Anthocyanin: A Natural Dye Extracted From Hibiscus Sabdariffa (L.) For Textile and Dye Industries

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Abstract

Environmental pollution is one of the major issues faced by all the countries throughout the world. To prevent the environment scarcity and crisis faced in day-to-day life due the increasing chemical industries, usage of chemicals and the effluents processed out after the treatment also consists of some trace elements in them. Hence the extraction of enzymes on natural basis forms an alternative criteria for the production of dye in order to reduce pollution which in turn helps to nourish and protect the environment for future generations. Hibiscus sabdariffa (L) has a rich source of anthocyanins which is further enhanced by callus production and synthesized by increasing the sucrose concentration. Anthocyanin pigments were extracted using acidified ethanol and the dye obtained was screened for GL-MS analysis and its dyeing process in textile industry. The study showed significance properties along with coloring nature on the clothes used. Color of anthocyanin pigment depends on pH maintained and also shows the adaptability towards the nature with varied environmental conditions

Highlights

- Extraction of anthocyanin by different solvent to improve the intense of coloration
- Phytochemical evaluation of plant sample to know the commercial values and medicinal values
- Plant tissue culture and callus assisted anthocyanin induction
- Amalgamation of plant oriented dye application (cloth dye, lip balm, dye sensitized solar cell).

Introduction

Hibiscus is the broad variety of genus which has more than 679 species and has been used in umpteen purpose. *Hibiscus sabdariffa* (L.) under the family of Malvaceae has the pigment called anthocyanin which is water soluble and their color may varies based on potential of hydrogen. Studies indicate the Roselle's different properties and commercial uses for their rich molecular pigmentation and it's also reported for their antioxidant and antimicrobial properties. Roselle cultivated due to their calyces and leaves which is exported to several countries for their edible parts and can make jelly like substances and pickles (Fig. 1). *Hibiscus sabdariffa* pigment can produce dye after some natural processing by the way it can be alternative for synthetic dye formulation also can be limited to environmental exposure. By grinding and soaking in water it can produce coloration, increasing and decreasing pH also the factor which influence color intense [1].

Some limited natural chemicals used to isolate and formulate the natural dye formation. Ancient people have been used dye formation by natural way for their clothes and their beauty care, they have already known about natural dye and their medicinal and pharmaceutical uses. There are different methods are equipped to extract the dye from Hibiscus sabdariffa plant parts especially from calyces which may be grinded or fully soaked in water, because of their water soluble pigment nature it able to produce dye without any additives or formulation, but intense of color may be varied.by studying *Hibiscus sabdariffa*

and their physical and chemical characteristics further we can make dyeing intense by periodical and spontaneous enhancement of potential of hydrogen [2, 3].

Plant tissue culture also involved to reduce the time period of plant maturation and simultaneously avoid the interference with food and other agricultural use. the anthocyanin pigments obtained was further studied for its applications by treating dye against various material ,especially on clothes, cosmetics due to their antioxidant property like lip stick, face cream, sunscreen lotion and dye sensitized solar cells [4].

Many researchers have found the dye application from roselle in regard to separate application. Phytochemical variation and fabric treated with dye extract using organoleptic attributes and mathematical evolution. Jian et al. reported cosmetic application of roselle through reversing UVB induced photoaging. Characterized dye sensing of solar cell using roselle dried extract and performed different instrumentation. All of these studies proved and performed only focused on future sustainability in their own motive [5, 6]. However, the green chemical environment important to bags towards future generation, parallely to fulfill the global requirements also important so, in this present study have evolved to bring the requirement as well as the natural sustainability by well-equipped manner [6].

Experimental Methods

Sterilization of explants

The explants were washed with sodium hypochlorite (1.0%) and mercuric chloride (0.05%) for different time duration. The explants were transferred to a beaker containing 1% sodium hypochlorite solution with rapid shaking for 20-25 min with changing the solution at 5 min interval. Then, the explants were washed with distilled water. Finally, the explants were treated with 0.05% mercuric chloride for 1-5 min. The surface sterilized explants were washed with sterile distilled water for 5 times (5 min each). The excess water on the explants was removed by using sterile tissue paper before culturing. The explants were cut into small pieces (0.5-1.0 cm) barring the cut ends and transferred to semi-solid culture medium under aseptic conditions in a laminar flow chamber.

Anthocyanin pigment extraction

Anthocyanin content of freeze-dried calyx was determined using the pH-differential method, with modifications. 50 mg of freeze-dried calli were steeped in methanol containing 0.1% trifluoroacetic acid (TFA) at 4 °C overnight. After calyx were ground, the samples were then centrifuged at 10,000 rpm for 5 min. The supernatants were used as the crude anthocyanin extract. 4.5 mL of potassium chloride buffer 0.025 M at pH 1.0 and 4.5 were placed into separate test tubes, and a 0.5 mL aliquot of supernatant (10 times diluted) was added and mixed well with vortex. The absorbance (A) was measured at 521 and 700 nm against the blank sample [7].

Gas chromatography/mass spectrometer

Plant extract was tested against GC/MS to confirm the presence of anthocyanin. Gas chromatography / mass spectrometry (GC/MS) is the marriage of two analytical methods into a versatile technique for the identification of complex volatile materials. Gas chromatography (GC) effectively separates the different constituents of the sample for subsequent analysis and identification by mass spectrometry (MS) [8].

