjournals.com

Luzardo-Ocampo, I., Ramírez-Jiménez, A. K., Yañez, J., Mojica, L., & Luna-Vital, D. A. (2021). Technological applications of natural colorants in food systems: A review. *Foods*, *10*(3), 1–34. https://doi.org/10.3390/foods10030634

Mohammad Azmin, S. N. H., Sulaiman, N. S., Mat Nor, M. S., Abdullah, P. S., Abdul Kari, Z., & Pati, S. (2022). A Review on Recent Advances on Natural Plant Pigments in Foods: Functions, Extraction, Importance and Challenges. *Applied Biochemistry and Biotechnology*, *194*(10), 4655–4672. https://doi.org/10.1007/s12010-022-04050-z

Mushtaq, A., Akbar, S., Zargar, M. A., Wali, A. F., Malik, A. H., Dar, M. Y., Hamid, R., & Ganai, B. A. (2014). Phytochemical screening, physicochemical properties, acute toxicity testing and screening of hypoglycaemic activity of extracts of eremurus himalaicus baker in normoglycaemic wistar strain albino rats. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/867547

Ngamwonglumlert, L., Devahastin, S., & Chiewchan, N. (2017). Natural colorants: Pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. *Critical Reviews in Food Science and Nutrition*, *57*(15), 3243–3259. https://doi.org/10.1080/10408398.2015.1109498

Odey M O, Iwara I A, Udiba U U, Johnson J T, Inekwe, Asenye M E, & Victor O. (2012). Preparation of Plant Extracts from Indigenous Medicinal Plants. In *International Journal of Science and Technology* (Vol. 1, Issue 12).

Oliveira-júnior, R. G. De, Grougnet, R., & Bodet, P. (2020). *Updated pigment composition of Tisochrysis lutea and purification of fucoxanthin using centrifugal partition chromatography coupled to flash chromatography for the chemosensitization of melanoma cells*. 1–34.

Poongothai, A. (2019). *QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF Lantana camara LEAF EXTRACT*. *6*(12), 603–607.

Quach, H. T., Steeper, R. L., & Griffin, G. W. (2004). An improved method for the extraction and thin-layer chromatography of chlorophyll a and b from spinach. *Journal of Chemical Education*, *81*(3), 385–387. https://doi.org/10.1021/ed081p385

Rodríguez-Mena, A., Ochoa-Martínez, L. A., González-Herrera, S. M., Rutiaga-Quiñones, O. M., González-Laredo, R. F., & Olmedilla-Alonso, B. (2023). Natural pigments of plant origin: Classification, extraction and application in foods. *Food Chemistry*, *398*(August), 133908. https://doi.org/10.1016/j.foodchem.2022.133908

Salimeh, S., Abandansarie, R., & Ariaii, P. (2019). *Effects of encapsulated rosemary extract on oxidative and microbiological stability of beef meat during refrigerated storage*. *September*, 3969–3978. https://doi.org/10.1002/fsn3.1258

Salminen, J.-P. (2003). Effects of Sample Drying and Storage, and Choice of Extraction Solvent and Analysis Method on the Yield of Birch Leaf Hydrolyzable Tannins. *Journal of Chemical Ecology*, *29*(6), 1289–1305. https://doi.org/10.1023/A:1024249016741

Srivastava, R. P., Dixit, P., Singh, L., Nagpoore, N. K., Pandey, S., Verma, P. C., Rawat, V., & Saxena, G. (2021). HPTLC fingerprinting, photopigments, and nutrient analysis of Selinum tenuifolium along the altitudinal gradient. *Journal of Liquid Chromatography and Related Technologies*, *44*(1–2), 87–94. https://doi.org/10.1080/10826076.2020.1848861

Tavlasoglu, M., Ozkan, G., & Capanoglu, E. (2022). Entrapment of Black Carrot Anthocyanins by Ionic Gelation: Preparation, Characterization, and Application as a Natural Colorant in Yoghurt. *ACS Omega*, *7*(36), 32481–32488. https://doi.org/10.1021/acsomega.2c03962

Westfall, A., Sigurdson, G. T., Rodriguez-Saona, L. E., & Giusti, M. M. (2020). Ex vivo and in vivo assessment of the penetration of topically applied anthocyanins utilizing atr-ftir/pls regression models and hplc-pda-ms. *Antioxidants*, *9*(6). https://doi.org/10.3390/antiox9060486

Zia, P., Sunita, M., & Sneha, S. (2021). *Extraction of Natural Colour from Beet Root (Beta vulgaris) its Phytochemical Analysis and Antibacterial Activity*. *1873*(4), 3–8. https://doi.org/10.36349/easjnfs.2021.v03i04.002