ESTIMATION OF NATURAL FOOD COLOR POTENTIAL OF SOME LOCAL PLANT SPECIES THROUGH ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES

SAMAN SHAHID^{1,2}*, WAJIHA ZAKA ANSARI^{2,3}, ZAINAB SHEHZADI², MEMUNA GAFOOR SHAHID¹, AMJID KHAN³

¹Department of Botany, Government College University Lahore, Punjab, Pakistan ²Department of Botany, Lahore College for Women University Lahore, Punjab, Pakistan ³Department of Botany, University of Mianwali, Mianwali, Punjab, Pakistan

*Corresponding author's Email: shahidsaman36@gmail.com

Abstract

The present study evaluated the dye extract from different cheap and waste sources such as Salmalia malabarica, Dahlia pinnata and Chrysanthemum grandiflorum. Color pigments such as anthocyanins and carotenoids were recognized to be the primary coloring agents that give distinct color to the samples. The highest yield obtained from all of these samples was given by Dahlia pinnata ethanolic flower extract i.e., 47.67% ad minimum yield was given by Salmalia malabarica ethanolic flower extract 29.76%. Room temperature was determined to be the best favorable temperature for the stability of color extracts among all temperatures tested. Antibacterial activity was performed agaist B. subtilis and S. aureus bacterial strains. The maximum zone of inhibition was found to be 28±0.4mm by Dahlia pinnata against B. subtilus. That is almost equals to the standard Erythromycin 22 ± 1.0 zones that reveal the intermediate activity of the extract. On the other hand, minimum zone was reported by the extract of Salmalia malabarica as 9±0.57mm. These outcomes with reference to standard indicate that the ethanolic extract of the Dahlia pinnata shows the better antibacterial activity as compared to that of Salmalia malabarica. The antifungal activity of color dyes extracted in ethanol against different fungal strains like Rhizopus stolonifer and Trichoderma viride. For this analysis dye extract of Dahlia pinnata used against Rhizopus solonifer and dye extract of Salmalia malabarica used against Trichoderma viride. Zone of inhibition was not developed. For analysis of antioxidant activity of dye extracts of Dahlia pinnata, Chrysanthemum grandiflorum, and Salmalia malabarica. Different test like DPPH, TPC, TAA and FRAP tests were performed. The antioxidant activity ranges from 92.92±0.08mm to 3.53±0.12mm. And all extracts demonstrated the good antioxidant activities within standard range. Between these extracts Dahlia pinnata found to be a potential antioxidant. The results obtained were directly compared with BHT as standard. The extracted colors were also used in the manufacturing of sweets, jellies, cakes and sugar syrup.

Keywords: Dye, extract, *Dahlia pinnata, Salmalia malabarica* and *Chrysanthemum grandiflorum*, antimicrobial activity, antioxidant activity, application of extracts.

Introduction

Dyes and colorants that are naturally sourced are currently gaining demand over the synthetic dye. The reason behind this actually the increase of awareness of consumer that is developed due to health and environmental issues (Nwoba et al., 2020). Basically, color is an important factor that increases the acceptability of consumer towards food product (Ghaffar et al., 2015). Consumer links the food color with other qualities which include food safety, freshness and ripeness (Jaberian et al., 2013). There are many food products are added to the food to make it more acceptable to consumers (Chung et al., 2017). The food colors that are synthetic, not obtain from natural sources are available in different colors and shades (Siva et al., 2011). There are many reports that are on toxicity of synthetic colors. In many cases the synthetic colors proved to be carcinogenic and toxic (Arora et al., 2017). This necessity is the result of regulatory measures and consumer focus on synthetic food colors. The use of high synthetic hues pollutes, perturbs the environment and poses health risks to humans (Sanda et al., 2020). Natural colors may be extracted from natural sources such as plants, animals, insects, and minerals (Jadhav et al., 2020). People who lives in major cities far from agricultural production, where more food processing and transportation is necessary, people know that the food has own natural colors (Yusuf et al., 2017). During the transportation and processing of food from production areas to big cities it may lose their qualities, so that some preservatives must be added for desirable appearance and safe for consumption so there are multiple reasons for food colorants are used. (Shetty et al., 2017). The food's natural color is mostly attributable to anthocyanins, carotenoids, concrete and chlorophyll. These pigments are enormous in the lives of people not because of their coloring

