

Phytomelanin - A potential antioxidant isolated from dark seed coat of certain plants

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ABSTRACT

Phytomelanins are dark phenolic derivatives found in various parts of plant. Phytomelanin has UV resistance, anti-inflammatory and protective action. Earlier phytomelanin was reported in several families. Since it has several potentials in cosmetic, food and pharmaceutical industry, search for more sources is inevitable. In the present work dark coloured seeds of different taxonomic families were extracted, purified and confirmed by precipitation and decolourization test. All isolated phytomelanin were positive for the confirmatory test. The UV-VIS spectroscopy of *Tamarindus indica*, *Citrullus lanatus*, *Ricinus communis*, *Carica*, *Mirabilis jalapa*, *Annona squamosa* and *Mucuna bracteata* had maximum absorption between 280-285nm. *Acacia concinna*, *Dimocarpus longan* and *Manikara sapota* melanin had absorption between 265-276nm. An appreciable free radical scavenging activity was observed in *Citrullus lanata*, *Ricinus communis*, *Tamarindus indica*, *Annona squamosa*, *Manikara sapota* and *Mucuna bracteata* at 50µl. The EC₅₀ of *Ricinus communis*, *Tamarindus indica* and *Mucuna bracteata* melanin was 25 µl, 15 µl and 40 µl respectively.

Keywords: Dark coloured seeds, Phytomelanin, Antioxidant activity, *Ricinus communis*, *Tamarindus indica*, *Mucuna bracteata*.

INTRODUCTION

Melanin is a black, insoluble pigment that is resistant to degradation by concentrated acids but can be whitened with oxidizing agents. It is found in plants, animals and microorganisms. In plants is called phytomelanin or phytomelan [1]. Phytomelanin is especially found in the seed coats of Asteraceae species. There are only a few reports on the structure, chemical composition and formation of phytomelanins in the last 50 years. It has been reported that black coated seeds survive for years under the soil, resistant to abiotic and biotic stresses [2].

Melanins have been isolated from a variety of phylogenetic sources: animals [3], plants (including seeds) [4][5], bacteria [6] and fungi [7]. Melanins are commonly represented as black and brown pigments, the high molecular weight heterogeneous polymers derived from the oxidation of monophenols and subsequent polymerization of intermediate o-

diphenols and quinones [8]. Melanins are types of pigments, possessing broad biological activities including antioxidant [9], radioprotective, thermoregulative, hypoglycemic [10], chemoprotective, antitumor, antiviral, antiicrobial [11], immunostimulating and anti-inflammatory. Based on these features, natural melanin has the potential to be of great value and application in the fields of pharmacology, cosmetics and functional foods [12]. Due to the unique features of melanin, such as its stable free radical state, ultraviolet-visible (UV-Vis) light absorption, and complexation and ion-exchange capacities, these pigments have attracted growing interest as materials for a broad range of biomedical and technological applications [13][14][15]. Hence search for novel sources of phytomelanins is essential. Therefore this study was carried out to investigate the sources and its antioxidant potentials.

MATERIALS AND METHODS

Selection Of Dark Coloured Seeds

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ricinus communis (Euphorbiaceae), *Dimocarpus longan* (Sapindaceae), *Acacia concinna* (Fabaceae), *Carica papaya* (Caricaceae), *Tamarindus indica* (Fabaceae), *Mirabilis jalapa* (Nyctaginaceae), *Manikara sapota* (Sapotaceae), *Annona squamosa* (Annonaceae), *Citullus lanatus* (Cucurbitaceae), *Mucuna bracteata* (Fabaceae) were selected for phytomelanin screening and extraction.

Isolation And Purification Of Phytomelanin:-

About 5gm of the above seeds were soaked in 0.1M and 1M Sodium hydroxide and extracted after 72 hours. The extracts of various seeds were treated with 2M Hydrochloric acid and allowed to precipitate. The contents were centrifuged at 4000 xg for 5 mins. The pellet was suspended in water twice and centrifuged at 4000 xg for 5 mins. The pellet thus obtained was collected and rinsed in ethylacetate and later ethanol to remove carbohydrates, proteins and lipids. The residue was collected and dried at 100°C. The dried powder was scraped and the yield of phytomelanin was estimated. Thus obtained phytomelanin was checked for its solubility in water, DMSO and alkali. The texture, colour and yield were recorded.

