Available online at: http://www.ijmtst.com/vol6issue06.html

International Journal for Modern Trends in Science and Technology

ISSN: 2455-3778 :: Volume: 06, Issue No: 06, June 2020

Synthesis of Natural Food Colour from Carotenoid using Flower Petals

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To Cite this Article

K Naganandhini and Radha Palaniswamyi, "Synthesis of Natural Food Colour from Carotenoid using Flower Petals", *International Journal for Modern Trends in Science and Technology*, Vol. 06, Issue 06, June 2020, pp.:151-160; https://doi.org/10.46501/IJMTST060630

Article Info

Received on 18-May-2020, Revised on 06-June-2020, Accepted on 15-June-2020, Published on 23-June-2020.

ABSTRACT

Colour is an important characteristic of food. Since the colours are obtained from synthetic origin, it shows some adverse effect to humans. So it is an alternative way to use natural food colour obtained in the form a carotenoid pigments along with health benefits, In this current study, natural food colours are obtained by means of a carotenoid pigments by using flower petals of Hibiscus rosa- sinesis, senna auriculata, Magnolia champaca and Ixora coccinea by using the solvent extraction method. During the extraction upper phase containing carotenoid pigments are separated. The extracted pigments are then subjected to confirmatory assessment of carotenoid pigments by UV spectrophotometer. Phytochemical analysis was done to each extract to see the bio active compound present in it. Extracted sample was studied for antioxidant activity, antibacterial activity for each extract was performed against Escherichia coli. To identify the mixture of compounds, it was subjected to Thin Layer Chromatography, then analysed and compared with the standard carotenoid. To identify molecular components and structure of the each extract and functional group present in it, FTIR was done. Each sample of extraction was checked for the physical parameters like stability and pH. The obtained natural carotenoid colour pigments were incorporated in food along phytochemical properties too.

KEYWORDS: *Food colour, carotenoid, antioxidant, antibacterial, colour pigments*

DOI: https://doi.org/10.46501/IJMTST060630

I. INTRODUCTION

Colour is an important characteristic of food. Food colours are pigment, dyes or in any other form of a compound which are incorporated in food products to make it look more attractive and gives a tempting feel [1].It is added to food for four reasons to replace the colour during processing, to enhance colour already present, to minimize batch to batch variations and to colour uncoloured food. To protect and increase the shelf life of food, additives are incorporated.

Normally food additive or food colorant from synthetic origin are mostly used in food industry[2].Colours like yellow, orange, red, green etc. are highly preferred in soft drinks, candies, bakery products etc. These colouring compounds are used in two ways both as synthetic and in natural form [3].The usage of natural food colour in food does not carry any harmful effect to human health, but still they are less stable, attractiveness will be less, cost effective and it is difficult to find the exact shade and variety of shades of colour which are required to add in food products [4].

Synthetic food colours are cheap, easy blending with uniformity, available in different shades and hence it is an inevitable use in food industry. Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, Sunset Yellow FCF are the examples of synthetic food colours commonly used.

Some synthetic flavouring additive are used in food like Benz aldehyde for replacing the cherry and almond flavour and monosodium glutamate, it is used as flavouring agent in many food products. But these synthetic colours have undesirable taste and harmful effect to humans like allergic reactions, lower the haemoglobin count in body etc., so consumers are moving towards the naturally derived colours in food because of their health promoting properties and as an alternate solution for the usage of synthetic colours in food products [5].

Natural colour are extracted from various sources like edible plant, fruits, vegetables, seeds, root and pigments like carotenoids, chlorophyll etc. An alternative is to use natural food colorant from natural sources in the form of carotenoids which can be incorporated into food with medicinal value or health benefits [6].

Carotenoids are the class of more than 600 naturally occurring pigments synthesized by plants, flowers, algae and photosynthetic bacteria. Carotenoids are classified as carotenes, xanthophyll, lycopene etc., which are oxygenated hydrocarbon compounds, lipid soluble, yellow orange red pigment found in all higher plants [7]. The most widely used carotenoids in food industry are β-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin and α-carotene [8]. In this study, flowers from different colour shades and along with medicinal properties are taken into account. The flower petals used in the study are *Hibiscus rosa-sinesis, senna auriculata, Magnolia champaca,* and *Ixora coccinea.*

II. MATERIAL AND METHODS

Sample collection & Preparation

The samples collected were *Hibiscus rosa-sinesis, Senna auriculata, Magnolia champaca and Ixora coccinea.* Identification of flower samples was authenticated in Botanical Survey of India, TNAU campus, Coimbatore and the authentication number is as follows: - *Ixora coccinea* (Voucher Number : BSI/SRC/5/23/2020/Tech /609), *Hibiscus rosa- sinesis* (Voucher Number : BSI/SRC/5/23/2020/Tech /610), *Magnolia champaca*(Voucher Number:BSI/SRC/5/23/2020/Tech/611), *Senna auriculata* (Voucher Number: BSI/SRC/ 5/23/2020/Tech /612)**.**They were washed thoroughly using sterile water and dried in the shade to get rid of the moisture and nearly two gram of fresh petals were taken for the extraction process.