qualities but because of their likely health consequences (Belemkar et al., 2015). They have been widely investigated in their occurrences in food along with the problems that impact the structure. Natural colors contain low oxygen, metal and lignin consistency, temperature, oxidation agents and pH (Kumar and Dhinakaran, 2017). The main objective of the study to extract natural colour dyes from flowers of Salmalia malabarica, Dahlia pinnata and Chrysanthemum grandiflorum. То examine the stability, antimicrobial and antioxidant activities of extracted natural color dyes and to explore the applications of color extract in various food items.

Materials and Methods

The current research was carried out for dyes extraction and to evaluate different parameters like antimicrobial activities and antioxidant activities. Different extraction techniques were used for the extraction of dyes.

Plant material collection

For the extraction of color dyes and for various experimental analyses, flowers of *Dahlia pinnata*, *Chrysanthemum grandiflorum* and *Salmalia malabarica* obtained from botanical garden, of Government college university Lahore. Sample (flowers) were placed in bags and brought to laboratory for further analysis.

Evaluation of Antimicrobial Activity

For the evaluation of antimicrobial potential of distinct color extracts, various bacterial and fungal strains were used (Menazea et al., 2020).

Bacterial strain and fungal strains

The bacterial strains (*Bacillus* subtilusand Styphylococcus aureus) and fungal strains (*Rhizopussolonifer* and *Trichodermaviride*) were collected from the institute of industrial biotechnology, GC University Lahore.

Extraction of plant pigment

The selected petals of *Dahlia pinnata* and *Chrysanthemum grandiflorum* were washed properly and then dried at room temperature. The weighed amount of flowers of *Salmalia malabarica* were blended with ethanol for 15-20 minutes and the ethanol gives highest color yield. Weighed number of petals of *Dahlia pinnata* and *Chrysanthemum grandiflorum* were dried and powder was made by grinding in pestle and mortar. The powder form was dipped in ethanol at room temperature. The pigments came out in dissolved form in ethanol and the extract was collected in beaker.

Very little amount of citric acid was introduced in extracted color liquids that act as a stabilizer. Then the extraction was done through Soxhlet apparatus in order to form paste, and were saved in sterilized jars. The formula below was used to calculate the crude dye's percentage (%) yield.

$$wdy = rac{wbe - wae}{wae} imes 100$$

Where, Wdy= % yield of crude dye Wbe= weight of plant material before extraction Wae= weight crude material after evaporation

Thermo-stability of extracted color dye

The extracts were stored at various temperatures within a specified range of temperature to determine the stability of the dye. To check the color stability, the samples were stored at varying temperatures for 15 days.

For this purpose, the weighed amount of dyes was taken in Petri plates and kept at following temperature ranges.

Sample1: color extracted was kept at room temperature $30-35^{\circ}$ C

Sample 2: Maintaining the color extract in the incubator at temperature 45-55° C

Sample 3: color extract was kept in freezer at -15 to -20° C

Sample 4: color extract was kept in incubator at 15 to20°C

The samples were reweighed after 15 days and the change in weight of dyes was noted. The change in color was also visually noted.

Estimation of Antimicrobial activity

Preparation of samples

For the analysis of antimicrobial activity 0.1g of dye was taken and volume was raised up to 10ml with ethanol (the solvent that was used during extraction) for making 10% solution. The vials containing the sample were stored at 20° C for subsequent use.