Dye preparation for clothes

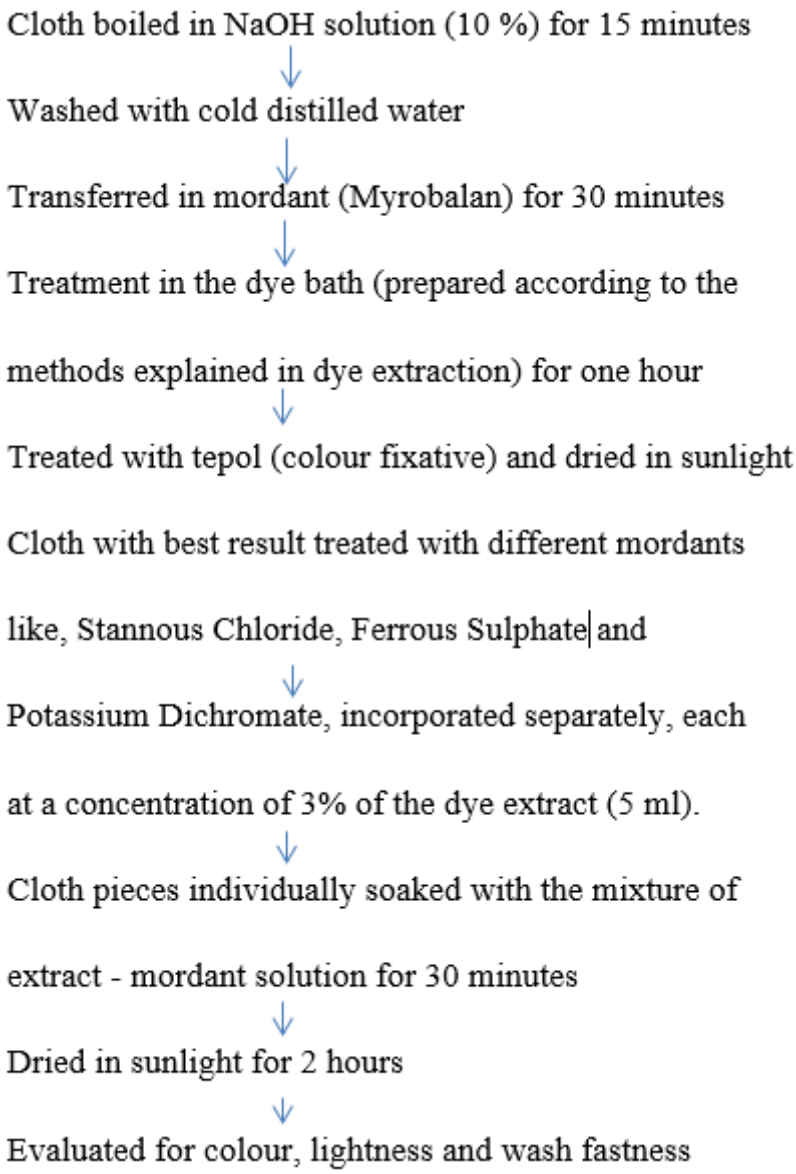
In dye extraction, the flowers (10 g) were crushed and put in an earthen pot to which 100 mL water was added. The pot was kept undisturbed for 20-25 days and extract was then filtered through a piece of cloth to yield the natural dye.

Dyeing procedure

The extracts obtained through above mentioned methods were filtered and used for dyeing. Cloth used for dyeing was boiled in NaOH solution (10%) for 15 minutes to remove starch from the cloth, then washed with cold distilled water. This cloth was then transferred in mordant (Myrobalan) for 30 minutes followed by treatment in the dye bath for one hour. Effect of dye without mordanting the fabric was also studied. Then the cloth was treated with tepol (colour fixative) and dried in sunlight.

Similarly effect of various mordants on colour of dye extracted from the flowers were also studied on the cloth found best in the above experiment. This was achieved by incorporating different mordants like, Stannous Chloride, Ferrous Sulphate and Potassium Dichromate separately, each at a concentration of 3% of the dye extract (5 mL). Cloth pieces were individually soaked with the mixture of extract-mordant solution. After 30 minutes of soaking the cloth was dried in sunlight for 2 hours. The sun dried cloth was further evaluated for its colour, lightness and wash fastness. Wash fastness was tested by washing with soap water (10% w/v) and heat resistance was tested by keeping the cloth at various temperatures, viz. 50, 60, 70°C for 30 minutes in the oven without water [9].

Flowchart for staining of anthocyanins on cloth



Application of Anthocyanins in the formulation of herbal lip balm.

Formulation of herbal lip balm:

The herbal lipstick was formulated as per method described. The ingredients used in the formulation of herbal lipstick are: Bee wax, Shea butter, Strawberry essence, anthocyanin pigment obtained from calyx of *H. sabdariffa* and Vanilla essence [10].

Results And Discussion

Plant Tissue Culture

MS medium supplemented with varying concentrations of sucrose of 20% and 50% along with combination of plant growth regulators such as 2,4-D (1.0 mg/L) + BAP (1.0 mg/L) showed maximum formation of callus with slight initiation of anthocyanin pigment formation in the medium containing

50% sucrose concentration (Fig. 2). The work carried out is supported by the earlier report stating that highest formation of callus was observed in the MS medium supplemented with 2,4-D (1.0 mg/L) and BAP (1.0 mg/L) after three sub culturing [7].