Extraction of carotenoid pigments

Carotenoid extraction from the petals was carried out by using solvent extraction method, the solvent used in the study are hexane, toluene, acetone, methanol. Two grams of flower petals were weighted and ground in mortar and pestle with 5ml of solvent and made up to 20ml with solvent and filtered by using what man No.1 filter paper. To this 20ml of distilled water was added and along with 10ml of 10% KOH. The mixture was shaken vigorously and kept undisturbed for separation. The upper phase containing carotenoid was obtained.

Spectroscopic screening and analysis of carotenoids

Carotenoids were quantified using Thermo UV-Vis spectrophotometer from 300-500 nm. Carotenoids in solution obey the Beer-Lambert law- their absorbance is directly proportional to the concentration. Carotenoids were quantified spectrophoto-metrically. Absorbance was measured using UV-Vis spectrophotometer. The λ max values of common carotenoids were obtained from Britton's 1995 compilation. The samples taken for the spectroscopic screening were acetone extraction of *Hibiscus rosa- sinesis* and *Ixora coccinea.* Hexane extraction of *Senna auriculata* and toluene extraction of *Magnolia champaca.*

Phytochemical screening

Phytochemicals are bioactive compounds present in the plants. It is formed during the normal metabolic process of plants. The extract were subjected to the phytochemical screening to detect the presence or absence of various phyto constituents like carbohydrates, steroids, cholesterol, proteins, alkaloids, terpenoids, flavonoids, tannins, saponins, anthocyanin coumarins and phenols. These phytochemical screening were subjected to four flowers extracts of *Hibiscus rosa- sinesis, Senna auriculata, Magnolia champaca* and *Ixora coccinea.* The protocol for screening in laboratory given below:

Test for Carbohydrates

To the 2ml of flower extract was taken and along with this 2 drops of Molisch's reagent was added and shaken well. 2ml of concentrated H_2SO_4 was

added on the sides of the test tube, a reddish violet colour ring appears at the junction of the two layers immediately indicates the presence of carbohydrates.

Test for Steroids

To 2ml of acetic anhydride was added to 0.5gm of flower extract was added to 2ml of concentrated H_2SO_4, the colour change from violet to blue or green indicated the presence of steroids.

Test for cholesterol

To 2ml of flower extract 2ml of chloroform was added in a dry test tube and along with this 10 drops of acetic anhydride and 3 drops of concentrated was added. A red rose colour changes to blue green colour which indicated the presence of cholesterol.

Test for proteins

To 2ml of flower extract, 2ml of biuret reagent was added and the formation of violet colour ring indicated the presence of proteins.

Test for Alkaloids

To the flower extract of 2ml 1% HCl and 6 drops of Mayer's reagent and Drangendroff's reagent was added. An organic precipitate indicated the presence of alkaloids.

Test for flavonoids

To the flower extract 5ml of ammonia solution were added and concentrated $H_2SO_4$was added. Formation of yellow colouration indicated the presence of flavonoids.

Test for Terpenoids

To 2ml of flower extract added 2ml of chloroform and 3ml of concentrated$H_2SO_4$to form a monolayer of reddish brown colouration showed the presence of terpenoids.

Test for Tannins

To 5ml of flower extract, a few drops of 1% of lead acetate was added. A yellow precipitate indicated the presence of tannins.

Test for Saponins

To the 5ml of flower extract 20ml of distilled water was added and agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Anthocyanin

To 2ml of flower extract 2ml of 2N HCL and ammonia was added. The appearance of pink red turns blue violet indicated the presence of anthocyanin.

Test for Coumarins

To the 2ml of flower extract 3ml of 10% NaOH was added. The formation of yellow colour indicated the presence of coumarins.

Test for phenols

To the 2ml of extract 3ml of ethanol was added and a pinch of FeCl3 was added which turns greenish yellow colour that indicated the presence of phenols.