Preparation of culture medium plates

Preparation of nutrient Agar and potato dextrose agar

For this purpose, 8g of Nutrient broth was dissolved in some amount of water and pH was adjusted to 6.8 with the help of 10% KOH and 10% HCl, 14g of agar was added and raised the volume up to 1000ml. The solution was mixed with stirrer until a homogenized mixture was obtained. Eventually the medium was poured in 2L conical flask and autoclaved at 121oC temperature under 15 lb pressure for 15 minutes. For the preparation of PDA medium 39g of potato dextrose was dissolved in 800ml of distilled water and pH was maintained of 5.6 using 0.01N NaOH and 0.01N HCl. The final volume made up to 1000ml. Eventually, for 15 minutes the medium was autoclaved at 121°C temperature at 15 (pa) pressure.

Preparation of PDA and Nutrient agar medium plates

Medium plates were prepared by pouring 15ml of media in each autoclaved petri plate. The plates were covered with cling film and kept in the refrigerator until they were used.

Bacterial strains and fungal strains

Under aseptic circumstances, 20ml of nutrient broth was poured into a test tube. A cotton plug was placed in the test tube holding the nutrient broth after the bacterial colony had been transferred there. The test tube was properly labeled and was stored in refrigerator at 4° C.In each test tube, the autoclaved PDA medium was poured under aseptic conditions. Fungal colonies which were to be cloned are transferred to PDA medium. The test tube was cotton plugged and sealed with cling film was placed in incubator at 25° C for 2 days.

Agar-well diffusion Method

Agar-well diffusion method was adopted by (Kirby et al. 1956). The inoculum was spread with the help of cotton bud on the surface of solid medium. Well, was made with the help of corkborer No. 4 in the center of plate. With the help of sterilized dropper, the color extract was poured in the well. Cling film was used to seal the Petri plates and were incubate at 37° C for bacterial strains and 25°C for fungal strains.

Measurement of Zone of inhibition

After the completion of incubation time, the Petri plates were observed and zone was measured. The wells were record with the help of scale

Antioxidant activity estimation

Antioxidant potential was determined by underneath variables:

- Total antioxidant activity (TAA) by phosphomolybdenum scheme
- Total phenolic contents (TPC) quantification
- DPPH radical scavenging action
- Ferric Reducing Antioxidant Power Assay (FRAP)



Figure.1: Showing a complete Experimental design for dye extraction from different plant sources, its thermo-stability, antimicrobial and antioxidant activities.

Total antioxidant Activity (TAA) by phosphomolybdenum scheme

For this 5.32g of sodium phosphate, 2.47g of ammonium molybdate was added and raised the volume with the help of distilled water up to 100 ml. By using pipette, 16.7 ml of H2SO4 was added. By using distilled water, the volume raised up to 500 ml.

1ml of pigment extract of 500µg/ml concentration was added in each test tube and 4ml reagent was poured in each test tube. Capped each test tube and labeled each tube properly.

The test tubes were incubating at 95°C in water bath for 80-90 minutes. After incubation period, test tubes were placed at room temperature to allow them to cool. After the room temperature has attained the final activity was recorded by using UV spectrophotometer at 695nm. BHT (Butylated Hydroxytoluene) was used as standard and reagent was used as blank, in this activity. The regression equation of the ascorbic acid calibration curve was used for the phosphomolybdenum method's computation of TAA (Total antioxidant activity). The results were documented as $ACC\mu g/mL$.

Total phenolic contents (TPC) quantification

For the TPC 0.5mg/ml plant dilution was prepared. For this preparation 0.001g of the colour extract was mixed with sample and mixed with the solvent and raised the volume up to 20 ml. The Method of (Makkar et al., 1993) was followed for determining the total phenolic contents (TPC) estimation. In order to prepare the test tubes, 1 ml of the colour extract with a consistency of 0.5 mg/ml was mixed with 2.8 ml of 10% Na2CO3 and 0.1 ml of 2N FC reagent, test tubes were incubated at 25° C for 40 minutes. The absorbance of the spectrophotometer was taken at 725nm. Different gallic acid reagent solutions were made in order to calculate TPC. The results were documented as GAE µg/ml.

