P: ISSN No. 0976-8602 RNI No. UPENG/2012/42622 VOL.-10, ISSUE-2, April 2021

E: ISSN No. 2349-9443 Asian Resonance

# Staining Potential of Selected Plant based Natural Dyes on Protein in Polyacrylamide Gel Electrophoresis

Paper Submission: 03/04/2021, Date of Acceptance: 15/04/2021, Date of Publication: 25/04/2021

**Nihar Ranjan Singh**
Assistant Professor,
Dept. of Botany,
Ravenshaw University,
Cuttack, Odisha, India

Dyes are important molecules for coloring different materials for better visualization. Synthetic dyes are harmful, non-ecofriendly and toxic to human health as well as surrounding environment. Synthetic dyes are usually discarded into the aquatic system and potentially expose humans and local biota to adverse effects. Therefore, to substitute the harmful effect of synthetic dyes, natural dyes are a better choice. Natural dyes are ecofriendly, non-toxic, non-allergic, non-mutagenic and cheaper in cost. Proteins in SDS-PAGE gels are stained with chemical dyes like coomassie blue, silver stain, zinc stain and fluorescent dye. These dyes are expensive and hazardous to health. All these synthetic dyes used to stain proteins in SDS-PAGE are more or less self-reactive, flammable, having mild to acute toxicity (oral, dermal, inhalation) and skin irritant properties. Alternatively, natural dyes can be a substitute, which is easily available and their making procedure is much cheaper in comparison to synthetic dyes. Protein sample stained with selected natural dyes were observed on SDS-PAGE and evaluated for being their effectiveness in binding protein. Further, isolation of natural dyes and their staining compounds can be considered an alternative to synthetic dyes in protein staining process.

**Keywords:** Natural Dyes, Staining, SDS-PAGE, Protein

**Introduction**

Dyes are very important for coloring different materials and molecules for better visualization. Biomolecules like DNA, RNA, proteins are very minute and they cannot be easily seen, unless they are stained with a specific colorant that has an affinity for the respective biomolecules to be visualized. In molecular biology study especially in proteomic study, it is very essential to stain proteins for their identification, characterization and to find out its functional roles. In the gel based proteomics approach, proteins are generally separated according to their size and charge ratio in polyacrylamide gel electrophoresis (PAGE) [1]. Two-dimensional gel electrophoresis (2DE) is another convenient gel based procedure to minutely separate proteins of same size according to their pH. This involves both isoelectric focusing point (IEF) and PAGE[2]. The stained proteins in the gel can be isolated by cutting the gel part, containing identified proteins and such proteins are further sequenced and analysed by mass spectrometry (MS).

Proteins can be track while running in the electric field by a very common dye bromophenol blue (BPB). Tracking dye is used to mark the direction of migrated protein samples travels from the well to the end of the gel during electrophoresis. BPB is a tetra-bromophenol sulfonephthalein, widely used as tracking dye. Since BPB carries a slightly negative charge at moderate pH, it will migrate in the same direction as DNA or protein[1-6].
Proteins are usually stained with Coomassie blue, silver nitrate and fluorescent staining. The dye molecules bind tightly to proteins and absorb light in the visible region of the spectrum. The choice of the dye depends mostly on the quantity of the protein to be detected.

CBB is the most popular and efficient protein stainer, employed for the first time in 1963 by Groth and his colleagues[4]. It is an anionic dye,

which non-specifically binds to proteins. The excess dye incorporated into the gel can be removed by distaining with the same solution without the dye. The proteins are detected as blue bands on a clear background. As SDS is also anionic, it may interfere with staining process. Therefore, large volume of staining solution is recommended, at least ten times the volume of the gel. Classically, Coomassie blue was the most widely used non-covalent dye for post-electrophoretic protein staining. Two forms of Coomassie brilliant blue are available, R-250 and G-250. R stands for reddish hue and G for greenish hue, the number 250 is an indicator number for dye strength. Typically, R-250 is used to stain SDS polyacrylamide gels and G-250 in the Bradford assay[5-7].