ANTIOXIDANT ACTIVITY

a) DPPH Method

The free radical scavenging activity of the extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (1,1- diphenyl- 2-picryl-hydrazyl). 4mg of DPPH was allowed to dissolve in 100ml of methanol, which was served as a standard during the OD reading. Each extract were taken in different concentration of 250ul, 500ul, 750ul and 1000µl. These samples were made up with methanol to 1ml and 5ml of DPPH was added to all the test tubes. Then the tubes were subjected to incubation for 20min in dark at room temperature, the OD reading at 517nm was taken. It was calculated using formula

$$\% \text{ inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

b) Estimation of Vitamin C

To estimate the vitamin C content from the flower extract. The standard and working standard solution were prepared by 4% of oxalic acid solution (4gm in 100ml of water), the working standard was prepared by taking 10ml of standard and make up to 90ml of water and it was pipetted out in 0.2, 0.4, 0.6, 0.8 and 1ml in boiling tube. The samples of different concentration 250ul, 500ul were taken. Volume was made up to 3ml with water. To this 1ml of DNPH (2, 4-Dinitrophenylhydrazine) was added and 1-2 drops of thiourea solution was added to it. It was kept in water bath for 20 minutes at 40℃ for incubation, after which orange red ozone crystals were formed, 7ml of H2SO4 was added. The OD readings at 540nm were taken.

ANTIBACTERIAL ACTIVITY

In this study, well diffusion method is followed for screening carotenoid pigments of flower extract and the antibacterial activity was determined and activity was tested against Escherichia coli. The culture was inoculated in nutrient broth and kept in shaker for 24hr. The culture was swab in nutrient agar plates and well

was made using sterile cork borer of 11mm. The samples were loaded on the well and kept for incubation for 24hrs and the zone of inhibition was observed.

Thin Layer Chromatography (TLC)

TLC plates were prepared by adding 20ml of water to 10gm of silica gel powder in a beaker and the resulting slurry was poured on to glass plate with the height of 10cm and with of 5cm with the help of gel applicator at a thickness of 1cm, resulting silica gel plate is oven dried at 60℃ for 1 hour for pre activation. The extracted carotenoid extract was spotted on the base line of the TLC plates at 1cm away from the bottom and allow to dry at room temperature. The samples applied on the TLC plates were kept undisturbed in the TLC chamber containing mobile phase (petroleum ether and acetone in the ratio 19:1). Then the chromatogram was developed by providing the environment up to ¾ of the slide. Then the plate were taken out and dried for few minutes. Using UV light, the developed spots were seen and taken out. The distance travelled by the each spot can be calculated by using Rf value.

Fourier Transform Infra-Red (FTIR)

FT-IR is the preferred method of infrared spectroscopy. The IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. For sample analysis, the present samples were subjected to FTIR in the Biotechnology Laboratory at St. Joseph College of Arts and Science, Trichy.

PHYSICAL PARAMETERS

Stability

Extracted carotenoid pigments were subjected to stability test which are exposed to different temperature at 30℃, 37℃, 40℃, 50℃, 60℃, 70℃ and 80℃. Effect of differenttemperature on the colour intensity of the extract was measured by reading the absorbance at 520nm.

pH test

The effect of pH were determined in a range from 3 to 8 in a flask containing different buffer solution like acetate buffer for up to 3 to 5.6 pH and Boric acid buffer for 7 to 9, samples were mixed along with buffer solution and kept for 2 weeks and measurement of absorbance was taken every day at 517nm.

III. RESULTS AND DICUSSION

The synthesis of natural food colour from carotenoid was carried out by using four different flower petals *Hibiscus rosa- sinesis, Ixora coccinea, Magnolia champaca* and *Senna auriculata.* The colour pigment was obtained in the form of carotenoid pigments by extraction method. The second part was carried out by taking the carotenoid pigments to further study of phytochemical analysis, antioxidant activity and antibacterial activity. The carotenoid pigments were subjected to the characterisation purpose in order to indicate the presence of carotenoid pigments, like UV spectrophotometer, FTIR, TLC.