Anthocyanin pigment extraction

After fine grinding of roselle calyces with acidified solvent such as 1N HCl, 1.5N HCl, 1% citric acid, 2% citric acid and absolute ethanol. 1.5N HCl showed maximum extraction of anthocyanin(1.976%) from the calyx of H. sabdariffa, followed by 1.790 in 1% citric acid ,1.635 in 2% citric acid, 1.214 in 1N HCl and 0.784 in absolute ethanol (Fig. 3). The result of absorbance of different acidified solvent and calyx. Solvent which gives the more absorbance was 1g of calyx extract and 5ml of 1.5N HCl that was 1.976 from this we can understand that 1.5N HCl has the higher color intense and available anthocyanin (Table.1). According to the reports, the results showed that all the lipsticks were stable and had a good force of application while the breaking point reached 76.67–106.67 g. The melting points of the lipsticks containing 50%, 60%, and 70% castor oil were 56, 55, 53.5 °C respectively, while the pH test resulted in 4.4, 4.7, and 5.2. In addition, the hedonic test showed that respondents liked the exciting color, fragrant smell, and oily texture of the lipsticks. The lipsticks themselves did not cause any irritation, so they were safe to wear [11].

Table. 1 Anthocyanin pigment extraction from calyx of *H. sabdariffa*

S.No.	Extraction and solvent	Absorbance
1	1g of calyx extract & 5ml of absolute ethanol	0.784
2	1g of calyx extract & 5ml of 1N acidified ethanol	1.214
3	1g of calyx extract & 5ml of 1.5N acidified ethanol	1.976
4	1g of calyx extract & 5ml of 1% citric acid	1.790
5	1g of calyx extract & 5ml of 2% citric acid	1.635

GC-MS of methanol extract of *H. sabdariffa*

GC – MS chromatogram of methanolic extract of H. sabdariffa showed 23 peaks indicating the presence of 23 compounds. The chemical compounds identified in the extract are represented in the table below. GC –MS analysis revealed the presence of compounds such as 1). Cyclotrisilixane, Hexamethyl, 2).α,β crotonolactone, 3).Pentanoic acid,4-oxo,ethyl ester, 4).2-oxopentanedioic Acid, 5).Butanedioic acid,Hydroxy-dieth,6)1,3-isobenzofurondione, 7).Butanedioic acid, Diethyl ester, 8).2-piperidinnemethanol, 9).Dodecanoic acid, 10).1,2-benzene dicarboxylic acid, Diethyl, 11).1,2,3-propane Tricarboxylic acid, 12).Butanedioic acid, 13).Butanedioic acid, 3-hydroxy-2,2-DI , 14).Hexadecanoic acid, Ethyl ester, 15).1-docosene, 16).9, 12, 15- octa Decatrienoic acid,Ethyl ester(z,z,z), 17). E, E-1,9,17-docasatriene, 18). Eicosane,2-methyl, 19). Cyclopentane,(4-octyldodecyl)-, 20). Dososanic acid Ethyl ester, 21) 4-decenoic acid Ethyl ester,(z)-, 22).1-heptacoanol, 23). 2-[(hexadecycloxy), Methy]oxirane (Fig. 4).

The GC-MS analysis provided different peaks determining the presence of fifteen compounds from *H. rosasinensis* flower. These compounds have biological activity namely Ethanimidic acid, ethyl ester (31.43%), Propanal, 2,3-dihydroxy (12.58%), 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6- methyl (10.69%), Ethylenediamine (6.71%), o-Methylisourea hydrogen sulfate (4.06%), Ethene, ethoxy- (3.63%), Methyl palmitate (2.99%), 7- Formylbicyclo[4.1.0] heptanes (2.80%), 2-Butanamine, (S)- (2.72%), 1,3,5-Triazine2,4,6-triamine (2.48%), N-Formyl-β-alanine (2.36%), (Z)6,(Z)9-Pentadecadien-1-ol (1.70%), 1,2-Ethanediamine, Butanedial (1.65%), N-methyl-1-Propanol, 2-methyl- (1.57%) and Methanecarbothiolic acid (1.08%) [12].

Application of dye to clothes

Where we take cotton clothes for the application of dye and procedure was given in the methods and materials. NaOH is used to manufacture variety of detergents and soaps used in the homes and commercial application.it bleach dirt and other unwanted material on the clothes. Cloth were soaked in dye for half day and it changes light brown color. Cloth were treated with Myrobalan + dye extract gives light reddish brown. Cloth were treated with dye + $K_2Cr_2O_7$ gives shaded brown. Cloth were treated with dye + $FeSO_4$ which gives shaded blackish brown. Cloth were treated with dye + $SnCl_2$ which gives light green + black (Fig. 5). According to a review, color of anthocyanin varies with pH which shows its adaptability to nature with varied environmental conditions Research showed that they possess antimicrobial properties. Advance research using Anthocyanin and its related gene, textile could develop with antibacterial and self-fluorescence properties. Anthocyanin is also known to protect plant in extreme weather conditions. This property could be used to develop super cloths [13]. The dye extracts applied to silk fabric with mordant free dyeing show different color under different pH conditions, changing between purple, blue, green, and yellow. However, the dyed colors is light and the dyeing rate is low. Metal mordant such as Sn in chelation enhances the dye depth and improves the fastness of the dyed silk fabrics, especially in silk fabrics dyed by premordanting and metamordanting [14].

Dye sensitized solar cell

Dye sensitized solar cell is an imitation of biological system which is exactly photosystem of plant cell. Plant cells are all ways synthesize their own food by photosynthesis, through this process they make carbohydrates from sun light, water and carbon dioxide which is given.

$$\text{Carbon dioxide} + \text{water} + \text{sun light} \longrightarrow \text{carbohydrates}$$

Overall process involves so many chemical combinations and bonding of atom of molecules, especially electrochemical process. Based on electron transfer mechanism dye sensitized solar cell works. It's economically and performance wise stable one to use (Fig. 6).