DPPH free radical scavenging activity

The absorbance was adjusted to 1.00 0.02 at 515 nm by diluting the stock solution of DPPH radical cation (25 mg/l) in methanol. After adding the appropriate amount of sample to 2.5 ml of this diluted reagent, the change in absorbance was measured every five minutes for 30 minutes. The formula was used to determine the percentage of the remaining DPPH radical. %DPPH Appropriate quantity of sample was added to 2.5 ml of this diluted reagent and change in absorbance was measured after every 5 minutes intervals for 30 minutes. The %age of DPPH radical remaining was calculated using the formula. (Jones *et al.*, 2020)

$$\%$$
 DPPH remaining $=\frac{Af}{Ao} \times 100$

In addition to calculating the EC50 value for each sample, kinetic curves were used to demonstrate how DPPH absorbance decreased with time.

Ferric Reducing antioxidant power assay (FRAP)

The FRAP reagent was prepared by mixing 25ml of 300mM acetate buffer (pH 3.6), 2.5ml of 10mM TPTZ solution prepared in 40mM HCl and 2.5ml of ferric chloride (FeCl3), 2990µl of FRAP reagent was mixed in 10µl of plant solution. The sample was placed in FRAP reagent could stand in dark for 30 minutes. In the meanwhile, the FRAP reagent was react with plant sample. The absorbance was noted in the spectrophotometer in at 593nm. The results were evaluated in micromoles of Trolox Equivalent (TE) per mL of sample by computing with standard curve constructed for different concentrations of Trolox (Spiegel et al., 2020). The following equation, which was generated from the standard calibration curve, was used to determine the FRAP values.

$$X = \frac{y - 0.069}{0.002}$$

Statistical Analysis

Data were analyzed using the analysis of variance (ANOVA) method with LSD p 0.05 using the Statistix tool, version 8.1. In all tables and figures, mean values were distinguished (p 0.05) and denoted by various letters along with the \pm standard error.

RESULTS

Percentage yield and physical conditions of plant extracts

By using different plant parts, various colour dyes were extracted, such as flowers. The percentage (%) of crude dye extraction yielded the highest amount of 45.67% of *D. pinnata*, 41.67% yield was shown by *C. grandiflorum*, whereas 29.76% yield was shown by *S. malabarica*.

At different temperature, the colour dye's thermo-stability was evaluated. The colour dyes were placed at freezer, incubator, and room temperature. The dye extract that was placed at incubator, there was a loss of moisture and pH value was varied. The extract that were kept at room temperature showed better thermo-stability because there was less loss or gain in weight and pH was not affected. all plant samples. Means followed by different letters are significantly different according to ANOVA, *LSD* at $p \le 0.05$ (Least significant difference).

ANTIMICROBIAL ACTIVITY

The activity was performed to determine the antimicrobial activity of extracted color dyes. For the analysis C. grandiflorum, D. pinnata and S. malabarica were used. The standard disc was used by taking into consideration that, microbial samples act as the positive control, while the extraction solvent acts as the negative control. The reference discs were used to check the bacteria used as the test organisms for their vulnerability. The test organisms could be classifying as Susceptibility, i.e. the microbes were repressed by the attainable antimicrobial agent's concentrations, intermediate, i.e. the reaction of microbes may be less than the susceptible and resistant, i.e., the bacteria showed negligible resistance to the concentration.



Figure 2: Showing percentage of crude dye from

| Antibiotic (standard discs) | Quantity (ml) | Zone of inhibition (mm) Styphylococcus aureus. | Zone of inhibition(mm) Bacillus subtilis |
|-----------------------------|------------------|---|---|
| Amikacin | 1.5 | 19 ^b ±0.4 | 17 ^b ±1.3 |
| Ampicillin | 1.5 | $22^{a}\pm1.0$ | $21^{a}\pm1.5$ |
| Tetracycline | 1.5 | 17°±0.6 | $16^{c}\pm 0.6$ |
| Final response | | Intermediate | Intermediate |

Table 1: Zone of inhibition (mm) against standard antibiotic discs produced by the strains of bacteria