EXTRACTION OF CAROTENOID PIGMENTS

The carotenoid extraction was carried out by using solvents like acetone, hexane and toluene. The acetone extraction of *Hibiscus rosa- sinesis* and *Ixora coccinea* were done. Extraction from *Senna auriculata* was done by hexane and *Magnolia champaca* by toluene. Two grams of flower petals and 25ml of solvent were taken for the extraction of carotenoid pigments using mortar and pestle. The mixture was shaken vigorously and kept undisturbed for separation. [9] has worked on the extraction of carotenoids from the marigold (*Tagetes*) flower petals by solvent extraction of the pellatilized meal using food grade hexanes to obtain carotenoids esters rich in marigold oleoresin. Hydrolysis of carotenoids esters with alkali after homogenising the oleoresin in absolute alcohol and precipitation of carotenoid crystals using water alcohol mixture was done. In another study by [10] has done the extraction of carotenoid from *Osmanthus fragrans,;* they have taken the powdered samples and performed methanol and hexane extraction and a solution of NaCl (10%, w/v) was added in order. The mixture was continuously shaken until the plant material was colorless. Pooled organic phases were dried under nitrogen stream and saponified overnight using a 6% KOH methanolic solution. The carotenoids were subsequently re-extracted with hexane: diethyl ether (3:1, v/v) repeatedly until the aqueous phase became colourless.

SPECTROSCOPIC SCREENING AND ANALYSIS OF CAROTENOIDS

The extracted carotenoid pigments were analysed with the help of UV-Visible spectroscopy. Here, the carotenoid compounds along with the solvent (sterile water) are placed at room temperature, after 10 min, the absorption spectrum of each compound can be analysed as various peaks. The carotenoid compounds of flower extract of *Hibiscus rosa- sinesis* shows 244nm, *Ixora coccinea* shows 243nm and 657nm, *Magnolia champaca* shows 242nm, 486nm and 656nm and *Senna auriculata* shows 242nm and 485nm as projected in Fig. 4.2.

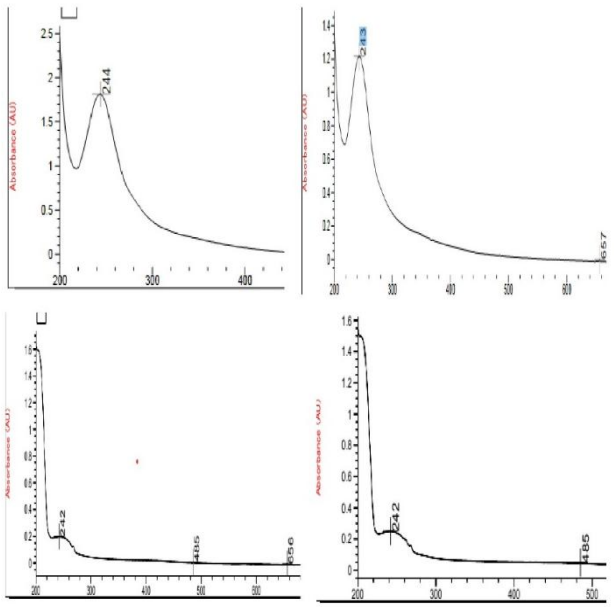


Fig 4.2: UV Spectroscopic analysis of A) *Hibiscus rosa- sinesis* B)*Ixora coccinea*C) *Magnolia champaca*D)*Senna auriculata*

The total carotenoids were determined by the Extract preparation from the strawberry, apricot and raspberry. The acetone-water mixture (4:1) was used as a solvent. The absorbance maxima were read at 663.6 nm for strawberry 646.6 nm for apricot and 470.0 nm for raspberry [11]. The sample of fungal biomass were taken for the extraction of the major carotenoid and the mixture of carotenoid isolated pigments were subjected to the UV visible spectrophotometer analysis shows that optical density of lycopene at the wavelength of 470 nm. β-carotene at the wavelength of 470 nm and the optical density of the mixed carotenoids in the mixture at the wavelength of 470 nm and the optical density of pure lycopene at the wavelength of 470 nm [12]. With the above supportive data, it can be clearly concluded that the UV spectroscopic values of the current study are similar to those obtained in the previous studies by several other scientific groups.

Phytochemical screening

The phytochemical analysis of each flower extract of carotenoid pigments were performed and reported as shown in Table 1.

