PHYTOPIGMENTS AS THERAPEUTIC LEADS: THE PHARMACOLOGICAL RELEVENCE OF NATURAL PIGMENTS

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ABSTRACT:

Phytopigments are naturally occurring bioactive compounds responsible for the coloration of plants and play vital roles in photosynthesis and protection against environmental stress.In recent years, these natural pigments have gained considerable attention as promising therapeutic leads due to their broad pharmacological relevance. Major classes of Phytopigments, including carotenoids, chlorophylls, anthocyanins, betalains, and flavonoids, exhibit diverse biological activities such as antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, and antidiabetic effects. These pharmacological actions are primarily mediated through modulation of oxidative stress, regulation of cellular signaling pathways, enzyme inhibition, and gene expression control. Increasing evidence from experimental and clinical studies supports the potential of phytopigments as safe and effective agents for the prevention and management of chronic diseases. Advances in extraction, standardization, and formulation technologies have further enhanced their therapeutic applicability. This review highlights the pharmacological significance of phytopigments and underscores their potential as natural, multifunctional drug leads in modern pharmaceutical and nutraceutical development.

Key words: Phytopigments, Natural pigments, Antioxidant, Pharmacological activity.

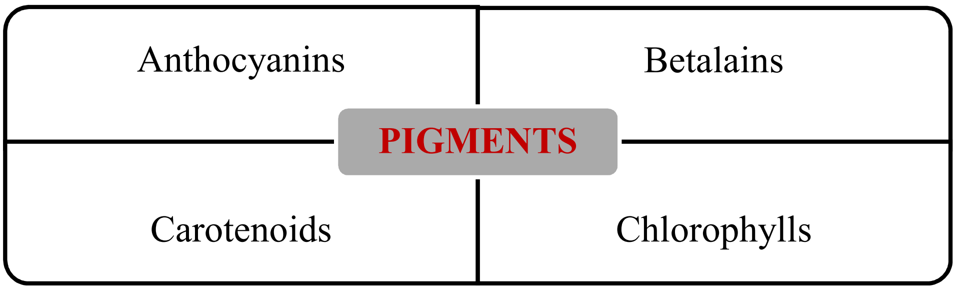
INTRODUCTION:

The colours we see throughout our lives are caused by pigments which are found all over the world and are mostly made by plants they can be present in fruits, vegetables, flowers, skin, eyes and other animal structures.In addition to bacteria and fungi a wide range of products including food, clothing, furniture, cosmetics and pharmaceuticals use both natural and artificial pigments[1]. Molecular pigments known as phytopigments or biochromes absorb and reflect specific light wavelengths during photosynthesis [2].Pigments are optical features found in plants [3].

DEFINITION:

A pigment is a chemical compound that absorbs light in the visible wavelength range.The colour it produces is produced by a structure specific to each molecule called a chromophore which absorbs energy and excites an electron from an external orbital to a higher orbital.The non-absorbed energy is then refracted and reflected to be captured by the eye and the resulting neural impulses are sent to the brain where they may be interpreted as a colour[1].

CLASSIFICATION OF NATURAL PIGMENTS:



ROLE OF PIGMENTS IN PLANTS

1)IN VEGETATIVE TISSUES:

Both carotenoids and chlorophylls are necessary for photosynthesis with chlorophylls serving as vital electron donors and light energy absorber.Carotenoids are the essential structural element that safeguards the photosynthetic system from photooxidation. In addition to anthocyanins which are commonly found in vegetative tissues.Plant pigments also influence radiation.The most notable example is their role in the autumnal colours of many deciduous species leaves which they produce in tandem with the retention of carotenoids and the loss of chlorophyll [4][5][6].In tissues that are not senescing their incidence is more variable some species store a lot of them in healthy leaves giving the foliage a reddish-purple colour.

In other cases, anthocyanin production is induced in leaves in response to stresses such as cold, high light levels, pest and pathogen attack or deficiency of nutrients such as phosphate and nitrogen. Anthocyanin colouration in leaves can vary with season, environment, between individuals of a population and between different leaves on a single plant. It is commonly thought that anthocyanins have a role in protecting the photosynthetic apparatus from damage in many of these situations and those tissues that show more anthocyanin accumulation are often at greater photo-inhibitory risk, e.g. during nutrient reabsorption in senescing leaves or in cold temperatures [5]. But, the details of how anthocyanins achieve this are not determined. One hypothesis is that anthocyanins help attenuate the light levels, modifying the quantity and quality of light incident on the chloroplasts and thus reducing excitation pressure [7].

2).IN REPRODUCTIVE TISSUES:

The most observable function of plant pigments is to provide colour to flowers and fruit for attraction of pollinators and seed dispersal agents. These colours arise predominantly from flavonoid and carotenoid pigments and a short guide to the likely pigments producing specific colours in flowers and fruits of plants. There are also few common pigments that generate colours in specific species. For angiosperms, colour is key to attracting pollinators, whether they are bees, butterflies, other insects or birds. Even if, it is frequently one of a number of factors, including fragrance, floral shape which combine to determine pollinator choice. Flavonoids are the most common flower colour pigments. The role of flavonoids in pollination was the subject of an extensive review by Harborne and Grayer[8].UV-absorbing pigments also affect floral choice for pollinators like bees that can detect light in the UV spectrum chalcone and flavonol-type flavonoids are the primary contributors to the flowers UV absorption flowers frequently have higher concentrations of flavonols than coloured pigments [8][9]. Fruit pigments serve as a signal to seed-dispersal agents.About the fruits, ripeness fruit colours generally originate by carotenoids and flavonoids seeds.It can also be coloured by flavonoid and carotenoid such as the purple flavonoid and yellow carotenoid found in maize kernel[10].

IMPORTANCE OF PIGMENTS IN HUMAN HEALTH:

Despite the fact that food colouring additions are strictly regulated[11], consumers often choose naturally tinted foods and food items enhanced with pigments derived from plants in addition to avoiding the possible health risks related to artificial chemical colorants.This is especially true given the negative press surrounding the consumption of some artificial colouring compounds.Due to their established biologically active qualities carotenoids, anthocyanins, chlorophylls and betalains are examples of natural plant colours.They are thought to have nutraceutical value and bioactive potential.