Silver staining is one of the most sensitive protein staining methods and could detect proteins at very low levels. However, it has certain disadvantage like difficulty in the operation and poor repetition rate. Moreover, silver staining shows poor mass-spectrometry (MS) compatibility compared with the traditional Coomassie blue staining because it includes glutaraldehyde in the sensitization solution[8]. Silver staining technique involves the deposition of metallic silver onto the surface of a gel at the locations of protein bands.

Silver ions (from silver nitrate in the staining reagent) interact and bind with certain protein functional groups. Two categories of silver staining were used to visualize proteins in gel: the acidic silver nitrate and the alkaline silver diamine complex of silver nitrate in alkaline environment, which differed on binding specificity, sensitivity, costs and safety risk. The strongest interactions occur with carboxylic acid groups. Silver staining increases the sensitivity typically 50 times more than the coomassie blue staining. Silver staining was introduced as a sensitive procedure to detect nanogram level of proteins in gels. The rationale of the silver staining is based on the binding of silver ions with the proteins that can be reduced under suitable condition and finally giving images[11,13].

Silver staining utilizes the protein binding properties of silver ions, which are then reduced to silver metal using a developing solution, creating a visible image. The primary benefit of silver staining is its high sensitivity, as it can detect less than 1ng of protein inside the gel[13].

The fluorescent staining method is compatible with the electroblotting of protein bands and also with the staining of the resulting blot with the covalent dye MDPF (2-methoxy-2, 4-diphenyl-3(2H)-furanone). These staining procedures are applied sequentially; there is no need to run a duplicate unstained gel for protein blotting. Furthermore, since only adduct formed by the reaction of MDPF with proteins is fluorescent, there is no need to destain the membrane after protein labelling. In addition, MDPF staining is compatible with further immune detection of specific bands with polyclonal antibodies. MDPF staining does not prevent the N-terminal sequence analysis of proteins in the protein bands. The stain can be visualized using a wide range of excitation sources commonly used in image analysis systems including a 302 nm UV-B transilluminator, 473 nm second harmonic generation (SHG) laser, 488 nm argon-ion laser, 532 nm yttrium-aluminum-garnet (YAG) laser, xenon arc lamp, blue fluorescent light bulb or blue light-emitting diode (LED). The sensitivity of SYPRO Ruby Protein Gel Stain is superior to colloidal Coomassie Brilliant Blue (CBB) stain or monobromobimane labeling and comparable with the highest sensitivity silver or zinc-imidazole staining procedures available[9,10].

Natural dyes are any coloring agents, which are derived from plants, invertebrates or minerals. Mostly available natural dyes are vegetable dye from plant sources like roots, bark, berries, leaves, wood and other organic sources such as fungi and lichens[14]. In nature, there is a wide range of colors available. Some plants may have more than one color depending upon the plant part is used[15-17]. The shades of the color of dye produced from plant vary according to the time of the year the plant is picked, how it was grown, soil conditions, etc. In India, there are more than 450 dye-yielding plants[12,18,20]. A list of uses of natural dyes is listed in Figure-1. Though there is a lack of availability of technical knowledge on the extraction and dyeing techniques, it has not commercially succeeded compared to synthetic dyes. Natural dyes of plant, mineral and animal sources are fascinating, beautiful and most of them produce very colorful effects that are so amazing to visualization.