Table 1: Phytochemical Analysis of Extracted Carotenoid Compounds

Phytochemical	*Hibiscus rosa-sinesis*	*Ixora coccinea*	*Magnolia champaca*	*Senna auriculata*
Carbohydrates	–	–	+	+
Steroids	–	–	–	–
Cholesterol	–	–	–	–
Proteins	+	+	–	+
Alkaloids	+	+	–	+
Terpenoids	+	+	-	–
Tannins	–	–	–	+
Saponins	+	–	+	+
Anthocyanin	–	–	–	–
Coumarins	+	+	–	+
Phenols	+	–	–	+
Flavonoids	+	–	–	+

symbol + indicated positive and – indicates negative

Flower extracts of *Ixora coccinea* contains ursolic acid and triterpenoids and have shown protective effect against systemic toxicity induced by cyclophosphamide and cisplastin. They are useful in dysentery, dysmenorrheal, leucorrhoea, haemoptysis, catarrhal bronchitis and opthalmopathy [13]. *Hibiscus rosa- sinesis*of flower extract contain alkaloids, saponins, protein, phytosterols and carbohydrate, has many pharmacological properties including antioestrogenic, anti-implantation, abortifacient, antipyretic, antispasmodic, hypotensive, embryotoxic, antispermatogenic, insect attractant, analgesic, antifungal and anti-inflammatory activities [14].Phytochemical screening revealed a rich number of phytoconstituent groups viz. flavonoids, alkaloids, tannins, glycosides, carbohydrates, amino acids, saponins, and phenolic compounds with ample health benefits [15].

ANTIOXIDANT ACTIVITY

a)DPPH (2, 2-Diphenyl-1- picrylhydrazyl) Assay: The obtained carotenoid compounds of each flower extract were taken in different concentration ranges from 250µl to 1000µl and their free radical scavenging activity was measured and reported as shown in Table 2. All the 4 extracts showed potential activity with *Ixora coccinea* which was comparatively lesser than the rest three.

Table 2: Antioxidant activity by DPPH method

Volume of Sample	*Hibiscus rosa-sinesis*	*Ixora coccinea*	*Magnolia champaca*	*senna auriculata*
250µl	46%	5.5%	27%	34%
500µl	50%	10%	39%	39%
750µl	56%	15%	49%	49%
1000µl	65%	18%	54%	54%

Hibiscus rosa-sinensis flowers was observed using different concentrations of the methanolic extract. The % inhibition of the methanol extract was

highest at 100µg/ml concentration, followed by 75µg/ml and 50µg/ml concentration and lowest with 25µg/ml concentration [15]. Studies on *Ixora coccinea* shows that the radical-scavenging activities of all the extracts increased with increasing concentration and the maximum scavenging activity showed was 20.86% [16]. Free radical scavenging activity of the different extracts of the flowers of *Magnolia champaca*shows the scavenging DPPH free radical of hexane, ethyl acetate extracts and the three isolated com-pounds 1-3 were found to be 250 µg/ml, 160 µg/ml, 200 µg/ml, 220 µg/ml and 150 µg/ml, respectively [17].

Antioxidant activity of Vitamin C

Scavenging of vitamin Cwas observed in *Hibiscus rosa-sinensis* shows 0.15µg and 0.21µg. *Ixora coccinea* shows 0.18µg and 0.36µg, *Magnolia champaca*shows 0.1µg and 0.2µg and *Senna auriculata* shows 0.24µg and 0.16µg. All 4 extracts showed good activity as indicated in Table 3.

Table: 3 Antioxidant activity by Vitamin C method

Sample	Concentration (µl)	Vitamin C (µg)	Total Antioxidant (µg)
Hibiscus rosa-sinensis	250 500	6 8	15 21
Ixora coccinea	250 500	3 10	10 20
Magnolia champaca	250 500	5 12	18 36
Senna auriculata	250 500	2 4	24 16

Previous studies have showed that ascorbic acid percentage in *Hibiscus rosa-sinensis* flower and *Citrus sinensis* fruit were significantly influenced by plant parts and their interactions. The ascorbic acid percentage in *Citrus sinensis* fruit was statistically higher 53.59mg/100g; as compared to *Hibiscus rosa-sinensis* flower 26.48mg/100g contained 27% lower ascorbic acid percentage [18]. The content of ascorbic acid determined by spectrophotometric method ranged between 617.07 and 1104 mg /100 g of dry sample for all investigated species. The highest content of ascorbic acid was measured in the flowers of *Crataegus monogyna* and *Crataegus subsphaericea,* 1104 mg and 1103 mg/100 g respectively [19].

ANTIBACTERIAL ACTIVITY

The antibacterial study to evaluate the antibacterial activity of the extracted carotenoid compound, the antibacterial activity was performed in agar well diffusion method against *Escherichia coli.* The well was made by using sterile cork borer of 11mm thickness. The zone of incubation was observed *Hibiscus rosa-sinensis* – 11mm, *Ixora coccinea* – 7mm, *Magnolia champaca* -12mm and *Senna auriculata* – 15mm. the antibiotic disk used against *Escherichia coli*is Gentamicin.