Table 2: Inhibition zone of fungi against standard antifungal discs, measured in millimeters

| Antifungal standard discs | Conc. (µg/mL) | Zone of inhibition (mm) Rhizopus solonifer | Zone of inhibition (mm) Trichoderma viride |
|---------------------------|------------------|---|---|
| Fungivin | 1.5 | 15 ^b ±2.4 | 42 ^a ±1.7 |
| Grisofluvin | 1.5 | 23ª±1.0 | 27 ^b ±1.5 |
| final response | | Intermediate | Intermediate |

Table 3: Solvent-negative control inhibition zone (mm) of bacterial and fungal strains

| Solvents | Quantity(ml) | Zone of inhibition(mm) R. soloniferand B. subtilis | Zone of inhibition(mm) <i>T. viride</i> and <i>S. aureus</i> |
|-----------------|--------------|---|---|
| Ethanol | 1.5 | 0±0 | 0±0 |
| Methanol | 1.5 | 0±0 | 0±0 |
| Distilled water | 1.5 | 0±0 | 0±0 |
| Response | | Negligible | Negligible |

Table 4: Zone inhibition (mm) produced by D. pinnata, S. malabarica and C. grandiflorum

| Plants | Conc. (µg)/mL | Zone of inhibition (mm) Ethanol | Zone of inhibition (mm) Methanol | Zone of inhibition (mm) Distilled water |
|-----------------|------------------|---------------------------------------|--|---|
| D. pinnata | 100 | $28^b \pm 0.57$ | $28.5^{a}\pm0.5$ | $28^{a}\pm1.68$ |
| S. malabarica | 100 | 24°±0.57 | $24.3^{\circ}\pm1.78$ | 27 ^b ±0.5 |
| C. grandiflorum | 100 | $30^{a}\pm1.15$ | $26.3^{b}\pm2.88$ | 26.3°±0.18 |

ANOVA, LSD at p 0.05 shows a significant difference between means expressed by various letters (Least

significant difference).

Antibacterial activity

The zone of inhibition created by the dye extracts of *S. malabarica, D. pinnata* and *C. grandiflorum* was calculated by ethanol against positive strain of *B. subtilis.* The reading was taken in mm by simple scale. The maximum zone of inhibition was shown by *D. pinnata.* That was 28 ± 0.4 against *B. subtilus.* Zone of inhibition of *C. grandiflorum* is 24.8±0.3 and minimum zone of inhibition shown by *Salmaliamalabarica* 9±0.57. These outcomes with reference to standard indicate that the ethanolic extract of *D. pinnata* shows the better antibacterial activity as compared to that of *C. grandiflorum* and *S. malabarica.*

Antifungal activity

The antifungal activity of colour dyes extracted in ethanol against different fungal strains like *R. solonifer* and *T. viride*. For this analysis dye extract of *S. malabarica*, *D. pinnata* and *C. grandiflorum* were used against the fungal strains. There was no zone of inhibition observed.

ANTIOXIDANT ACTIVITY

The percentage (%) of DPPH free radical quenching potential of the dye extract was evaluated. For this analysis colour extracts of *S. malabarica, D. pinnata* and *C. grandiflorum* were used. The antioxidant activity was ranges from 92 ± 0.08 to 3.53 ± 0.12 . And extracts demonstrated the good antioxidant activities within standard range. The results obtained were directly compared with the standard BHT as standard.

These results indicate that all the extracts demonstrated the good antioxidant activity within the standard range.

Total phenolic content (TPC)

Total phenolic contents (TPC) were examined on the extracted colour dyes. For this analysis *S. malabarica*, *D. pinnata* and *C. grandiflorum* were used. Among these samples maximum value of 27.6 ± 0.85 was shown by *S. malabarica*, minimum value of 23.00 ± 0.23 shown by *Dhaliapinnata*. The results indicate that *S. malabarica* dye has a maximum total phenolic content value.

For this analysis *S. malabarica*, *D. pinnata* and *C. grandiflorum* were used. Between these samples, maximum values of 4.989 ± 0.17 was shown by *D. pinnat extract*. Whereas, minimum value was 3.53 ± 0.12 , that was given by *S.malabarica*. The results indicated that *D. pinnata* has a maximum total antioxidant potential.