These pigments have been scientifically linked to strategies for preserving health in humans, preventing the occurrence serious illnesses, or restoring wellness by mending damaged tissues.Once illness has taken hold because the biosynthetic pathways of the pigments are being thoroughly studied, it is now possible to enhance or alter the pigment content in a food crop using either strategy[12].A variety of bioassays and tests have been forwarded to establish the biological efficacy of natural pigments in the treatment of humans including in vitro(laboratory bench top) bioassays, in vivo (animal trials) epidemiological studies of the population and less frequently clinical (human intervention) trials. Almost all early support for the roles of pigments in biological systems was based on an eclectic set of anecdotal and/or epidemiological evidence, not always well documented; however, increasingly, with the surge of interest in the functional /natural foods for health the evidence is shifting towards replicated in vivo animal models and clinical trials especially for the most widely researched pigments like anthocyanins and some carotenoids[13].

PIGMENTS	SOURCE	COLOR	HEALTH BENEFITS	REFERENCE
Anthocyanins	✓ Plants(fruits, vegetables)	✓ Red ✓ Blue ✓ Purple	✓ High antioxidants activity ✓ Reduceinflammatory insult anticancer modulate immune response	[14][15]
Betalains	✓ Plants(flowers) ✓ Fungi	✓ Red/Violet ✓ Yellow/Orange	✓ Antioxidants ✓ Anticancer ✓ Antilipidemic ✓ Antimicrobial	[16]
Carotenoids	✓ Plants ✓ Marine algae ✓ Microorganisms	✓ Yellow ✓ Orange ✓ Red	✓ Improve immune system ✓ Antioxidant ✓ Provitamin A activity	[17][18]

			✓ Preventcardiovascular disease ✓ Anticancer property	
Chlorophylls	✓ Algae ✓ Cyanobacteria ✓ Higher plants	✓ Majorly green	✓ Antioxidants ✓ Antimutagenic ✓ Anti-inflammatory	[19]

ANTHOCYANINS:

The term anthocyanin is derived from the Greek words meaning flower and blue. Anthocyanins are natural pigments belonging to the flavonoid family. They are responsible for the blue, purple, red and orange colour of many fruits and vegetables. More than 500 different structures have been identified [20][21][22][23]. Anthocyanins are present in different plant organs, such as fruits, flowers, stems, leaves and roots [24]. These pigments are normally found dissolved uniformly in the vacuolar solution of epidermal cells. However, in certain species, the anthocyanins are localized in discrete regions of the cell vacuole, called anthocyanoplasts [25].

NAME OF THE PLANT	SOURCE	FAMILY	REFERENCE
Blue Berry	*Ericaceae Vaccinium*	Rosaceae	[26]
Black Berry	*Rubus fruticous L.*	Rosaceae	[27]
Cherry	*Prunus avium L.*	Rosaceae	[28]
Plum	*Prunus domestica L.*	Rosaceae	[29]
Pomegranate	*Punica granatum L.*	Lythraceae	[30][31]
Egg Plant	*Solanum melongena L.*	Solanaceae	[33]
Carrot	*Daucus carota L.*	Apiaceae	[34]
Hibiscus	*Hibiscus sabdariffa*	Malvaceae	[35]
Rose	*Rosa*	Rosaceae	[36]
Lavender	*Lavandula angustifolia*	Lambiaceae	[37]
Moringa Glory	*Ipomoea marginata*	Convolvulacea	[38]
Dianthus	*Dianthus caryophyllus L*	Caryophyllaceae	[39]

CAROTENOIDS:

The most prevalent type of pigment found in plants microbes and marine algae is called a carotenoid which consists of eight isoprenoid units and forty carbon atoms.It is divided into two categories according to oxygen fixation and has superior antioxidant qualities. Its colour is either yellow or yellow-orange.Due to its advantages for human health including enhanced immunity antioxidant capacity and provitamin A activity.Carotenoid pigments like beta-carotene, lycopene, lutein, astaxanthin, beta-cryptoxanthin, fucoxanthin, zeaxanthin and capsant capsorubin are utilized in numerous sectors[40].

NAME OF THE PLANT	SOURCE	FAMILY	REFERENCE
Mango	*Mangifera indica L.*	Anacardiaceae	[41]
Papaya	*Carica papaya Linn*	Caricaceae	[42]

Orange	*Citrus sinensis*	Rutaceae	[43][44]
Apricot	*Prunus armeniaca L.*	Rosaceae	[45]
Pumpkin	*Cucurbita pepo*	Cucurbiteae	[46]
Yellow Capsicum	*Capsicum annuum*	Solanaceae	[47]
Sweet Potato	*Ipomoea Batatas*	Convolvulaceae	[48]
Yellow Tomato	*Solanum lycperscum L.*	Solanaceae	[49]
Corn	*Zea mays Linnaeus*	Poaceae	[50]
Sunflower	*Helianthus annuus L.*	Asteraceae	[51]
Marigold	*Tagetes erecta Linn*	Asteraceae	[52]
Calendula	*Calendula officinalis Linn*	Asteraceae	[53][54][55]

CHLOROPHYLL:

Higher plants chlorophylls are fat and oil-soluble pigments found solely in algae and cyanobacteria through photosynthesis chlorophyll which has a greenish hue and a porphyrin ring with Magnesium (Mg) as the central atom is involved in the synthesis of complex biomolecules such glucose from carbon dioxide as well as water.Its side chain is composed of 20 hydrocarbon phytol that is esterified to one pyrrole ring.The porphyrin ring and phytol combine to form the chlorophyll molecule which is further divided into chlorophyll A, chlorophyll B, chlorophyll C and chlorophyll D. The most common sources of chlorophyll are chlorella also known as blue-green algae, spirulina, thread spinach,Enteromorpha, sea cucumber stem and a few coloured plant sources photosynthesis a process that produces sugars depends on the pigment chlorophyll for instance the growth of the aquatic species. Enteromorpha produces colouring agents with potent antimutagenic and anti-inflammatory properties[56].