Length of the FTO glass = 5cm

Breadth of the FTO glass = 9nm

Cell in this experiment measured 5cm by 9nm.

Area of the glass which is equals to

Area (A) = $5\times (9\times10^{-7} = 0.0000045\ m^2$

Solar cell power

$P_S = Si \times A$

$Si = 1000\ w/m^2$ $A = 0.0000045\ m^2$

$P_S = 1000\ w/m^2 \times 0.0000045\ m^2$

$Ps = 0.0045\ W$

Dye sensitized solar cell from H.sabdariffa dye gives the given result,

$V_{OC} = 1.1$

$I_{SC} = 0.37$

$P_{max} = V_{OC} * I_{SC}$

$= 0.87 * 0.37$

$= 0.2109W$

The maximum theoretical efficiency E, of the solar cell is estimated to be

$E = P_{Max} / P_S$

$E = 0.2109W / 0.0045W = 46.86\%$

The maximum theoretical efficiency of solar cell is 46.86%.

Earlier study have reported that the dyes have shown absorption in broad range of the visible region (400–700 nm) of the solar spectrum and appreciable adsorption onto the semiconductor (TiO2) surface. The DSSCs made using the extracted dyes have shown that the open circuit voltages (Voc) varied from 0.430 to 0.610 V and the short circuit photocurrent densities (Jsc) ranged from 0.11 to 0.29 mA cm−2. The incident photon-to-current conversion efficiencies (IPCE) varied from 12–37%. Among the four dyes studied, the extract obtained from teak has shown the best photosensitization effects in terms of the cell output [15].

Anthocyanin pigment for Lip balm formation

Lip balm or lip stick and any other cosmetic materials are now a days necessarily required for all the humans, especially for women. Cosmetic and other kind of perfume which are need to be tested for several kind of test for toxicological and stability test.

Table. 2 Ingredients for formulation of lip balm using anthocyanin pigment obtained from *H. sabdariffa*

S.NO	Ingredients	Importance	Quantity (gm)
1	Bee wax	Glossy & hardness	12g
2	Shea butter	Blending properties	25g
3	Strawberry essence	Flavoring agent	1.5g
4	*H. sabdariffa* sample	Coloring agent, Antioxidant agent	3ml
5	Vanilla essence	Preservative	0.1g

Double heating system was used to improve their ability to form an emulsion. It was also to prevent the loss of chemical and physiochemical property of added samples. There is need to test sample pH after adding all ingredients added, all cosmetics has optimal of 5.8-7. With an increase in the acid base balance, the irritating potential for skin is greatly elevated. The result showed that application of herbal dye adopted lip care product using anthocyanin rich *H.sabdariffa* (L) gives different color when adding different pH of dye ,where 1.5N HCl , 1% citric acid and 2% citric acid were show pH of 5.6 to 6 and its show no irritation when applied on skin (Fig. 7).

Conclusion

Hibiscus sabdarrifa (L.) is a rich source of anthocyanins and a medicinal plant used to make variety of home products and commercial products. Anthocyanins obtained from *H. sabdarrifa* was studied for dye applications to reduce the usage of chemical dyes in the fields of fabrics, automobiles etc. Most of the studies were reported on the acidified solvents to extract and make intense coloration from the plant materials as source. The use of plant tissue culture forms as another boon to the synthesis of anthocyanins and production of the pigments through callus induction and thereby treating the callus with increased sucrose content.

Different types of explants of *H. sabdariffa* were inoculated on MS medium supplemented with different hormone concentrations and hence initiation of callus was obtained. The callus obtained were further treated with increased sucrose concentration for the synthesis of anthocyanin pigment in the callus. Thus the obtained anthocyanins from calli and calyx of *H. sabdariffa* was extracted by acidified solvent which showed maximum extraction of the pigment. The pigment was used as a dyeing agent on various

applications as solar cell basis, lip balm and coloration as dye for the cloths. Thus, these kind of aspects helps to prevent the environment from the scarcity and crisis which we face every day. As chemical industries are increasing and the usage of chemicals and the effluent processed out after the treatment also consists of trace elements of chemicals in it. Hence the extraction of enzymes on natural basis forms an alternative for dye production to reduce pollution to some extent to nourish and protect the environment for the future generations.

Declarations

Author Contribution B.S. and B.L. contributed in data writing and designing of the study. B.S. and S.C. contributed in the preparation of the manuscript. M.S.A. contributed in revision of the manuscript. All the authors are responsible for the final manuscript.

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Data Availability Not Applicable

Ethical Approval and Consent to participate Not applicable

Consent to publish Not applicable

Conflict of Interest The authors report no competing interest.

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Figures

Figure 1

a. Habitat of Hibiscus sabdariffa L. b. calyx of H. sabdariffa L.