Table: 4 Antibacterial activity

S.No	Samples	Zone of inhibition
1	*Hibiscus rosa-sinensis*	11mm
2	*Ixora coccinea*	7mm

3	*Magnolia champaca*	12mm
4	*Senna auriculata*	15mm

Previous studies related to antibacterial activity showed that the ethanolic crude extract and the ethyl acetate crude extract of all the flower extract of Hibiscus and Red jungle flame showed good antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli.* The zone inhibition was observed and shows 15mm and 12mm [20]. Carotenoids isolated from *Rhodotorula glutinis* showed the antibacterial activity against *Streptococcus pyogenes* and *Escherichia* coli which was performed by using disk diffusion method at 0.5mg concentration which showed 9.1mm and in 1.5mg shows 11mm the zone of inhibition.

THIN LAYER CHROMATOGRAPHY

The obtained carotenoid pigments from the flower petals of *Hibiscus rosa- sinesis, Ixora coccinea, Magnolia champaca* and *Senna auriculata* were done using the mobile phase of petroleum ether and acetone in the ratio 19:1 and obtained chromatogram was calculated using Rf value which are listed below as shown in Table 5.

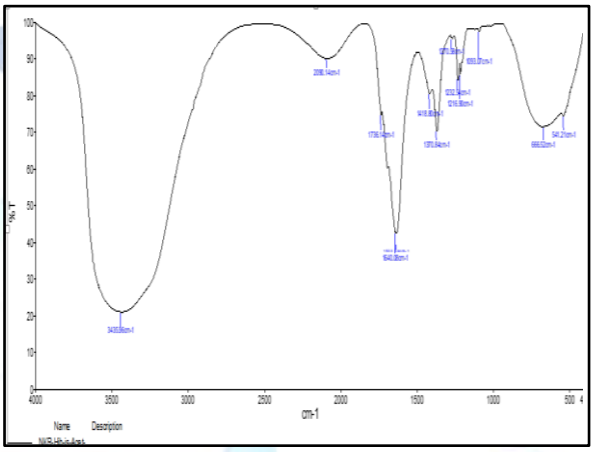
Table 5: Thin Layer Chromatography

S. No	Sample	Rf factor
1	*Hibiscus rosa- sinesis*	0.92
2	*Ixora coccinea*	0.94
3	*Magnolia champaca*	0.89
4	*Senna auriculata*	0.94

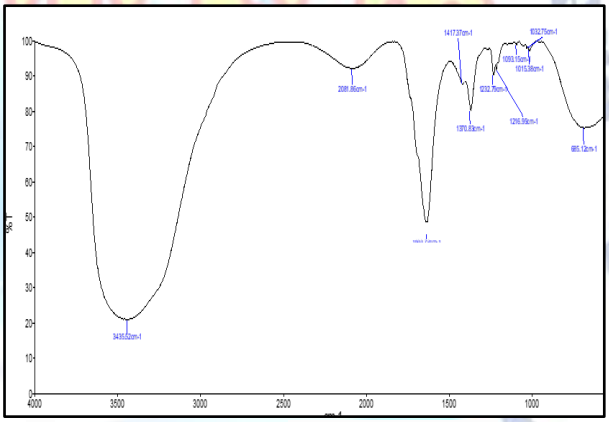
Carotenoid pigments from of extract of *Hibiscus rosa- sinesis* ethyl acetate and chloroform showed 0.95 0.94, *Ixora coccinea* extract of ethyl acetate and chloroform showed 0.97, 0.95 Rf value [22].The crude leaf and flower extracts of *Peltophorum pterocarpum* the purified carotenoid pigments and the standard were subjected to TLC. The mobile phase was hexane and acetone in the ratio of 6:4. Their respective Rf values were leaf carotenoid pigment was 0.96 and the flower carotenoid pigment was 0.91[23].