Confirmatory Test For Phytomelanin:

- 1). The extracts of all the seed coats were subjected to Hydrogen peroxide and observed for decolourization and the observations were recorded and photographed.
- 2). To the extract Ferric chloride solution was added and observed for precipitation.
- 3). To the extract Potassium permanganate solution was added and observed for decolourization of purple colour.

Uv -Vis Spectroscopy

The purified phytomelanin was suspended in Phosphate buffer pH-8, filtered and diluted 20 times. The absorbance was recorded in UV-Visible Shimadzu spectrophotometer between 200 and 400 nm.

Antioxidant Assay-DPPH Free Radical Scavenging Activity

The synthesized compounds were studied for their free radical scavenging activity against DPPH (2, 2-diphenyl-1-picryl hydrazyl). Extracts of plant crude prepared at various concentrations (10, 20, 30, 40 and 50 µg/mL) in methanol were taken in small tubes. The extracts were made up to 1 ml using methanol and 1 ml of DPPH was added to each of the tube. The same solution of DPPH in methanol was used as the control and Butylated Hydroxyanisole (BHA) was used as the reference. After 30 minutes of incubation in dark at room temperature, the absorbance of the solution was read at 517 nm. The percent inhibition was calculated using the following formula:

$$\text{Effective concentration \%} = (\text{Control Absorbance} - \text{Test Absorbance}) / \text{Control Absorbance} \times 100$$

Statistical Analysis- All experiments were performed in triplicate, and the data are presented as mean $\pm$ standard error (SE).

RESULTS AND DISCUSSION

Isolation Of Phytomelanin

Phytomelan was isolated from dark coloured seeds. The physical properties such as colour and texture were recorded (Fig No-1). Among the seeds investigated, a higher yield was observed in *Mucuna bracteata* (Fabaceae), followed by *Citrullus lanatus* (Cucurbitaceae) and *Annona squamosa* (Annonaceae). Phytomelanin has also been reported earlier in dicotyledon families such as Campanulaceae, Umbelliferae^[16], Compositae, Agavaceae, Aloaceae, Amaryllidaceae, Hyacinthaceae, Convolvulaceae etc. Earlier work by Anitha and Geetha^[17] had reported the presence of phytomelanin in edible seed coats of members belonging to Fabaceae, Leguminosae, Pedaliaceae and Linaceae. In such families, phytomelanin appears in the epidermis or the hypodermal layer. Taxonomic significance of presence of phytomelan layer in seeds has been elucidated by Huber^[18]. Phytomelanin was reported earlier in watermelon^[19] sunflower^[20], buckwheat^[21], grape^[22], tomato^[23], fragrant olive^[24], night jasmine^[25], sesame^[26], ipomoea^[27], black



mustard ,rape ^[28], chestnut ^[29], garlic ^[30], oat ^[31] and barley ^[32].



Fig.No-1 Purified phytomelanin isolated from dark seed coat of certain plants

1. *Dimocarpus longan* (Light brown, Powdery) 2. *Ricinus communis* (Dark black, Flaky Crystalline) 3. *Tamarindus indica* (Dark Red, Flaky Crystalline) 4. *Annona squamosa* (Brown, Powdery) 5. *Manikara sapota* (Dark brown, Powdery Crystalline) 6. *Mirabilis jalapa* (Brown, Powdery) 7. *Senegalia rugata* (Dark brown, Powdery Crystalline) 8. *Carica papaya* (Black, Powdery Crystalline) 9. *Citullus lanatus* (Light brown, Powdery) 10. *Mucuna bracteata* (Dark black, Powdery Crystalline)

Confirmatory test for Phytomelanin

The purified phytomelanin was confirmed using Hydrogen peroxide, Potassium permanganate and Ferric chloride. All the extracted phytomelanin were positive for the above tests. Phytomelanin was found to be soluble only alkaline solution and insoluble in water and alcohol. Earlier. Phytomelanin isolated from plant sources were sparingly soluble in DMSO ^[17].

UV-VIS Spectroscopy

UV-Visble spectroscopy of the isolated phytomelanin showed maximum absorption between to 272-288nm in *Tamarindus indica* and *Citrullus lanatus*. *Ricinus communis*-284nm, *Carica papaya*-283nm, *Acacia concinna*-265nm, *Mirabilis jalapa*-284nm, *Annona*

squamosa -286nm, *Manikara sapota* -276nm, *Dimocarpus longan* -272nm, *Mucuna bracteata* -285nm, Phytomelanin is report to have maximum absorbance between 190-390nm ^[33] and towards visible spectrum the absorbance decreases. In the present study maximum absorbance was observed between 260-280nm. The conjugated molecules in melanin absorbs and scatters UV ^[34]. Phytomelanins from the sunflower seed coat had a maximum absorption at 280 nm.