NAME OF THE PLANT	**SOURCE**	**FAMILY**	**REFERENCE**
Green apple	*Malus domestica*	Rosaceae	[57]
Kiwi	*Actinidia deliciosa*	Actinidiaceae	[58]
Guava	*Psidium guajava*	Myrtaceae	[59]
Green Grapes	*Vitis vinifera*	Vitaceae	[60]
Avocado	*Persea Americana Mill*	Lauraceae	[61]
Spinach	*Spinacia Oleracae Linn*	Chenopodiaceae	[62]
Green Beans	*Phaseolus vulgaris*	Fabaceae	[63]
Cucumber	*Cucumis sativus L.*	Cucurbitaceae	[64]
Lettuce	*Lactuca sativa L.*	Asteraceae	[65]
Kale	*Brassica oleracea*	Brassicaceae	[66]
Chrysanthemum Flower	*Chrysantheum*	Asteraceae	[67]
Broccoli	*Brassica oleraceae*	Brassicaceae	[68]

BETALAINS

Betalains are aromatic indole dyes that dissolve in liquids predominantly have red or purple and yellow or orange in hue depending on colour.They can be separated into two different classifications betaxanthins and the pigment betacyanins. The combination of the betalamic acid with cyclo-d DOPA facilitates the synthesis of betacyanins a derivative of betanidin consequently. Betaxanthins are produced when betalamic acid and amino acids are combined these betaxanthins which consist of betanin, isobetanin, probetanin, neobetanin, amaranthine, isoamaranthine and phyllocactin. They are produced by fungi and plant basidiomycetes additionally flowers like bougainvillea glabra which are found practically everywhere in India except from mountainous areas may be used to make betalain[69].

NAME OF THE PLANT	SOURCE	FAMILY	REFERENCE
Beetroot	*Beta vulgaris L.*	Chenopodiaceae	[32]
Dragon fruit	*Hylocereus*	Cactaceae	[70]
Star Fruit	*Averrhoa carambola L.*	Oxalidaceae	[71]
Jack fruit	*Artocarpus heterophyllus*	Moraceae	[72]
Rambutan	*Nephelium lappaceum var*	Sapindaceae	[73]
Litchi	*Litchi chinensis*	Sapindaceae	[74]
Tomato	*Lycopersicon esculentum Mill*	Solanaceae	[75]
Turmeric	*Curcuma longa*	Zingiberaceae	[76]
Amaranthus	*Amaranthus tricolor*	Amaranthaceae	[77]
Radish	*Raphanus sativus*	Brassicaceae	[78]
Bougainvillea	*Bougainvillea Spectabilis Willd*	Nyctaginaceae	[79]
Lotus	*Nelumbo nucifera*	Nymphaeaceae	[80]

CHEMICAL NATURE

ANTHOCYANINS:

The pigment known as aphenolic ingredient that consist of initially hydrogenated derived products of flavylium salts or glycosides of methoxy compounds. agricultural products have various kinds of anthocyanins that include cyanide and delphinidin which are antioxidants the adherence that is organic acids and groupings of to the sugar moieties within the matrix of the amino acids.Pelargonidin, malignant, peonidin as well as and the mineral petunidin determinesthe predominant anthocyanin associated with is cyanidin 3-glucoside[81].Based on its ionic structure's anthocyanins colouring in solution depends on pH[82].They have blue hues when the solutions become closer to a balanced pH and the hue of red hues when it is the solutions become stronger in acidity lower pH values. donate anthocyanins more stability due to increased protonation brought on by an increase in pH-coloured pigments become more soluble in water at low pH.Furthermore, it has been demonstrated that pigment stability and level increase color stability by reducing polymerization processes at neutral pH stable quinonoid anions with a purple hue are created [83]

.BETALAINS:

The nitrogencarrying colouring substances known as betalainsyellow-orange betaxanthins and violet-red betacyaninsare created when tyrosine is divided into two fundamentals groups.The composition of the coloured substance of betalamic acid which all colors made from betalain [84].The pigments categorized as the pigment betacyanin or betaxanthin depends on the kind of betalamic acid addition residue.The several ways that the betalain subgroups manifest among the betacyanins are a sign of structural differences.The condensation of the cyclodopa residue with its closed structure extends the electric resonance to the diphenolic aromatic ring in cyclo-34-dihydroxyphenylalanine unique replacement. Different betacyanins are produced by glycosylation and acylation patterns of one or both hydroxyl groups at positions 5 or 6 of betanidin most of these are 5-o-glucosides.However, 6-o-glucosides have been additionally found betanidin is the aglycone of the majority of betacyanins.Additionally, esterification with the acid hydroxycinnamic and glycosylation of the 5-ob-glycoside caused by cyclo-dopa are the most common forms of betacyanin.Red beetroot main pigment is a substance called betanin which depending on the solvent has two absorption maxima one in the visible range at (535-540 nm) and another in the UV at (270-280 nm). The red and violet hues are caused by distinct betacyanin substitution patterns, glycosylation of betanidin typically results in a hypsochromic shift of roughly 6 nm, but a second sugar moiety linked to the first one did not seem to significantly alter the colorwhile aliphatic acyl moieties do not change the spectra [88][89].Acylation with hydroxycinnamic acids produces a third maximum at (300-330 nm)[89].Betaxanthins have distinct side chains of amines or amino acids hypso or bathochromic alterations are caused by structural changes in betaxanthins[85].

CAROTENOIDS:

Carotenoid are naturally occurring in natural colorants that give plants their yellow, orange, red and even purple colours. They are present in the photosynthetic microbes, seaweed, fungi, higher plant and even humans.Most carotenoid are made up of eight isoprene units that depending on how the polyene chain is modified and whether oxygen is present in the end groups form a 40-carbon backbone.Their molecular structure is usually made up of a long polyene chain with multiple linked double bonds and terminal end groups on both sides.Lycopene and beta-carotene are two well-known examples of carotenes which are hydro carotenoids,carbon molecules with over fifty variants found in nature hydrocarbon carotenoids and oxygen-containing carotenoids are the two main categories of carotenoids.Terminal portions of pigments in addition to having more than fifty distinct types or pigments present in organic matter namely lycopene and beta-carotene polymeric chain topologies classify them into two groups these substances also contain xanthophylls and carotene[90].However, carotenoids pigments such as plant yellow pigments red pigments brown pigments ocean pigments carotenoid composed of molecular oxygen such furan oxide group, hydroxy carbonyl aldehyde and carboxylic epoxide respectively the discovery of around 800 different forms of xanthophylls that exist proteins mixtures containing certain xanthophylls such as the glycoside[90][91].Most carotene molecules have forty carbon particles in their internal structures however some have 45 or 50 links apocarotenoids also known as breakdown products of c40 carotenoids are found in some plant and animal species and are composed of carbon structures with fewer than 40 carbons[90][91].