FRAP assessment

The FRAP evaluation was done to calculate the possible potential decrease of several extracted dyes. The evaluation was expressed in TE (M/mL). Trolox's standard curve was created to accurately represent the results. For this analysis, S. malabarica, D. pinnata and C. grandiflorum were used. The maximum ferric reduction potential was indicated by Salmaliamalabaricaas 9.37± $0.86\mu g/mL$. D.pinnata showed 7.785±1.04 value.

| Plants | Solvent | Total phenolic contents Absorbance at 765nm | Total phenolic contents (µg/ml of Gallic acid) |
|-----------------|---------|--|---|
| S. malabarica | Ethanol | 1.23 ^c ±0.19 | $27.6^{a}\pm0.85$ |
| D. pinnata | Ethanol | 1.79 ^b ±0.31 | 23°±0.23 |
| C. grandiflorum | Ethanol | $2.65^{a}\pm0.11$ | $25.7^{b}\pm 0.55$ |
| LSD | | 0.55 | 0.689 |

Table 5: Total phenolic content of S. malabarica, D. pinnata and C. grandiflorum.

Table 6 showing total antioxidant potential by S. malabarica, D. pinnata and C. grandiflorum

| Plants | Solvents | Total antioxidant activity Absorbance at 695nm | Total antioxidant activity (mg/ascorbic acid) |
|-----------------|----------|---|---|
| S. malabarica | Ethanol | $0.78^{\circ} \pm 0.19$ | 3.53 ^b ±0.12 |
| C. grandiflorum | Ethanol | $1.0^{b}\pm 0.21$ | 2.45°±0.15 |
| D. pinnata | Ethanol | $1.13^{a}\pm0.31$ | 4.98 ^a ±0.17 |
| LSD | | 0.391 | 0.586 |

Table 7 FRAP assessment of different dyes extracted

| Plants | Solvent | FRAP | |
|-----------------|---------|---------------------|----------------------------|
| | | Abs. at 593nm | GAE (µg/mL of Gallic acid) |
| S. malabari | Ethanol | $0.121^{b}\pm 0.99$ | 9.37 ^a ±0.86 |
| D. pinnata | Ethanol | 0.101°±0.72 | $7.78^{b}\pm1.04$ |
| C. grandiflorum | Ethanol | $0.238^a \pm 0.62$ | $8.23^{\circ} \pm 0.76$ |
| LSD | | 0.682 | 0.585 |

ANOVA, LSD at p 0.05 shows a significant difference between means expressed by various letters (Least significant difference).

DPPH radical scavenging activity

The effectiveness of dye extracts for scavenging % DPPH radicals was measured to assess the antioxidant potential. It had a range of 32.720.56 to 92.920.08a%, with the highest activity being reported by ethanol extract of *Dahlia pinnata* and minimum by methanolic extract of antioxidant.

The reported results, which were performed in triplicates, were presented as mean \pm standard error. Least Significant Difference is referred to as LSD. Different alphabets represent changes in the mean that are statistically significant (p <0.05).

| Plant | Solvents | % DPPH |
|-----------------|-----------------|--------------------------|
| | | remaining |
| S. malabarica | Ethanol | $76.75^{b}\pm0.09$ |
| | Methanol | $77.70^{a}\pm0.72$ |
| | Distilled water | 74.92°±0.32 |
| C. grandiflorum | Ethanol | $66.65^{a} \pm 0.53$ |
| | Methanol | $52.20^{\circ} \pm 0.06$ |
| | Distilled water | $59.20^{b} \pm 0.20$ |
| D. pinnata | Ethanol | 92.92 ^a ±0.08 |
| | Methanol | $32.72^{\circ} \pm 0.56$ |
| | Distilled water | 83.13 ^b ±0.35 |
| LSD | | 0.204 |

Table 8: DPPH radical scavenging activity of different dye extract with different solvents