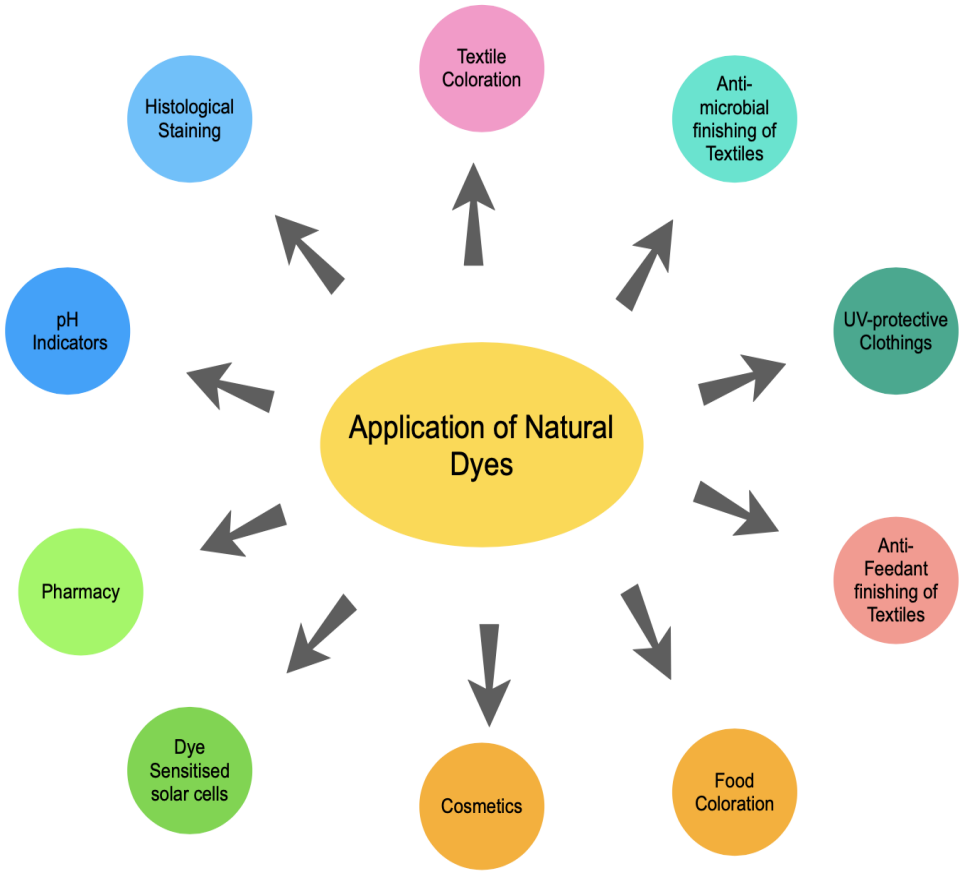


**Figure-1: The application of natural dyes**

In this work, seven plants are chosen for the extraction of natural dyes. These include *Bixa orellana* (Annota dye), *Tectona grandis* (Teak), *Beta vulgaris* (Beetroot), *Lawsonia innermis* (Henna), *Curcuma longa* (Turmeric), *Punica granatum* (Pomegranate), *Tridax procumbens* (Tridax). Annatto dye was extracted from B. *orellana* and first used as natural dye in protein tracking dye in SDS-PAGE. Earlier investigators have reported the dyeing property of annatto seeds for use in protein staining[3,20]. The seeds of this plant produce one of the dyes most frequently used worldwide, not only in food products but also in the textile, paint, and cosmetic industries. The annatto tree belongs to the family Bixaceae and the genus Bixa. Bixin, a red-colored carotenoid, is the pigment present in high concentration in the annatto seed aril. It is the main substance responsible for the dyeing characteristics of seeds, where its concentration can be as high as 5.0%. The rhizome of *C.longa* L. belonging to family Zingiberaceae. It has an aromatic odour and somewhat a bitter taste. The dried and ground rhizome of *C. longa* is called turmeric. It has been in use for centuries as a dye and as a component of curry powder, and more recently as a pH indicator; therefore, it is a common commercially used substance[19,22]. The chief reagent of turmeric is curcumin (diferuloylmethane) that is present together with smaller quantities of di-caffeoyl methane, caffeoyl feruloyl methane and dihydro curcumin. The volatile oil (It contains about 5% diaryl heptanoic colouring materials known as curcuminoids, about 5%) contains sesquiterpenes (e.g. zingiberene, 25%), sesquiterpene alcohols and ketones, and also monoterpenes[23]. Beetroot (*B. vulgaris*) is botanically classified as an herbaceous biennial from Chenopodiaceae family and has several varieties with bulb shape, colours ranging (from yellow to red). Betanin, the conspicuous red pigment of the root of the beet and has been classified as an anthocyanin, related to the pigments of the petals of many flowering plants. The intense red colour of beetroots derives from high concentrations of betalains, a group of phenolic secondary plant metabolites. Betalains are used as natural colorants by the food industry, but have also received increasing attention due to possible health benefits in humans, especially their antioxidant and anti-inflammatory activities[24]. Other benefits include the inhibition of lipid peroxidation increased resistance to the oxidation of low-density lipoproteins and chemo-preventive effects. The betalains that are mainly found in beetroot are betacyanins and betaxanthins[25]. The botanical name of Henna is *Lawsonia inermis* L., belonging to the family Lyrthaceae. Henna is a medium sized herb