CHLOROPHYLL:

Chlorophyll molecules using a single reduction double-bonds defined by chlorine in the central region comprising the chlorine bonds that is attached to the tetrapyrrole rings are an example of the porphyrin ring structures.Plant chlorophyll supplements which are comprised of a hydrophilic porphyrin with the head of the group and a lipophilic hydrocarbon tail part are frequently believed to be intractable in polar solutions[93].A lengthy hydrophobic alkyl chain connects the four pyrrole rings (C_4H_4NH) that make up the porphyritic structures of chlorophyll molecules which are subsequently coordinated by a magnesium ions [92].In the middle of themplants,algae and cyanobacteria all contain chlorophylls which are green oil-soluble amphiphilic pigments with two different structures. ChlorophyllA($C_{55}H_{72}MgO_5N_4$) has a methyl (–CH_3) group at the carbon-7 position while chlorophyll b($C_{55}H_{70}MgN_4O_6$) has an aldehyde (–CHO) group at the same position[94].The two chlorophylls distinct structural characteristics result in their different colors in plants, chlorophyll A is blue-green and chlorophyll B is blue-yellow the ratio of chlorophyll A to chlorophyll B is 3:1 [95].

EXTRACTION OF NATURAL PIGMENTS

ANTHOCYANINS

Extraction

Anthocyanin, like flavonoids in general, have aromatic rings containing polar substituent groups (hydroxyl, carboxyl, and methoxyl) and glycosyl residues that all together produce a polar molecule. Consequently, they are more soluble in water than in nonpolar solvents, but depending on the media conditions anthocyanins could be soluble in ether at a pH value where the molecule was unionized [96]. Conventional methods of pigment extraction usually employ dilute hydrochloric acid in methanol.

Methanol containing 0.001% HCl was the most effective, but HCl is corrosive, and methanol produces toxic effects after human exposition; consequently, food scientists frequently prefer the use of other extraction systems. Among other solvents, one finds ethanol and water, 80 and 27% as effective as methanol, respectively. Additionally, it must be taken into account that aromatic acyl acid linkages are relatively stable in dilute HCl/MeOH mixtures, but aliphatic Di carboxyl acyl groups (malonic, malic, oxalic) are susceptible to diluted acids, and different methodologies must be considered [97][98][99][100].

Separation

Nowadays, thin layer chromatography (TLC) is widely used, because this technique has shown continuous innovations. For preparative work, droplet counter current chromatography has been applied to separate the anthocyanins of black currant. On the other hand, a general patent for the purification of anthocyanins involves selective absorption on a finely divided oxide such as silicic acid, titanium oxide or alumina, which is coated with a styrene polymer [98]. However, undoubtedly, the main developments of recent years in the research of anthocyanins are the introduction of HPLC for their separation and quantitation. Interestingly, it is possible to distinguish zwitterionic anthocyanins by their HPLC chromatographic separation [99].

Characterization

Spectrophotometry is typically used to assess colour [98]. UV-visible spectroscopy has been used to study isolated pigments. Anthocyanins exhibit strong absorption between 520 and 560 nm (visible region), while all flavonoids exhibit strong absorbance between 250 and 270 nm (UV region). It has been proposed that ring A may be primarily responsible for UV absorption, while ring B and the pyran may be more apparent. Glycosylation on Bring can also be detected via UV-visible spectroscopy because the spectra exhibit a hypsochromic shift with respect to the un-glycosylated Bring. This technology also detects anthocyanin acylation, only when the 3′- and 5′-OH groups are free (nonacylated) does a bathochromic shift occur in the presence of AlC13[100].

Furthermore, the best method for observing the co pigment effect is visual absorbance. Anthocyanin visible spectrum exhibits a bathochromic shift that is due to a solvation phenomenon and the hyperchromic effect that increases the intensity of this maximum, producing a highly colourful species [101][99][102]. The anthocyanin substitution pattern has been demonstrated using Raman spectroscopy; the presence of phenyl ring substitutions on benzopyrylium results in distinct spectrum changes [102][103]. As previously said with carotenoids, the tentative identification of the separated anthocyanins has been made possible by the integration of the diode array detector dad into the HPLC approach.In addition, with the introduction of innovated methodologies such as NMR and mass spectrophotometry, anthocyanin compounds have been identified conclusively. Proton NMR has been used to study the self-association of anthocyanin molecules, while carbon-13 NMR spectroscopy has been used to define the sequence, position, and configuration of sugar residues in flavonoid glycosides[104]. In addition, mass spectrometry increased its potential with the introduction of the FAB-mass detector, which permitted observing an intense peak for the molecular ion, something difficult with the previously designed detectors; anthocyanins are unstable and with low volatility [105].