Fourier Transform Infra-Red

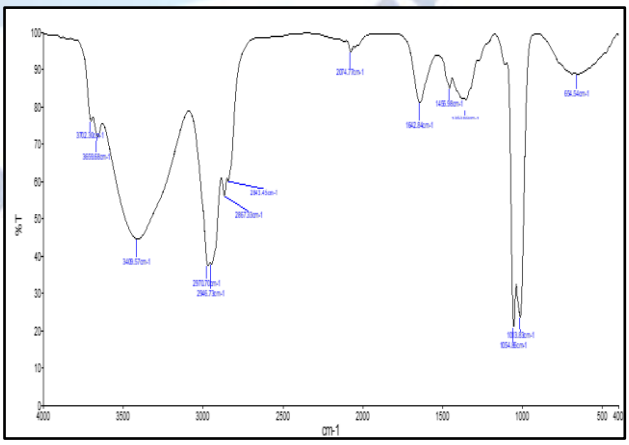
The spectral data were in the wavelength range of 4000–500 cm−1. The FTIR spectrum of flower extract of carotenoid pigments showed peaks for *Hibiscus rosa- sinesis*- 3435.56cm, 2090.06cm, 1736.84cm, 1640.90cm, 1418.52cm, 1370cm. *Ixora coccinea*- 3435.52cm, 2081.86cm, 1370.83cm, 1232.79cm, 1216.9cm. *Magnolia champaca*- 3669.98cm, 3409.57cm, 2970.7cm,2867.33cm, 2843.45cm, 2074.77cm, 1054.86cm, 654.54cm. For *Senna auriculata*- 3435.55cm, 3010.12cm, 2257.14cm, 14163.20cm, 1015.22cm, 675.87cm.



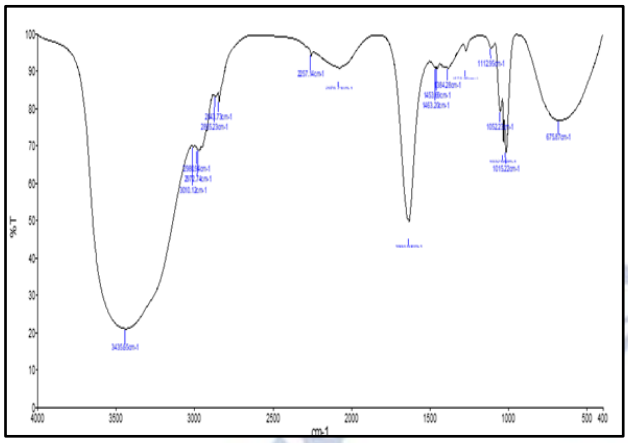
A) FTIR analysis of *Hibiscus rosa- sinesis*



B)FTIR analysis of *Ixora coccinea*



C)FTIR analysis of *Magnolia champaca*



D)FTIR analysis of *Senna auriculata*

Table: 6 FTIRanalysis for carotenoid compounds

Sl.No	SAMPLE	FREQUENCY cm-1	BOND	FUNCTIONAL GROUP
1	*Hibiscus rosa-sinesis*	3435.56 2090.06 1736.84 1640.90 1418.52 1370.22	C-H Stretching N=C=S Stretching C=O Stretching C=N Stretching O-H Bending S=O Stretching	Alkyne Isothiocyanate Aldehyde Imine loxime Alcohol Sulphonamide
2	*Ixora coccinea*	3435.52 2081.86 1370.83 1232.79 1216.9	O-H Stretching N=C=S Stretching - S=O Stretching S=O Stretching	Alcohol Isothiocyanate - Sulphate Sulfonyl chloride
3	*Magnolia champaca*	3659.68 3409.57 2970.07 2867.33 2843.45 1642.84	O-H Stretching N-H Stretching C-H Stretching C-H Stretching C-H Stretching C=C Stretching	Alcohol Aliphatic primaryamine Alkene Alkene
4	*Senna auriculata*	3435.55 3010.12 2257.14 14163.20 1015.22	O-H Stretching C-H Stretching N=N=N Stretching C-H Stretching CO-O-C O Stretching	Alcohol Alkene Thiocyanate Alkane anhydride

Literature shows that studies related to the FTIR analysis with water extract of *Hibiscus* flower exhibited the characteristic bands at 3307.11cm-1 indicating the presence of alcohol and phenol (O-H) groups, and at 1635.90 cm-1 for carbonyl (C=O) group [23].

FTIR test result for *Ixora coccinea* flower extract shows the important wavelengths and the functional group found in the flower extract are alcohol group at of 3386 cm-1 wavelengths with O-H bond then functional group of alkanes with C-H bond at 2935 cm-1. C=C bond provided by alkene with 1616 cm-1 and Ester functional group which has C-O bond is also shown at 1285 cm-1 and 1077 cm [24].The methanol extract of *S. auriculata* showed characteristic absorption bands at 3390 cm -1 and 1055 (C-O) for a hydroxyl (-OH) group 2929 cm -1 (for C-H stretching) and at 1627 cm-1 for C=C group [25].