with many branches. Lawsone (2-hydroxy-1, 4-napthoquinone) is the main colouring constituent of the henna and is obtained by the degradation of hennosides A, B and C. Lawsone is also known as hennotannic acid[26,29].

**Materials and Methods**

**Preparation of Dye from Plant Materials**

Seven different varieties of plant including *B. orellana, T. grandis, B. vulgaris, L. inermis, C.longa, P. granatum, T. procumbens* are chosen from the garden of the Botany Department, Ravenshaw University, Cuttack for yielding dyes. From all the above plants, natural dyes are prepared by slightly modifying the extraction method of Siva et al. 2008. For *B. orellana,* 50 gm of mature raw Bixa seeds having red dusts are taken in two conical flasks; one with 50 ml of distilled water and another with 50 ml of 50% ethanol. The filtered extracts are centrifuged at 12500 rpm for 10 min at room temperature. A reddish colored supernatant of water extracts and reddish orange colored supernatant of ethanol extracts are recovered. The exact protocol from above method is also followed for *T. grandis, B. vulgaris, L. innermis, C.longa, P. grannatum* and *T. procumbens.* For the leaf based samples, an added step of shade drying is included before the dye extraction. The water extracts and the ethanolic extracts of young dry leaves of *T. grandis* shows a mild reddish-brown color and a deep reddish colour supernatant. A mild reddish pink color dye was developed from water extracts and a deep dark pink colour dye was developed from ethanol extracts of Beetroots. Faint reddish color for water extracts and dark reddish color for ethanol extracts are seen from *L. innermis* dry leaves. The water extracts with dark yellow and the ethanol extracts with slight reddish yellow appeared in the supernatant, extracted from of the dry rhizome of *C.longa*. A deep dark orange color extract from water solution and a mild orange colour dye from the dry rind of *P. granatum* are obtained. From *T. procumbens* dry leaves, a dark brownish colored supernatant was developed in both water and ethanol extracts.

**Performing SDS-PAGE with Plant based Dye**

SDS-PAGE electrophoresis was done by taking bovine serum albumin (BSA) as our standard protein and for staining purpose coomassie brilliant blue (CBB) was used. This set was considered as control. To test the efficacy of natural dyes in staining, the BSA protein containing gel was stained with the extracted natural dye to see the binding of natural dye with the protein. Also to evaluate the tracking of the migration of the protein in the running gel, bromophenol blue (BPB) was used in the control gel where as the same natural dye was used in case of gels that was meant for staining with the natural dye.

**Results and Discussions**

Each individual gel is run for individual dye. Every Each individual gel is used to evaluate for one plant-based natural dye. Every gel contains 15 µl of BSA treated with BPB as tracking dye and followed by CBB solution as staining as seen in lane 1 and 2, which is considered as control. Other lanes were run with 15 µl BSA treated with plant based natural dye for tracking as well as staining dye.The seeds of B. orellana were taken for staining experiment. The images of natural dye staining the protein are shown in Figure 2.