CAROTENOIDS

Extraction

Carotenoids are soluble in lipids or in nonpolar solvents, except when they form complexes with proteins and sugars. Hence, they are extracted with nonpolar solvents. If the tissue is previously dried, then water-immiscible solvents are used such as petroleum or ethyl ether; with the fresh materials acetone or ethanol are used, which have two functions, extracting and dehydrating solvents. The extraction process consists of the removal of hydrophobic carotenoids from a hydrophilic medium. The use of nonpolar solvents is not recommended because of penetration through the hydrophilic mass that surrounds pigments is limited, while slightly polar solvents dissolve poorly carotene in dried samples and solubility diminish in fresh samples. Thus, it was postulated that complete extraction can be reached by using samples with low moisture, and slightly polar plus nonpolar solvents[106]. Currently, industrial extraction consists of pressing and solvent extraction of materials: material is milled and pelleted, mixed with hexane, and heated in a special recipient covered with a vapor jacket; hexane is eliminated in a film evaporator and afterward by vacuum distillation, and the main problems to be solved are to diminish pigment degradation, increase extraction performance, and solve safety and environmental problems. More than 50% of the pigments is lost during this extraction process; oil extraction industries emitted into the environment 210 to 430 million liters of hexane that together with other organic compounds can produce nitrogen oxides and other pollutants. These products in the last stage generate ozone and other highly dangerous photochemical oxidants[107].With these perspectives, several researchers have proposed alternative extraction solvents. Heptane has been used, and the following characteristics have been mentioned: lower volatility than hexane, similar extraction efficiency, and good quality product (oil)[108]. When mixtures of heptane-isopropanol or only isopropanol are used, more antioxidants are extracted and oils with enhanced stability are obtained, and considering that isopropanol has lower flammability than hexane, this solvent could be a good alternative in oil extraction[109][110]. Supercritical fluid extraction (SFE) is the other alternative method for extraction. Up to 1986, only two works using SFE had been reported, but in the period 1986-mid-1989 26 works were published; this large increment could be explained, because this technique shows the following advantages: rapidity, solvent strength can be controlled, and the solvents used are gases friendly to the environment and with low toxicity. Interestingly, with this method the concentration stage is avoided because solvents are eliminated immediately under environmental conditions[111][112][113]. SFE has been used commercially to obtain caffeine from coffee and hop oil. Lutein and carotene were extracted from protein leaf concentrate by using CO2 as a solvent in SFE, and it was shown that conditions can be manipulated to make a selective extraction[114].

Separation

Separation techniques can be divided into two categories non-chromatographic and chromatographic. The non chromatographic method uses mainly phase partition for example by using petroleum ether and aqueous methanol (90%).Carotenoids are dissolved and nonpolar compounds recovered in epiphase petroleum ether.In chromatographic methods adsorbents have been used such as sucrose, cellulose, starch, CaCO3, Ca3(PO4)2,Ca(OH)2, CaO, MgCO3, MgO, ZnCO3, Al2O3, Silicic acid, silica gel, kieselguhr, Microcel C, and mixtures[115]. These days, special flash opens columnar chromatography are used as a typical method for obtaining a pure carotenoid[116][117].High-pressure liquid chromatography is the preferred columnar chromatography to perform both qualitative and quantitative analyses of carotenoids. Open columnar chromatography is used as a repurification stage to separate groups of carotenoids with similar characteristics [118][119][120].

Characterisation

Mass spectroscopy is the method most frequently employed in carotenoid analysis mostly due to the extremely tiny sample quantity needed for analysis mass spectroscopy offers information on carotenoid MW and the fragmentation 5 pattern aids in determining the carotenoid structure [121]. However, because to carotenoid instability it is crucial to select suitable MS equipment fast atom bombardment is one of the first and most effective MS ionization methods(FAB)[122][123]. these days new methods have been developed by taking into account intrinsic carotenoid instability and using an electrospray detector that is 100 times more sensitive than traditional methods HPLC paired with a MS detector lcms is a very practical methodology (picomolar)[120]. The ionization of chemicals at atmospheric pressure (APCI)[124]. It isanadditional ionizing method carotenoid in biological systems can now be studied thanks to RAMAN spectroscopy and circular dichroism [122][125]. Spectroscopy using photoacoustics by identifying peaks or shoulders in the light spectrums region that correspond to carotenoids or chlorophylls paprikas pigment content was ascertained using PA with the information supplied it was feasible to differentiate between various paprika samples[126].

BETALAINS

Extraction

Betalain pigments are commonly extracted from plant tissues or cell cultures after maceration or grinding of the raw material. Although extraction can be performed using distilled water under cold or ambient conditions, complete pigment recovery generally requires aqueous organic solvents such as methanol or ethanol at concentrations ranging from 20–50% (v/v)[127]. In certain cases, aerobic fermentation of plant juices using microorganisms like Saccharomyces cerevisiae or Aspergillus Niger has been reported to lower free sugar content and enhance betacyanin concentration[128].To prevent enzymatic degradation, the extract may be subjected to brief thermal treatment (approximately 70 °C for 2 minutes); however, this process can result in partial pigment loss. Betacyanins can be selectively precipitated by mild acidification using hydrochloric acid or acidified ethanol (0.4–1% HCl). Subsequent addition of 95% aqueous ethanol facilitates the recovery of betaxanthins[127].

Separation

Ion-exchange and column chromatography

A rapid separation approach involves stirring the plant extract with ion-exchange resins such as Dowex 50W-X2, DEAE-Sephadex A25, or similar materials, which bind betalains primarily through non-ionic interactions. After adsorption, the resin is rinsed with dilute hydrochloric acid (0.1% v/v), followed by pigment elution using water. Further purification is achieved by column chromatography employing supports like polyamide, Polycarp-AT, polyvinylpyrrolidone, or Sephadex G-15/G-25. The chromatographic and electrophoretic behaviour of pigments isolated from unknown sources can be compared with documented standards for identification[127].

Electrophoresis and thin-layer chromatography

Paper electrophoresis using pyridine-based solvent systems combined with formic or acetic acid, or cellulose media, is widely used for betalain analysis. Betacyanins exhibit characteristic migration patterns, appearing as zwitterions at (pH 2), monoanions between (pH 2–3.5), and bisanions above (pH 3.5) up to neutral pH. Betaxanthin mobility is typically evaluated relative to Indica xanthin, while betacyanin mobility is compared with betanin standards[127].Electrophoresis is commonly performed with pyridine–citric acid buffer at a voltage gradient of 5.6 V/cm and maintained at 4 °C. More recently, capillary zone electrophoresis (CZE) has emerged as an effective technique for betalain analysis, particularly in Beta vulgaris[129]. Using fused-silica capillaries operated at 15 °C and −22 kV, CZE allows the resolution of betanin, isobetanin, and their corresponding aglycones. Preparative thin-layer chromatography has also been described using cellulose-coated plates (0.5 mm thickness) and solvent systems composed of isopropanol, ethanol, water, and acetic acid in different volumetric ratios[130].