DISCUSSION

In the present study, different samples of plants extracts were used to be isolating various Pigments. For this, easily available and comparatively cheap sources were procured from the local fruit and vegetable market, so that extract could be economical. The parts that are usually discarded due to lacFk of knowledge (flowers of *Salmalia malabarica*, *Chrysanthemum grandiflorum* and *Dahlia pinnata*) were used in the study. Two Gram-negative (*Escherichia coli* and *pseudomonas aeruginosa*) and grain positive (*Staphylococcus aureus* and *Bacillus subtilis*) were used for the measurements of antibacterial potential of plants extracts. Similarly, two fungal strains (*Rhizopus stolonifer* and *Trichoderma viride*) were employed for testing antifungal potential of the samples. DPPH radical scavenging activity, TPC, TAA, FRAP were estimated using assorted methodologies. With 1:1 material to liquor ratio and 30% pre-mordanting the jelly with extracts from *S. malabarica*, *C. grandiflorum* and *D. pinnata*. This work clearly indicates both extracts from *S. malabarica*, *C. grandiflorum* and *D. pinnata* could be used as source for natural color for cupcakes, jelly and hence will result in better utilization of *S. malabarica*, *C. grandiflorum* and *D. pinnata* waste from the food processing sector as a value-added component (Kumar and Dhinakaran, 2017).

The study of antimicrobial activity of S. malabarica aqueous excerpts in vitro was measured against some microorganisms using the agar disc diffusion method. Samples of S. malabarica were composed from tree source. Antimicrobial activity of S. malabarica was accepted out with different strains of bacteria such as Salmonella typhii, Staphylococcus aureus, Shigella dysentrae, and Escherichia coli. The distance of inhibition zone was used as pointers of antimicrobial activity. Comment based on diameter h5 of zone of inhibition on the petri dishes was renowned & the zone was considered and assessment was done. S. aureus and S. typhii were having a good zone of inhibition fluctuating between 15-17 mm in diameter whereas S. dysentrae and E. coli were having no zone of inhibition. The study exhibited that S. malabarica operative in the studied attentiveness in S. aureus and S. typhii and not in S. dysentrae and E. coli (Aggarwal, 2021).

The food and beverage manufacturing release substantial amount of waste which

comprise natural dyes. (Ayele *et al.*, 2020) Such waste could attend as source for the withdrawal of natural dyes like lemon and orange peels for food manufacturing procedure like extracted colors were employed in candies making, sugar syrup making and used in coloring various food stuffs (Dusemund, 2020). The consequences provide the potential of such left-over as a source of natural source for natural dye stuff withdrawal. (Kothari *et al.* 2021) Colors were obtained from different natural sources, such as barks, berries, and fruits, and they were used to colour a variety of foods, including butter, sweets, cakes, bakery goods, and sugar syrups.

CONCLUSION

From the present study, it could be concluded that solvent extraction method was the best method for the extraction of dye extract. It is advisable to extract natural dye colors from this technique. The results suggested that the plant extract can be used as the biological pesticides to kill certain harmful microorganisms. Antibacterial and antifungal potential of the dye extracts and be used as for the formation of some antimicrobial drug 'and are beneficial for eating purpose. The samples of dye extracts were found to be very beneficial for health purpose, as they are found to be very good antioxidants. The consequence of the %DPPH radical scavenging assay, total antioxidant activity, FRAP and total phenolic contents estimation of dye extracts had provided that the dyes had significant antioxidant potential which is comparable to the standard

antioxidants. Because they contain active components, these extracts can be used to make traditional or herbal medicines. These natural extracts can be used as food colouring, agents in jellies, candies, cakes and sugar syrup etc. These colour extracts are cheap and almost harmless for human beings.

Conflict of Interest: The authors have no conflict of interest to declare.