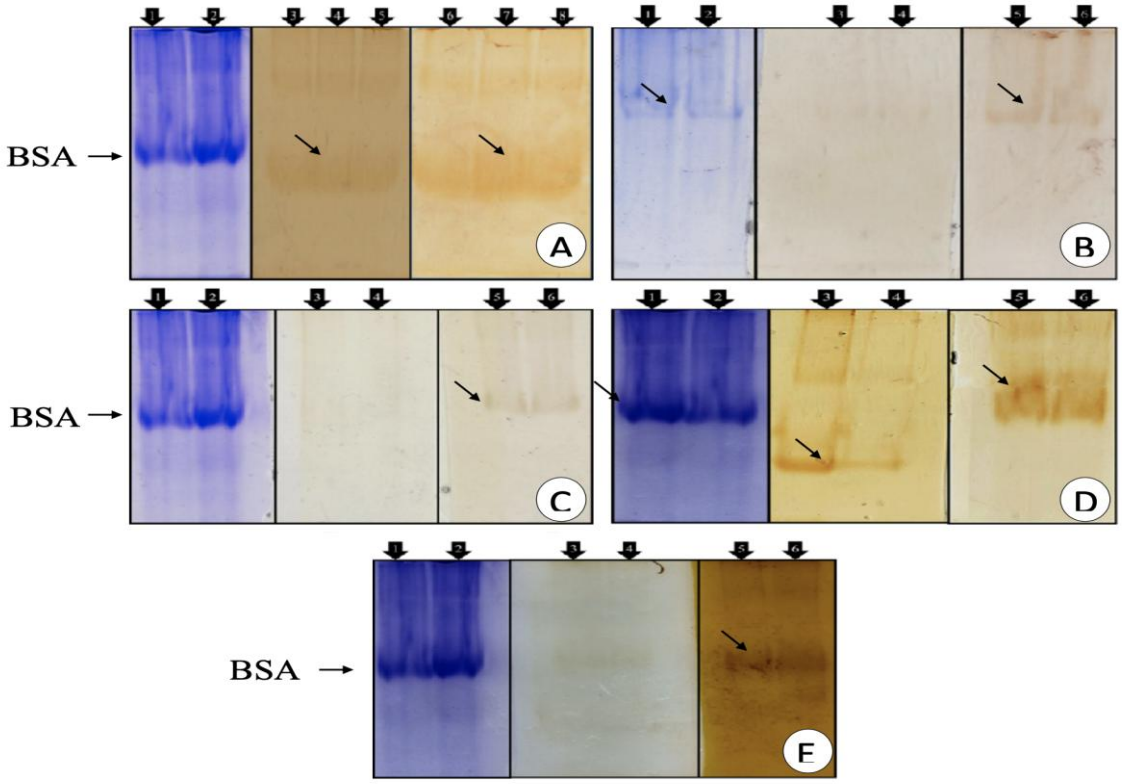

Figure- 2: The gel image of BSA stained with various natural dyes. Lane 1 & 2 are loaded with BSA and stained with CBB as control (A) Lane 3,4,5 stained with *B. orellana* extracted with water & lane 6, 7, 8 stained with *B. orrellana* extracted with 50% ethanol. (B) Lane 3 & 4 stained with *T. grandis* extracted with water & lane 5 & 6 stained with *T. grandis* extracted with 50% ethanol. (C) Lane 3 & 4 stained with *B. vulgaris* extracted with water & lane 5 & 6 stained with *B. vulgaris* extracted with 50% ethanol. (D) Lane 3 & 4 stained with *L. innermis* extracted with water & lane 5 & 6 stained with *L. innermis* extracted with 50% ethanol. (E) Lane 3 & 4 stained with *C. longa* extracted with water water & lane 5 & 6 stained with *C.longa* extracted with 50% ethanol.

Water based annatto dye was orange in color and alcohol based annatto dye was dark reddish orange in color. Positive results were found for both water and alcohol based dye for tracking protein in the gel (not showing figure). As staining dye, alcohol based dye gave more intense colored bands than water based dye as shown in figure 2(A). The water extraction of T. grandis colored as reddish color and the 50% ethanol extract colored as deep reddish. The ethanol extracted showed a clear staining whereas the water extracted showed a faint staining as seen in figure 2(B). *B. vulgaris* in water gave a maroon color, but in ethanol gave deep maroon color. Interestingly, while staining to BSA, the water extracted had no interaction with protein, but the ethanol extracts gave a distinct protein band as shown in figure 2(C). Conventionally, *L. innermis* used for color crafting body parts especially hands due to its reddish-brown coloring effects. The water and ethanol extract showed positive results. But the intensity of interaction is less in water than ethanol as observed in figure 2(D). *C. longa* is a common spice in cooking. Water based turmeric dye was yellow in color and alcohol based turmeric was dark brownish yellow in color. Turmeric for both cases (water and alcohol dye) failed as tracking dye (not shown in figure). Both dyes gave positive result towards staining of protein bands, but these bands were very insignificant in case of water based dye. The alcohol-based dye gave a better visual effect to protein bands as seen in figure 2(E).. The extraction of dye from *P. granatum* and *T. procumbens* did not respond to the gel with proteins. It clearly showed