Characterization

Simple qualitative tests have been established to differentiate betacyanins from anthocyanins based on their color responses to variations in pH and temperature[131]. Analytical characterization of betalains, like other natural pigments, relies heavily on UV–visible spectrophotometry. Betacyanins typically display absorption maxima around 540 nm, whereas betaxanthins absorb near 480 nm. Consequently, UV–visible spectroscopy has been fundamental in early betalain identification studies and remains useful for monitoring structural changes and degradation processes[127].

ANTHOCYANINS (Fruits, vegetables, flowers)

NATURAL PIGMENTS	PHARMACOLOGICAL ACTION	MECHANISM OF ACTION	APPLICATION	REFERENCE
1.Blueberry	Anticancer	**Blueberry** (Anthocyanins) Pyruvic acid ↓ Inhibits the proliferation of cancer cells ↓ **Anticancer activity**	✓ Cardiovascular and heart diseases ✓ Anti-inflammation ✓ Anti-obesity ✓ Anti diabetes ✓ Antimicrobial Activities	[132] [133]
2.Blackberry	Anti-inflammatory	**Blackberry** (Anthocyanins) ↓ Supress the production of NO ↓ **Anti-inflammatory activity**	✓ Fever ✓ Bleeding ✓ Discharge of vagina inflammation ✓ Slow wound healing ✓ Ulcers of mouth ✓ Mouth inflammation	[134] [135] [136]
3.Cherry	Antioxidant	**Cherry** (Anthocyanins) ↓ Inhibit copper triggered low density lipoproteins oxidation ↓ **Antioxidant activity**	✓ Inflammation ✓ Carcinogenesis ✓ Apoptosis ✓ Cell proliferation angiogenesis	[137] [138] [139]
4.Plum	Antihyperlipidemic	**Plum** (Anthocyanins) ↓ Decrease plasma and LDL cholesterol in mild hypercholesterolemic actions ↓ **Anti-hyperlipidemic activity**	✓ Acid dyspepsia ✓ Nausea ✓ Vomiting ✓ Bilious fevers ✓ Dysmenorrhea ✓ Leucorrhea ✓ Miscarriage ✓ Asthma and fever	[140] [141] [142]

5.Pomegranate	Antibacterial	**Pomegranate** (Anthocyanins) ↓ Inhibit the growth of gram positive and gram-negative bacterial strains ↓ **Antibacterial activity**	✓ Cardiovascular disorders ✓ Diabetes ✓ Manage obesity	[143] [144]
6.Eggplant	Hypolipidemic Action	**Eggplant** (Anthocyanins) S. melongena ↓ Reduced the blood levels of total and LDL cholesterol ↓ **Hypolipidemic activity**	✓ Antioxidant ✓ Anti-diabetic ✓ Bronchitis asthma ✓ Cardiac activity ✓ CNS depressant activity ✓ Spasmogenic Activity	[153] [154]
7.Carrot	Anti-bacterial	**Carrot** (Anthocyanins) ↓ Inhibitory effect on the entero pathogen, campylobacter coli and C. lari strains ↓ **Anti-bacterial activity**	✓ Wound healing ✓ Lung cancer ✓ Breast cancer ✓ Colon cancer ✓ Glowing skin ✓ Healing of sunburns	[155] [156] [157] [158]
8.Morning glory	Antinociceptive activity	**Morning glory** (Anthocyanins) ↓ Exhibit a good antinociceptive activity Ipomoea pes-caprae Treat dolorous processes ↓ **Antinociceptive activity**	✓ Inhibition of platelet aggregation ✓ Diarrhoea ✓ Vomiting ✓ Diuretic ✓ Laxative properties	[159] [160] [161]

9.Dianthus	Antiviral	**Dianthus** (Anthocyanins) → Dianthin antiviral proteins anti-HIV activity. → Suppress translation process of viral proteins → **Antiviral activity**	✓ Gum infections ✓ Gastro intestinal disorder ✓ Throat infections ✓ Cardiotonic ✓ Diaphoretic ✓ Vermifuge	[162] [163] [164]
10.Hibiscus	Anti-bacterial	**Hibiscus** (Anthocyanins) → Antibacterial activity against different gram-positive and gram-negative bacteria → **Anti-bacterial Activity**	✓ Menstrual cycle ✓ Ailing infants ✓ Gonorrhoea ✓ Anti tussive ✓ Contraceptive ✓ Bronchial catarrh ✓ Hypertension	[165]
11.Rose	Anti-inflammatory	**Rose** (Anthocyanins) → Inhibition of the NF-kappaB signalling pathway → Decrease the production of pro-inflammatory cytokines → **Anti-inflammatory activity**	✓ Astringent ✓ Antidiarrhoeic ✓ Diuretic ✓ Antiscorbutic ✓ kidney stones	[166] [167] [168] [169]

12.Lavender	Anti-microbial	**Lavender** (Anthocyanins) → Effective against all gram positive and gram-negative bacteria, active against E. coli → **Anti-microbial activity**	✓ Neuro psychiatric ✓ Antimicrobial ✓ Antineoplastic ✓ Cardio plastic ✓ Insomnia ✓ Postoperative pain	[170] [171] [172] [173]

CAROTENOIDS (Fruits, Vegetable, Flowers)

NATURAL PIGMENTS	PHARMACOLOGICAL ACTION	MECHANISM OF ACTION	APPLICATION	REFERENCE
13.Mango	Anti-cancer	**Mango** (Carotenoids) → Antiproliferative effect → Inhibiting AGS cancer cell proliferation → **Anti-cancer Activity**	✓ Dentifrice ✓ Antiseptic ✓ Astringent ✓ Leucorrhoea ✓ Wound healing ✓ Liver disorders	[174] [175]
14.Papaya	Anti-malarial	**Papaya** (Carotenoids) → Inhibit the immune mediated destruction of platelets → Suppress the bone marrow → Stabilize the membrane of infected cells → **Anti-malarial activity**	✓ Dyspepsia ✓ Gastrointestinal tract digestion issues ✓ Anti-fungal	[176] [177]