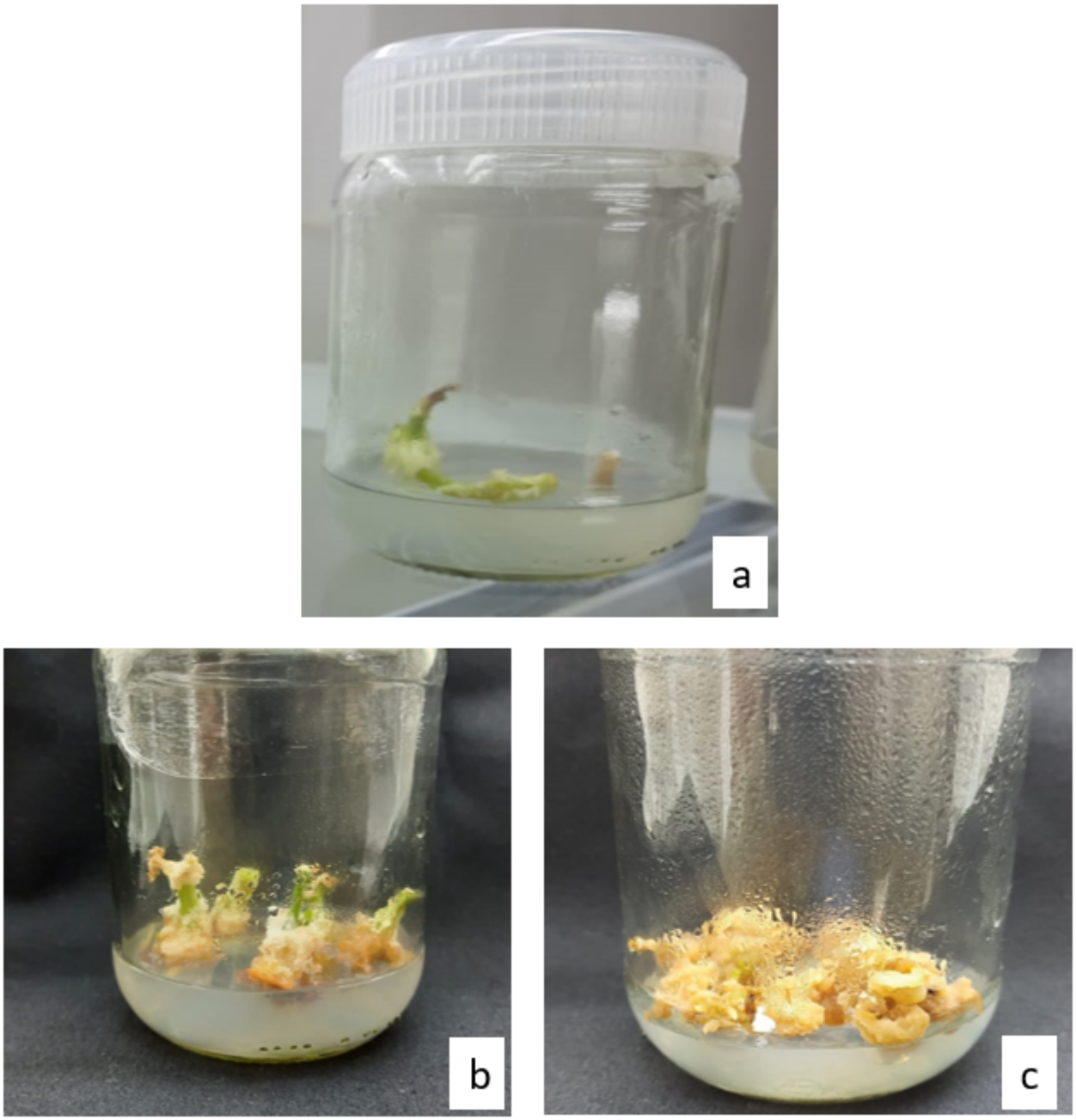

Figure 2

Callus formation from leaf explant of H. sabdariffa a. Callus formation on 10th day b. Callus formation on 15th day c. Mass culture on 25th day

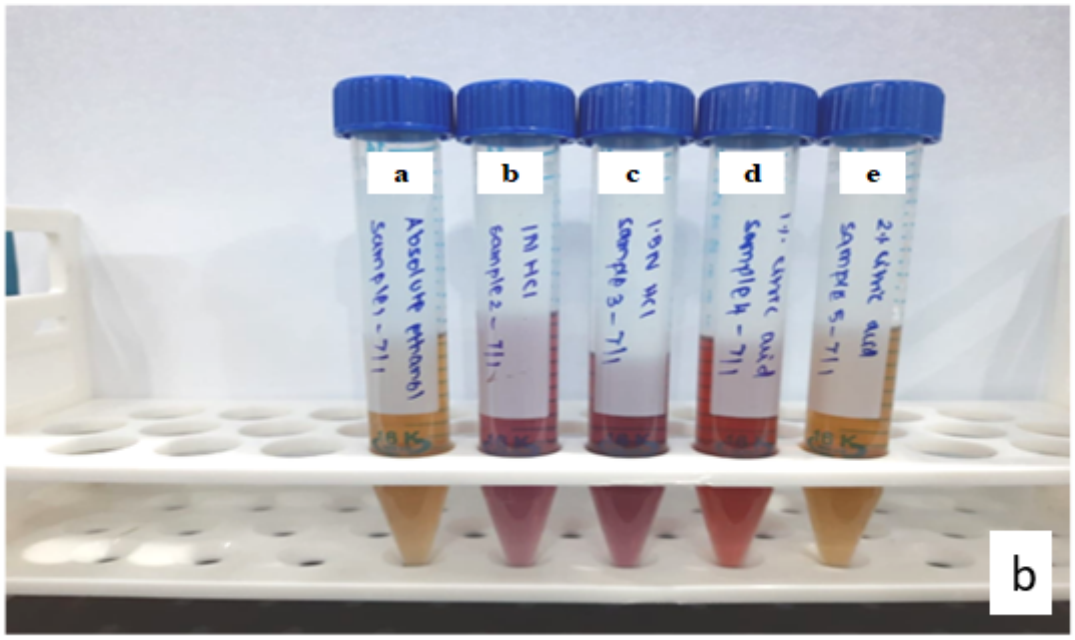

Figure 3

a. Dried calyx of H. sabdariffa b. Anthocyanin extraction from calyx of H. sabdariffa