There are many coloring substances in nature. Many synthetic dyes are synthesized and commercially purchased. But due to health risk and non-eco-friendly nature, now-a-days natural dyes are being given more preference[27].

In biochemistry staining involves adding of class-specific dye to a substrate (DNA, proteins, lipids, carbohydrates) to qualify or quantify the presence of a specific compound. SDS-PAGE has remained essentially unchanged since its introduction four decades ago. It is routinely used to probe the presence, relative concentration, and purity of proteins, their approximate molecular mass, and in conjunction with immunochemical methods or mass spectrometry, their identity and possible covalent modification. There are many commercial dyes are available for protein staining in gel electrophoresis. To add the number of dyes used in SDS-PAGE, some plant based natural dyes can be taken in to consideration. These dyes are easily available, cheap and non-toxic. Some natural dyes can serve as staining as well as tracking dye for protein as seen earlier.

Here BSA protein was taken as standard protein and CBB as control for staining experiment. Different natural dyes for staining of protein in SDS-PAGE were used. Some points were noticed during experiment are as follows: All dyes gave more intense color after mixing with alcohol than water. Dyes have reactive chemical properties which can bind with protein molecules to give visible appearance. *T. procumbens* known to have photo sensitizer properties but did not show any dyeing properties in binding proteins. So, it cannot bind with protein bands in SDS-PAGE[28].

**Conclusion**

There is increasing awareness among people towards natural products. Due to their non-toxic properties, low pollution and no side effects, natural dyes are used in many ways. More detailed studies and scientific investigations are needed to assess the real potential and availability of natural dye yielding plants, other sources. Biotechnological and other modern techniques are required to improve the quality and quantity of dye production. Natural dyes are not only having dyeing property but also having the wide range of medicinal properties.

However, out of seven different plant parts, five showed a clear potential for detecting proteins. *B. orellana, T. grandis, B. vulgaris, L. inermis*and*C.longa*have interacted with proteins and gave bands inside the gel. These plants are very common and can be found everywhere. Two plants, *P. granatum* and *T. procumbens*did not show any bands in gels. The natural dyes extracted from different tree parts with a very less investment have a potential to stain proteins in the PAGE and can be commercially investigated.

**References**

1. *Roy, Suvra, and Vikash Kumar. "A practical approach on SDS PAGE for separation of protein."Int. J. Sci. Res 3.8 (2014): 955-960.*
2. *Klose, Joachim. "Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues."Humangenetik 26.3 (1975): 231-243.*
3. *Siva, R., et al. "An alternative tracking dye for gel electrophoresis."Current Science (00113891) 94.6 (2008).*
4. *Groth, S. Fazekas de St, R. G. Webster, and A. Datyner. "Two new staining procedures for quantitative estimation of proteins on electrophoretic strips."Biochimica et biophysica acta 71 (1963): 377-391.*
5. *Meyer, T. S., and B. L. Lamberts. "Use of coomassie brilliant blue R250 for the electrophoresis of microgram quantities of parotid saliva proteins on acrylamide-gel strips."Biochimica et biophysica acta 107.1 (1965): 144-145.*