15.Orange	Antiosteoporotic	**Orange** (Carotenoids) Citrus ↓ Activity against osteoporosis ↓ **Antiosteoporotic activity**	✓ Constipation ✓ Obesity ✓ Menstrual disorder ✓ Hypertension ✓ Tuberculosis ✓ Bronchitis ✓ Depression	[178] [179]
16.Apricot	Hepatoprotective effect	**Apricot** (Carotenoids) ↓ Increase in serum constituents such as albumin ↓ **Hepatoprotective effect**	✓ Asthma ✓ Bronchitis ✓ Emphysema ✓ Constipation ✓ Leprosy ✓ Leukoderma ✓ Vaginal infections	[180] [181]
17.Pumpkin	Anti-hyperglycaemic	**Pumpkin** (Carotenoids) ↓ Glucose homeostasis Tocopherol isomers Alleviation of diabetes ↓ **Anti-hyperglycaemic activity**	✓ Wound healing ✓ Tumour growth inhibition ✓ Vermifuge ✓ Kidney stones	[182] [183] [184] [185] [186] [187] [188] [189]
18.Yellow capsicum	Reduces the risk of Cardiovascular	**Yellow capsicum** (Carotenoids) ↓ Avert the clotting of the blood, resulting in strokes ↓ **Reduces the risk of Cardiovascular**	✓ Obesity ✓ Hypertension ✓ Diabetes ✓ Atherosclerosis	[190] [191] [192] [193]

19.Sweet potato	Anti diabetic	**Sweet potato** (Carotenoids) ↓ β-pancreatic cells Insulin secretion ↓ **Anti diabetic activity**	✓ Oral infections ✓ Inflammatory diseases ✓ Management of diabetic conditions	[194] [195] [196] [197]
20.Yellow tomato	Anthelmintic	**Yellow tomato** (Carotenoids) methanolic extract ↓ Causing paralysis and death of earthworms ↓ **Anthelmintic activity**	✓ Antioxidants ✓ Diabetes ✓ Cell damage ✓ Lower cholesterol ✓ Combating stroke ✓ Treatment of vasodilatation	[198] [199] [200]
21.Corn	Anti-oxidant	**Corn** (Carotenoids) ↓ Prevent oxidation ↓ Eliminating free radicals ↓ **Anti-oxidant activity**	✓ Lowering blood pressure ✓ Decrease prostate inflammation ✓ Edema ✓ Obesity ✓ Promote relaxation	[201] [202] [203]
22.Sunflower	Anti diabetic	**Sunflower** (Carotenoids) ↓ Preventing diabetes ↓ **Anti diabetic Activity**	✓ Heart disease ✓ Pulmonary infections ✓ Coughs and colds ✓ Whooping cough	[204] [205] [206]

23.Marigold	Antibacterial	**Marigold** (Carotenoids) ↓ Inhibit the growth of bacteria ↓ **Antibacterial activity**	✓ Epileptic fits ✓ Astringent ✓ Carminative ✓ Liver complaints	[207] [208] [209] [210]
24.Calendula	Cardio-protective effect	**Calendula** (Carotenoids) ↓ Altering antioxidant and anti-inflammatory pathways ↓ **Cardio-protective effect**	✓ Gynaecological issues ✓ Gastro-intestinal disorders ✓ Skin injuries and certain burns	[211] [212] [213]

CHLOROPHYLL (Fruits, Vegetables, Flowers)

NATURAL PIGMENTS	PHARMCOLOGICAL ACTION	MECHANISM OF ACTION	APPLICATION	REFERENCE
25.Green apple	Anti-obesity	**Green apple** (Chlorophyll) ↓ Inhibit the oxidation of lipoprotein cholesterol ↓ **Anti-obesity activity**	✓ Hepatic cholesterol	[214] [215]
26.Kiwi fruit	Cardioprotective activity	**Kiwi fruit** (Chlorophyll) ↓ Platelet aggregation ↓ Increased blood pressure&oxidation of cholesterol ↓ **Cardioprotective activity**	✓ Blood clotting ✓ Absorption of Vitamin D ✓ Depression ✓ Anaemia ✓ Cancer ✓ Blood pressure ✓ Diabetes	[216] [217] [218]

27.Guava	Anthelmintic	**Guava** (Chlorophyll) ↓ Pro-oxidative activity ↓ Increase in total proteins ↓ **Anthelmintic activity**	✓ Wound-healing capabilities ✓ Regulation of blood glucose levels	[219] [220] [221]
28.Avacado	Analgesic effect	**Avacado** (Chlorophyll) ↓ Increase the threshold of pain ↓ **Analgesic effect**	✓ Anti-ageing ✓ Breast cancer ✓ Cosmetics	[222] [223]
29.Green grapes	Anti-diabetic	**Green grapes** (Chlorophyll) ↓ Inhibiting the activities of amylase and alpha-glucosides ↓ **Anti-diabetic activity**	✓ Laxatives ✓ Carminatives ✓ Cold and flu ✓ Anemia ✓ Allergies ✓ Bronchitis	[224] [225]
30.Green peas	Anti inflammatory	**Green peas** (Chlorophyll) ↓ Preventing hydrolysis ↓ **Anti inflammatory Activity**	✓ Treat liver problems ✓ Gastrointestinal issues	[226] [227]

31. Spinach	Anticancer	**Spinach** (Chlorophylls) → Inhibition of cough → **Anticancer Activity**	✓ Asthma ✓ Leprosy ✓ Biliousness ✓ Treatment of urinary calculi	[228] [229]
32. Broccoli	Anticancer	**Broccoli** (Chlorophyll) → Interacts with GSH to form SFN-GSH → Accumulation of SFN was encouraged → **Anticancer activity**	✓ Antihypertensive Activity ✓ Anti-inflammatory Activity ✓ Anticancer Activity ✓ Antimicrobial Activity ✓ Antioxidant Activity	[230]
33.Green Beans	Antioxidant	**Green Beans** (Chlorophyll) → Inhibited the production of free radicals → Activated antioxidant enzymes in the liver and kidneys → **Antioxidant activity**	✓ Diabetes Mellitus	[231] [232]
34.Cucumber	Antihepatotoxic	**Cucumber** (Chlorophyll) → Reduce the effect of carbon tetrachloride → **Anti hepato toxic activity**	✓ Anti-acne Activity ✓ Lower blood pressure ✓ Prevent Diabetes	[233] [234] [235] [236]