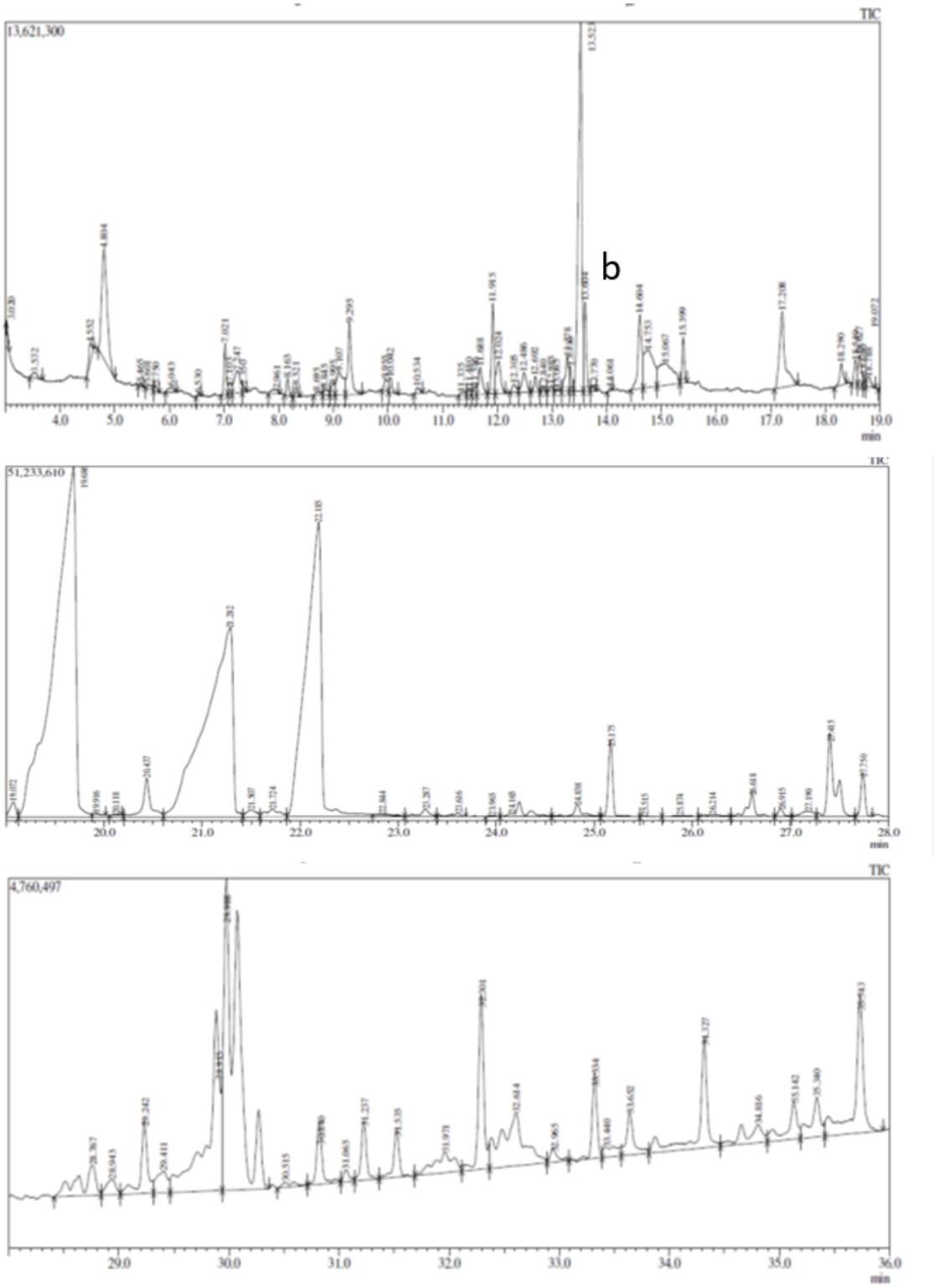

Figure 4

GC-MS of methanol extract of H. sabdariffa

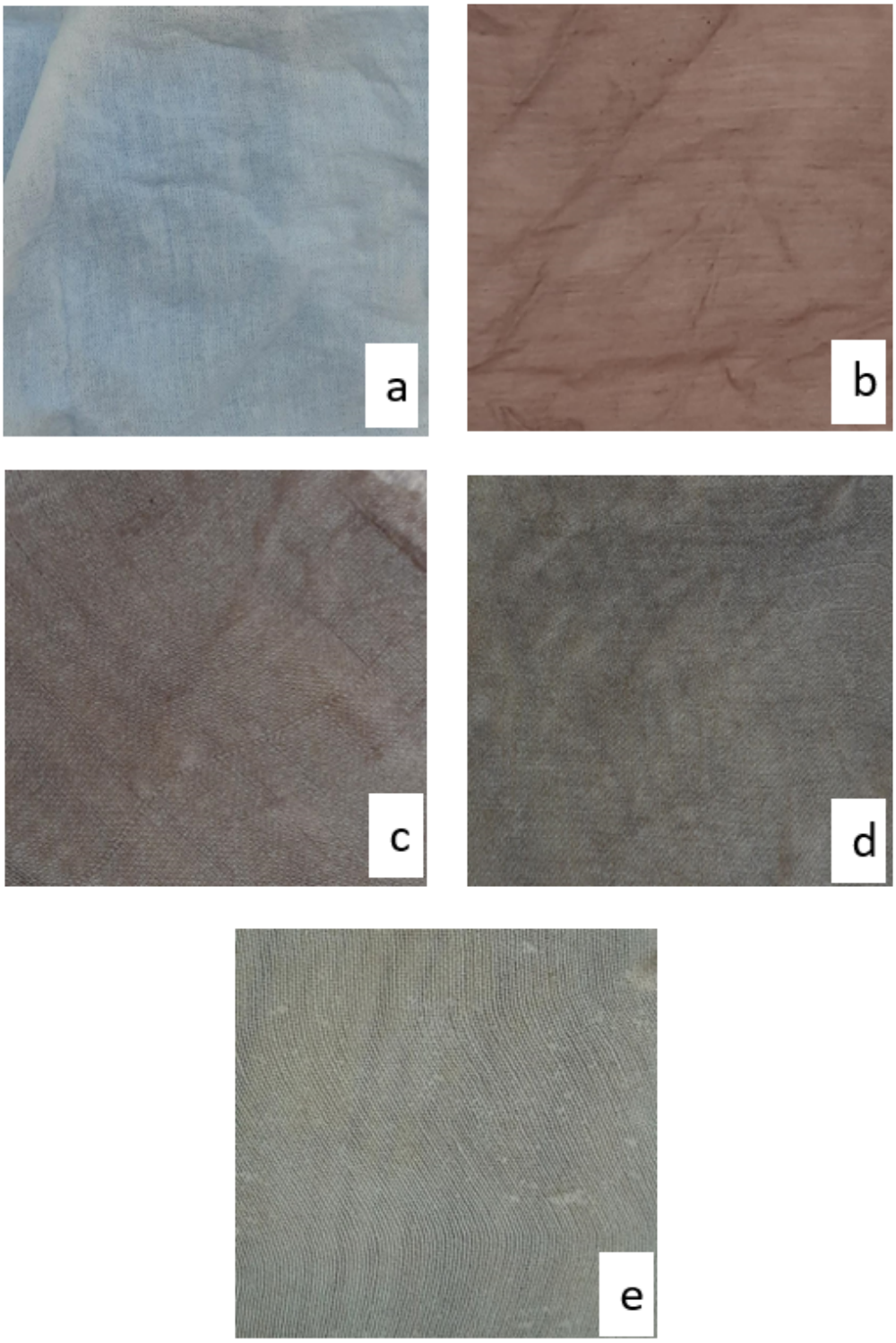

Figure 5

Application of Anthocyanin pigment treated on cloth a. Treated with 10% NaOH + H2O b. Treated with myrobalan + Anthocyanin c. Treated with K2cr2O7 + Anthocyanin dye d. Treated with FeSO4 + Anthocyanin dye e. Treated with SnCl2 + Anthocyanin dye