6. Diezel, W., G. Kopperschläger, and E. Hofmann. "An improved procedure for protein staining in polyacrylamide gels with a new type of Coomassie Brilliant Blue."Analytical biochemistry 48.2 (1972): 617-620.
7. Chevalier, François. "Standard dyes for total protein staining in gel-based proteomic analysis."Materials 3.10 (2010): 4784-4792.
8. Dong, Wei-Hua, et al. "Simple, time-saving dye staining of proteins for sodium dodecyl sulfate–polyacrylamide gel electrophoresis using Coomassie blue."PLoS One 6.8 (2011): e22394.
9. Fernandez-Patron, Carlos, et al. "Understanding the mechanism of the zinc-ion stains of biomacromolecules in electrophoresis gels: Generalization of the reverse-staining technique."Electrophoresis 19.14 (1998): 2398-2406.
10. Berggren, Kiera, et al. "Background-free, high sensitivity staining of proteins in one-and two-dimensional sodium dodecyl sulfate-polyacrylamide gels using a luminescent ruthenium complex."ELECTROPHORESIS: An International Journal 21.12 (2000): 2509-2521.
11. Chevallet, Mireille, Sylvie Luche, and Thierry Rabilloud. "Silver staining of proteins in polyacrylamide gels."Nature protocols 1.4 (2006): 1852.
12. Chengaiah, B., et al. "Medicinal importance of natural dyes-a review."International Journal of PharmTech Research 2.1 (2010): 144-154.
13. Rabilloud, Thierry. "A comparison between low background silver diammine and silver nitrate protein stains."Electrophoresis 13.1 (1992): 429-439.
14. Verenkar, Nikita GS, and Krishnan Sellappan. "Some potential natural dye yielding plants from the State of Goa, India."Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)] 8.4 (2018): 306-315.
15. Korankye, Obenewaa. Extraction and application of plant dyes to serve as colourants for food and textiles. Diss. 2010.
16. Kulkarni, S. S., et al. "Cotton Dyeing with Natural Dye Extracted from Pomegranate (Punica granatum) Peel."Universal Journal of Environmental Research & Technology 1.2 (2011).
17. Kiernan, John A. "Classification and naming of dyes, stains and fluorochromes."Biotechnic & histochemistry 76.5-6 (2001): 261-278.
18. Gulrajani, M. L., and Deepti Gupta, eds. Natural dyes and their application to textiles. Department of Textile Technology, Indian Institute of Technology, 1992.
19. Kurien, Biji T., Yaser Dorri, and R. Hal Scofield. "Spicy SDS-PAGE gels: curcumin/turmeric as an environment-friendly protein stain."Protein Electrophoresis. Humana Press, Totowa, NJ, 2012. 567-578.
20. Mala, Kripanand Sathiya, et al. "Studies on application of annatto (Bixa orellena L.) dye formulations in dairy products."Journal of food science and technology 52.2 (2015): 912-919.
21. Devi, Meena, V. N. Ariharan, and P. N. Prasad. "Annato: eco-friendly and potential source for natural dye."International Research Journal of Pharmacy 4.6 (2013): 106-108.
22. Bhatti, Ijaz A., et al. "Influence of gamma radiation on the colour strength and fastness properties of fabric using turmeric (Curcuma longa L.) as natural dye."Radiation Physics and Chemistry 79.5 (2010): 622-625.
23. Evans, W. C. "Pharmacopoeial and related drugs of biological origin."Trease and Evan's Pharmacognosy. London: WB Saunders Co. Ltd 17.11 (1996): 327-332.
24. Georgiev, Vasil Georgiev, et al. "Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot Beta vulgaris cv. Detroit dark red."Plant foods for human nutrition 65.2 (2010): 105-111.
25. Gandía-Herrero, Fernando, and Francisco García-Carmona. "Biosynthesis of betalains: yellow and violet plant pigments."Trends in plant science 18.6 (2013): 334-343.
26. Abulyazid, I., Elsayed ME Mahdy, and Ragaa M. Ahmed. "Biochemical study for the effect of henna (Lawsonia inermis) on Escherichia coli."Arabian Journal of Chemistry 6.3 (2013): 265-273.
27. Gurr, Edward. Synthetic dyes in biology, medicine and chemistry. Elsevier, 2012.
28. Rajkumar, S., and K. Suguna. "Analysis of natural sensitizers to enhance the efficiency in dye sensitized solar cell."International Journal of Engineering Research and Applications 6.5 (2016): 41-46.
29. Boubaya, Anissa, et al. "Chemical diversity analysis of Tunisian Lawsonia inermis L. populations."African Journal of Biotechnology 10.25 (2011): 4980-4987.