35.Lettuce	Anti -Diabetic	**Lettuce** (Chlorophyll) ↓ Inhibit α-amylase and α-glucosidase activities ↓ Reduce post-prandial hyperglycemia ↓ **Anti -Diabetic activity**	✓ Anti-aging ✓ Skin treatments ✓ Bone calcification	[237] [238]
36.Kale	Anticarcinogenic	**Kale** (Chlorophyll) ↓ Inhibitory effect on cell viability with concentration ↓ **Anti carcinogenic activity**	✓ Colorectal Cancer ✓ Anticancer Activity	[239]
37.Bottle gourd	Central Nervous System activity	**Bottle gourd** (Chlorophyll) ↓ Reduces spontaneous motor activity ↓ **Central Nervous System activity**	✓ Cooling effect ✓ Constipation ✓ Skin disease ✓ Diabetes ✓ Weight loss	[240]
38.Green chrysanthemum	Anti-inflammatory activity	**Green chrysanthemum** (Chlorophyll) ↓ Inhibited the output of inflammatory mediators ↓ **Anti-inflammatory activity**	✓ Diabetes ✓ Fever ✓ Cold ✓ Increasing metabolism ✓ Boost immune system	[241] [242]

BETALAINS (Fruits, Vegetables, Fruits)

NATURAL PIGMENTS	PHARMACOLOGICAL ACTION	MECHANISM OF ACTION	APPLICATION	REFERENCE
39.Beetroot	Anti-Carcinogenic	Beetroot (Anthocyanins) → Interruption of cell inflammation → Proliferation of human tumour cells are inhibited → **Anti-Carcinogenic activity**	✓ Hepatic, and renal protection ✓ Regulate the oxidative stress ✓ Apoptosis activity ✓ Maintain plasma, NO2 level ✓ Endothelial function ✓ Improve physical and cognitive performance	[145] [146] [147] [148] [149] [150] [151] [152]
40.Dragon fruit	Anti inflammation	**Dragon fruit** (Betalains) → Inhibited the synthesis of reactive oxygen and nitrogen species → **Anti-inflammatory activity**	✓ Hypoglycaemic ✓ Wound disinfectant ✓ Diuretic ✓ Dysentery ✓ Tumour dissolution ✓ Healing agent	[243]
41.Star fruit	Antiulcer	**Star fruit** (Betalains) → Scavenge ROS such as peroxidase → **Antiulcer activity**	✓ Fever ✓ Sore throat ✓ Cough ✓ Asthma ✓ Chronic headache ✓ Skin inflammation	[244] [245] [246]

42.Litchi	Anticancer	**Litchi** (Betalains) ↓ Inhibit several cycles of cancer cell ↓ DNA damage prevention ↓ **Anticancer activity**	✓ Breast and colon cancer ✓ Hepatoprotective agents ✓ Regeneration of liver cells ✓ Immunotherapy	[247]
43.Jack fruit	Antiviral	**Jack fruit** (Betalains) ↓ Increased risk of cardiovascular disease ↓ **Antiviral activity**	✓ Antioxidant properties ✓ Fights inflammation ✓ Malarial fever ✓ Antibacterial and anthelminthic properties	[248] [249]
44.Rambutan	Anti hyperlipidemic	**Rambutan** (Betalains) ↓ Inhibiting the recapture of serotonin ↓ **Anti hyperlipidaemic activity**	✓ Anti-obesity	[250]
45.White radish	Anticancer	**White radish** (Betalains) ↓ Activation of apoptosis ↓ **Anticancer activity**	✓ Colon cancer ✓ Breast cancer ✓ Lung and prostate cancer	[251]

46.Turmeric	Anti inflammatory	**Turmeric** (Betalains) → Anti- inflammatory action with specific lipoxygenase and COX-2 inhibiting properties → **Anti-inflammatory activity**	✓ Purifies blood ✓ Used as tonic to brain and heart ✓ Enlargement of spleen ✓ Gonorrhoeal discharge	[252]
47.Tomato	Anti carcinogenic	**Tomato** (Betalains) → Prevent the reduction of free radicals → **Anti carcinogenic activity**	✓ Antioxidant ✓ Helps to lower cholesterol ✓ Treatment of vasodilatation	[253]
48.Amaranth	Antioxidant	**Amaranth** (Betalains) → form stable complexes with ferrous ions → decrease the extent of free ions to Fenton's reaction → **Antioxidant activity**	✓ Treatment of piles ✓ Blood disorders ✓ Bladder distress ✓ Tooth ache ✓ Dysentery ✓ Astringent ✓ Diuretic ✓ Haemorrhage ✓ Hepatoprotective agent	[254]
49.Bougainville	Anti inflammatory	**Bougainville** (Betalains) → Inhibits the MAP kinase, NFKb → **Anti-inflammatory activity**	✓ Titration indicator ✓ Cosmetic products ✓ Insecticidal medicines	[255]

50.Lotus	Antioxidant	**Lotus** (Betalains) ↓ Inhibition of total ROS generation by kidney homogenates ↓ **Antioxidant activity**	✓ Diarrhoea ✓ Cholera ✓ Fever ✓ Gastric ulcers	[256] [257]

CONCLUSION:

Phytopigments constitute a promising class of natural compounds with significant therapeutic potential due to their wide-ranging pharmacological activities and favourable safety profile. Their ability to modulate oxidative stress, inflammation, and key molecular pathways involved in disease progression highlights their value as multifunctional therapeutic leads. While substantial preclinical and emerging clinical evidence supports their role in the prevention and management of chronic and lifestyle-related disorders, limitations such as poor bioavailability, stability challenges, and insufficient large-scale clinical validation persist. Addressing these challenges through advanced formulation approaches, mechanistic studies, standardization, and well-designed clinical trials is essential. Overall, phytopigments offer a sustainable and effective foundation for the development of novel pharmaceutical and nutraceutical agents, bridging natural product research with modern therapeutic innovation.

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