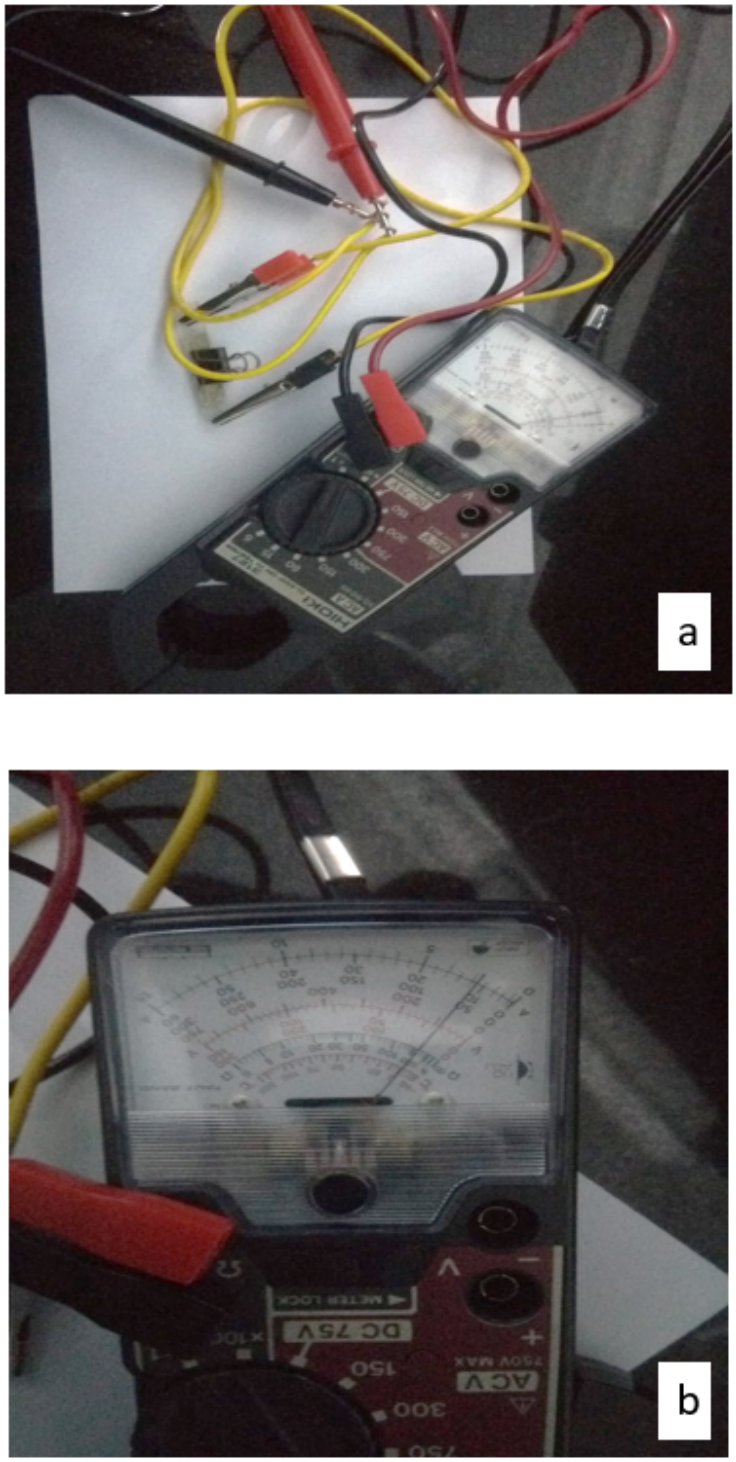

Figure 6

Solar sensitized cells treated with Anthocyanin pigment obtained from calyx of H. sabdariffa

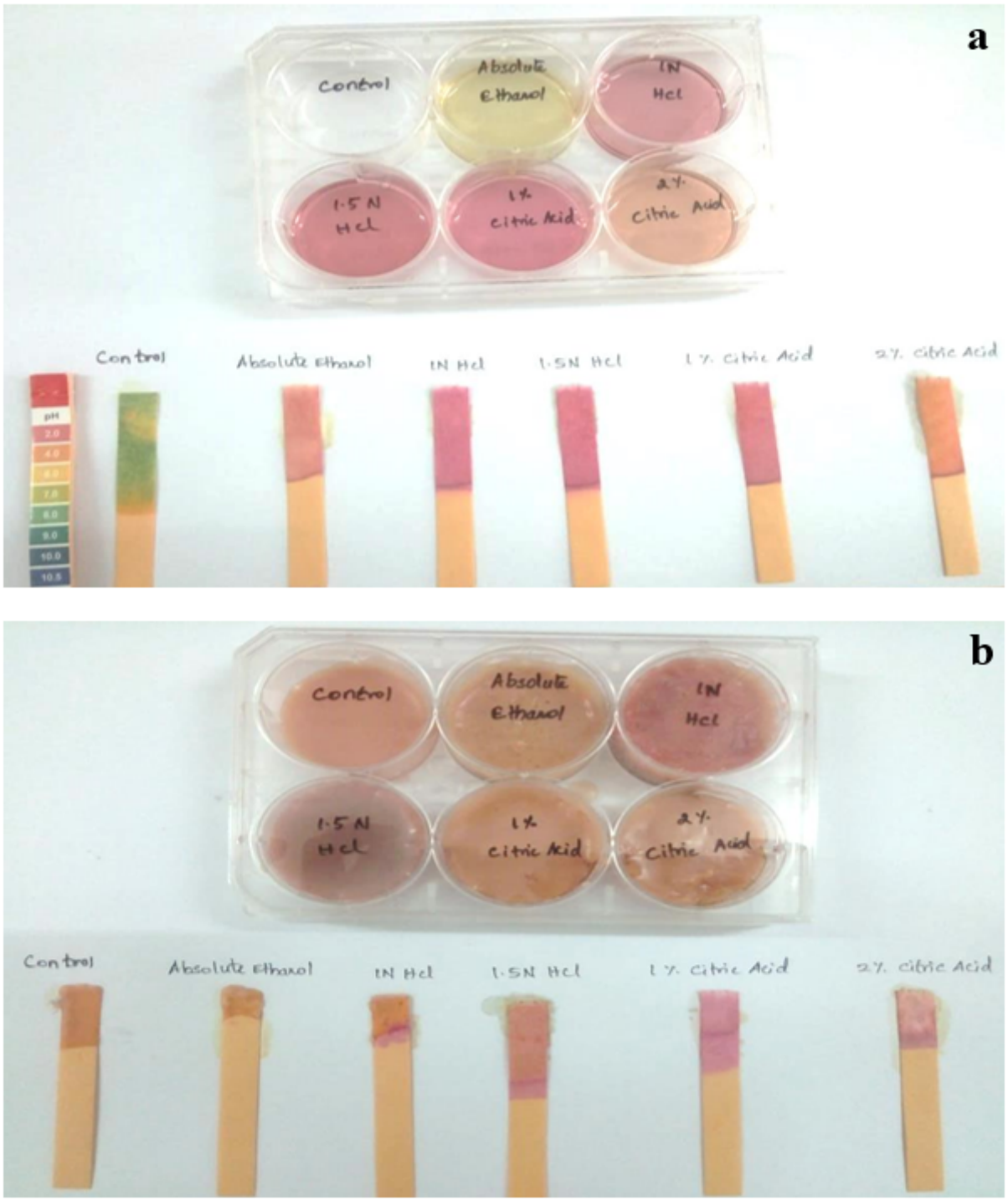

Figure 7

Lip balm prepared using Anthocyanin pigment extract from calyx of H. sabdariffa a. Before usage of wax b. After usage of wax