References

- Aggarwal, S. 2021. Indian dye yielding plants: Efforts and opportunities. *Natural Resources Forum*, 45(1): 63-86.
- Arora, J., Agarwal, P., and Gupta, G. 2017. Rainbow of natural dyes on textiles using plants extracts: Sustainable and eco-friendly processes. *Green and Sustainable Chemistry*, 7(01): 35.
- Ayele, M., Tesfaye, T., Alemu, D., Limeneh, M., and Sithole, B. 2020. Natural dyeing of cotton fabric with extracts from mango tree: A step towards sustainable dyeing. Sust. Chem. Pharm., 17: 100293.
- Belemkar, S., and Ramachandran, M. 2015. Recent trends in Indian textile industry-Exploring novel Natural dye products and resources. *Int. J. Text. Eng. Proc.*, 1(3): 33-41.
- Chung, C., Rojanasasithara, T., Mutilangi, W., and McClements, D. J. 2017. Stability improvement of natural food colors: Impact of amino acid and peptide addition on anthocyanin stability in model beverages. *Food Chem.*, 218: 277-284.
- Dusemund, B. 2020. Risk assessment of food components with botanical

origin. Reg. Toxicol., 1-15.

- Ghaffar, A., Yameen, M., Kiran, S., Kamal, S., Jalal, F., Munir, B., and Jabbar, A. 2015. Chemical composition and *invitro* evaluation of the antimicrobial and antioxidant activities of essential oils extracted from seven Eucalyptus species. *Molecules*, 20(11): 20487-98.
- Jaberian, H., Piri, K., and Nazari, J. 2013. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. *Food Chem.*, 136(1), 237-244.
- Jadhav, R. V., and Bhujbal, S. S. 2020. A review on natural food colors. *Pharm. Resonance*, 2(2): 12-20.
- Jones, A., Acquaviva, A., Suktham, T., Dennis, G. R., Shalliker, R. A., and Soliven, A. 2020. Total Antioxidant capacity with peak specificity via reaction flow chromatography and the ferric reducing antioxidant power assay. *Food Anal. Meth.*, 13(3): 608-616.
- Kirby, K. S. 1956. A new method for the isolation of ribonucleic acids from mammalian tissues. *Biochem. J.*, 64(3): 405.
- Kothari, S., and Khan, S. 2021. Natural dyes using plant palette: a brief review. J. *Global Biosci.*, 10(4): 8582-8605.
- Kumar, C. S., and Dhinakaran, M. 2017. Extraction and application of natural dyes from orange peel and lemon peel on cotton fabrics. *Text. Res. J.*, 80: 2117-2123.
- Makkar, H. P., Blümmel, M., Borowy, N. K., and Becker, K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agr.*, 61(2): 161-165.

- Menazea, A. A., Eid, M. M., and Ahmed, M. K. 2020. Synthesis, characterization, and evaluation of antimicrobial activity of novel Chitosan/Tigecycline composite. *Int. J. Biol. Macromol.*, 147: 194-199.
- Nwoba, E. G., Ogbonna, C. N., Ishika, T., & Vadiveloo, A. 2020. Microalgal pigments: a source of natural food colors. *Microalgae Biotechnology for Food, Health and High Value Products*, pp. 81-123. Springer, Singapore.
- Sanda, M., Badu, M., Awudza, J. A., Boadi, N. O. 2020. Development of TiO₂-based dye-sensitized solar cells using natural dyes extracted from some plant-based materials. *Chem. Int.*, 7(1): 9-20.
- Shetty, M. J., Geethalekshmi, P. R., and Mini, C. 2017. Natural pigments as potential food colorants: a review. *Trends in Biosci.*, 10(21): 4057-4064.
- Siva, R., Palackan, M. G., Maimoon, L., Geetha, T., Bhakta, D., Balamurugan, P., and Rajanarayanan, S. 2011. Evaluation of antibacterial, antifungal, and antioxidant properties of some food dyes. *Food Sci. Biotech.*, 20(1): 7-13.
- Spiegel, M., Kapusta, K., Kołodziejczyk, W., Saloni, J., Żbikowska, B., Hill, G. A., and Sroka, Z. 2020. Antioxidant activity of selected phenolic acids– ferric reducing antioxidant power assay and QSAR analysis of the structural features. *Molecules*, 25(13): 3088.
- Yusuf, M., Shabbir, M., and Mohammad, F. 2017. Natural colorants: Historical, processing and sustainable prospects. *Nat. Prod. Bioprospecting*, 7(1): 123-145.