Antioxidant Activity

Phytomelanin is a phenolic derivative and hence has antioxidant activity ^[9]. Antioxidants protect the cells from invading pathogens . *Citrullus lanata*, *Ricinus communis* , *Tamarindus indica* , *Annona squamosa* , *Manikara sapota* and *Mucuna bracteata*

Phytomelanin showed 100% inhibition of free radicals at 50 μ l concentration. *Ricinus communis*, *Tamarindus indica* and *Mucuna bracteata* melanin had an IC₅₀ values of 25 μ l, 15 μ l and 40 μ l respectively (Fig No-2). Earlier reports showed that Black sesamum had 82% antioxidant activity at 0.5mg/L. [26]. Similarly sunflower seed husk was reported to possess antioxidant activity [20]. Antioxidant activity of crude and purified phytomelanin from watermelon seed at 1 mg/ml was reported to be 79 and 92 % respectively [7]. Alam et

al. [35] reported antioxidant activity of phytomelanin in *Phoenix dactylifera* fruit pulp. The free radicle scavenging activity IC₅₀ of Phytomelanin ranged from 63.6–75.1 μ g/mL. Antioxidants are molecules that scavenge free radicals and reactive oxygen and nitrogen species. Potential antioxidants are those that are effective at lower concentration. Tamarindus phytomelanin had an IC₅₀ value of 15 μ l /ml. Tamarindus phytomelanin can find varied applications upon further invivo studies.

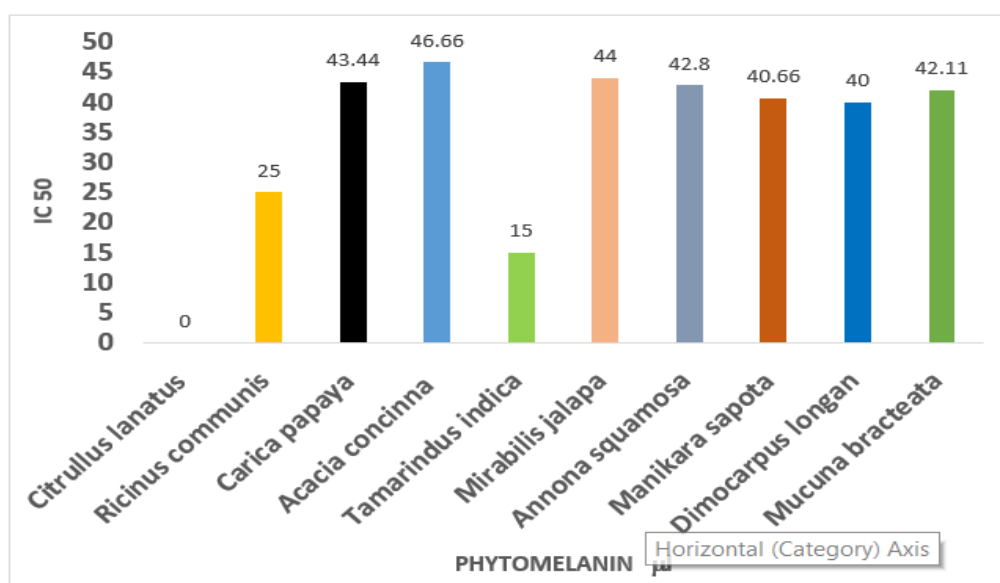


Fig No-2 IC₅₀ of antioxidant activity of phytomelanin extracted from dark seed coat. *Tamarindus indica* melanin most potent antioxidant with IC₅₀ of 15 μ l /ml followed by *Ricinus communis* with IC₅₀ value of 25 μ l /ml.

CONCLUSION:

Phytomelanins are Dark, phenolic compounds with wide application in phamecueticals and cosmetics .It has been isolated from few families alone. In the present study phytomelanin was isolated and confirmed from dark coloured seeds of many families. It also revealed maximum antioxidant activity in *Tamarindus indica* at lower concentration. The phytomelanin from these seed coats could be used as colouring and antioxidant agent in various food industry and pharmaceutical industry. It could also be explored for potential use as nutraceutical and supplements, however further studies is required on these lines.

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