Physical Parameters
Stability

Colour stability test results interpreted that *Hibiscus rosa- sinesis* shows at 37℃ -0.55, 40℃-0.61, 50℃-0.61, 60℃-0.60, 70℃-0.62, and 80℃-0.61. *Ixora coccinea* shows at 37℃-1.16,

40℃-1.07, 50℃-1.11, 60℃-1.15, 70℃-1.16, and 80℃-1.16. *Magnolia champaca* shows at 37℃-0.20, 40℃-0.19, 50℃-0.20, 60℃-0.21, 70℃-0.23, and 80℃-0.22. *Senna auriculata* shows at 37℃-0.06, 40℃-0.06, 50℃-0.11, 60℃-0.11, 70℃-0.11, and 80℃-0.11.due to the stability of the compound it doesn't show much difference and it can be referred to the table 7.

Table: 7 stability test for the carotenoid compounds

S.No	Sample	TemperatureRange in Celsius					
		37	40	50	60	70	80
1	*Hibiscus rosa-sinesis*	0.55	0.61	0.61	0.60	0.62	0.61
2	*Ixora coccinea*	1.16	1.07	1.11	1.15	1.16	1.16
3	*Magnolia champaca*	0.20	0.19	0.20	0.21	0.23	0.22
4	*Senna auriculata*	0.06	0.06	0.11	0.11	0.11	0.11

It was observed that increasing temperature from 100°Cto180°C caused to increase the degradation of lycopene compared with the treatment at25°C. These results may be due to its presence of carotene the form of complexes with protein or lipo-proteins, submicro-scopic structure may be also a factor in their outstanding stability. The highest degradation of carotenoids (lyco-red) extracted from tomato peel was observed at 100°C followed by 90 and80°C, respectively. Therefore, the carotenoids (lyco-red) from tomato peel were more heat stable between 40°Cto70°C but the lower, degradation rate was noticed at 80°C [26].

pH

The obtained carotenoid compounds of flower extract of *Hibiscus rosa- sinesis, Ixora coccinea Magnolia champaca* and *Senna auriculata* were tested for the pH from acidic to base pH within the range of 2.0 to 10.0. The pH of the extracted carotenoid compounds is shown in Table 8.

Table: 8 pH of the carotenoid compounds

S.No	SAMPLE	pH
1	*Hibiscus rosa- sinesis*	8
2	*Ixora coccinea*	8
3	*Magnolia champaca*	9.2
4	*Senna auriculata*	8.5

Similar studies have carried out for the pigment isolated from the tomato peel shows the highest stability and alkaline pH, No loss of carotenoid was observed at pH 9.0 and 10.0. On the other hand, the degradation of colour did not exceed than from pH 7.0 and 10.0. For instance, the highest stability and less degradation of carotenoids extracted from tomato peel were noticed at alkaline media from pH 7.0 to 10.00 [26]. Fresh astaxanthin nanodispersion (1 mL) that was prepared with a plain aqueous phase was added separately to various citrate-phosphate buffer solutions (4 mL each) with pH values ranging from 2 to 10 The astaxanthin nano dispersion had a smaller mean particle size at neutral and alkaline pH than at acidic pH. Because the particles carry zero net surface charge when the pH equals the isoelectric point of the adsorbed stabilizer, the largest particle size was observed at pH 4–5 [27].

IV. CONCLUSION

Food colour are widely used in a food industries, to enhance the organoleptic (colour, flavour, appreance, taste and texture) quality to food. An alternative is to use natural food colorant from natural sources in the form of carotenoids which can be incorporated into food with medicinal value and health benefits too. Carotenoids are a group of phytochemicals from which yellow, orange, red and violet colours can be obtained from source like fruits and flowers which can be used as a natural food colorant and having an important role in maintain good health for humans without adverse sight effect.With the above study we conclude that the obtained carotenoid pigments from the flower extract can be used as a natural food colorant and it has been confirmed that presence of carotenoid compounds by various characterisation study and the other analysis. The above study have concluded that the carotenoid compounds can be used as a natural food colorant, since it is an

alternative way for the use of synthetic food colours in food.

V. ACKNOWLEDGEMENT

The authors would like to acknowledge the support rendered by The Management, Principal, and Deans – Research and Development and department of Biotechnology for having materialized this article. The communication number is DrNGPASC2020-21 BS 003.

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