Formulation and Evaluation of Herbal Gulal using Different Temple Waste Flowers

Meenu Verma[1], Vaishali Mishra[1], Akanksha Singh[1], K.J. Singh[2], S.K. Tewari[2], Mahesh Pal[1 *]

1. *Phytochemistry Division, CSIR- National Botanical Research Institute, Lucknow- 226001, India.*
2. *Botanical garden Division, CSIR- National Botanical Research Institute, Lucknow -226001, India.*

***Corresponding Author:**
Mahesh Pal:
Phytochemistry Division, CSIR- National Botanical Research Institute, Lucknow 226001, India (drmahesh.nbri@gmail.com).

Abstract

Every day, tonnes of flowers offered by devotees in temples are simply thrown as waste in rivers which contributes to pollution in some or the other way. The flowers of *Rosa indica, Tagetes erecta*, *Hibiscus rosa-sinensis* and leaves of *Aegle marmelos* are offered by devotees in large quantity in almost all the temples. Instead of throwing these flowers as waste we may utilize them as a source of natural colour for various purposes. Thus, this research work aimed to develop a cost-effective and eco-friendly technology for the formulation of herbal gulal (a coloured powder, used mostly during holi festival) from temple floral waste. The formulated herbal gulal was evaluated on different parameters such as solubility, angle of repose, sensitivity, colour stability, water wash ability, pH and antibacterial activity. The developed formulation was found safe with no harmful health effects compared to harmful and hazardous synthetic dye based gulal, available in the market with lots of chemical compounds and heavy metals. Thus chemical free herbal gulal of different colours were developed from temple waste having colour stability, water washability, antibacterial activity, zero sensitivity and pH suitable for skin.

Key words: temple floral waste, pollution, cost effective, eco-friendly, herbal gulal, natural dyes, synthetic dye

Introduction

India is a land of diverse cultures and religions, where we can see people with different cultural values, customs and traditions according to their religions. One of the most important festivals of Hindus in India is Holi, also known as the 'festival of colors'. People throw colored powder into the air and splash them on others. These colors provide the festival a sense of beauty, ritual and tradition but if these colors are synthetic dye-based then they adversely affect the human health. Today, we employ synthetic dye based colors to celebrate holi which contain harmful chemicals. These chemicals are very injurious to the human body and if unfortunately, they enter inside our body, they may cause fatal reactions. Oxidized metals and harmful synthetic dyes are the most common colors available in the market. These substances have the potential to seriously damage both the environment and human health (1). Nowadays synthetic colouring agents are added to cosmetics. Dermal contact, with special consideration for areas near mucous membranes (like the eyes or lips, where colourful makeup is applied daily), is the primary way that humans are exposed to synthetic dyes in cosmetics. Triarylmethanes are able to reach the bloodstream following systemic absorption in cosmetics (2). Natural dye-based products are the potential substitute for these synthetic dye based products because of their safe, non-toxic and eco-friendly nature.

Therefore, in light of the aforementioned situation a process technology has been developed, to produce herbal gulal as a natural product from temple floral waste. The process provides an opportunity for the preparation of different colors of herbal gulal like yellow, pink, orange, green, using temple floral waste. The floral waste degradation is a very slow process as compared to the degradation of kitchen waste (3). About 23000 temples have been estimated in Varanasi where tonnes of flowers are thrown as waste every day (4). These temple wastes are rich in marigold, rose, hibiscus flowers and leaves of stone apple (baelpatra). Therefore, flowers of *Rosa indica* (rose), *Tagetes erecta* (marigold), *Hibiscus rosa-sinensis* (hibiscus) and leaves of *Aegle marmelos* (baelpatra) were principally used as source of natural dye for production of herbal gulal of different colors.

R. indica L. is a perennial shrub with flowers. Numerous rose formulations are utilized in the Indian medical system to treat conditions like gallstones, bacterial infections, enlarged tonsils, heart problems, and sore throats. The phytochemicals reported in the petals of *R. indica* are Quinic acid, cyanidin-3,5-diglucoside, pelargonidin-3,5-diglucoside, Pyrogallol, 5-Hydroxymethylfurfural, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, and Levoglucosan (5). The annual herb *T. erecta* L. has solitary inflorescences of yellow or orange flowers. The plant has mostly been researched for its carotenoid representatives. Its flowers contain phytochemicals like gallic acid, quinic acid, syringic acid, ellagic acid derivatives, glycosides of quercetagetin, quercetin, kaempferol, patuletin and lutein (6). The Malvaceae family plant *H. rosa-sinensis*is indigenous to China. It is a shrub that is frequently grown as an ornamental plant in the tropics. In many herbal teas, hibiscus serves as a key component and has medicinal qualities as well. The petals contain cyanidin-3,5-diglucoside, cyanidin-3-sophoroside-5-glucoside, quercetin-3,7-diglucoside, quercetin-3-diglucoside (7). Another important temple waste is the leaves of *A. marmelos*. It is commonly known as baelpatra, Indian quince and stone apple.

It is the most significant medicinal plant grown in India, the chemical constituents reported in the leaf include coumarins (mermenol and praeltin), O-(3,3-dimthylally) halofordinol, N-4-methoxystyryl cinnamide, N-2-methoxy-2- [4-(3',3'-dimethyl allyloxy) phenyl] ethyl cinnamide (8). Baelpatra is said to have exceptional antibacterial, anticancer, antiviral, anti-inflammatory, and anti-fungal properties that give it biological activity against a wide range of pathogenic organisms. The serum insulin level is raised by *A. marmelos* leaf extract, which lowers blood sugar levels (9).The current work intends to establish a practical and affordable approach technology for herbal gulal manufacture employing natural colours. Thus the objective of this study was utilization of temple waste for colour extraction, preparation and evaluation of herbal gulal.

Material and methods

Plant material and preparation of crude extract

Flowers of *R. indica, T. erecta, H. rosa-sinensis* and leaves of *A. marmelos* were collected from the local temples of Lucknow, Uttar Pradesh, India in the month of July 2021. The petals were separated from the flowers and weighed. The temple floral waste (flowers and leaves) was shade-dried for 2-3 days. The extraction process of *R. indica, T. erecta, H. rosa-sinensis* and *A. marmelos* was carried out with the help of different methods and different solvents as demonstrated in table 1. The extraction process of dried rose petals was carried out by maceration and decoction using methanol and water respectively as a solvent. The dried petals of marigold were also subjected to maceration and decoction using acetone and water respectively as a solvent. The extraction of hibiscus flowers and baelpatra (bael leaves) was carried out by maceration using methanol as a solvent. The extracts obtained were concentrated under reduced pressure. The concentrated extracts were then weighed for the calculation of their extractive yield.

Extract yield (%) = $(W_1/W_2) \times 100$

where W_1 is the weight of extracted plant residues in grams, and W_2 is the weight of raw material (flowers and leaves) in grams.

Table 1. Extraction methods and percentage yield of extracts of *R. indica, T. erecta, H. rosa-sinensis and A. marmelos*

S. No	Raw material	Method of extraction	Solvent used	Temperature of extraction (ºC)	Extract obtained	% Yield of extract
1	Fresh rose petals	Maceration	Methanol	27 (Room temperature)	E1	14.0
2	Fresh rose petals	Decoction	Water	80	E2	15.6
3	Shade-dried petals of marigold	Maceration	Acetone	27 (Room temperature)	E3	19.8
4	Shade-dried petals of	Decoction	Water	80	E4	16.2

	marigold					
5	Shade-dried petals of hibiscus	Maceration	Methanol	27 (Room temperature)	E5	17.6
6	Shade-dried leaves of bael	Maceration	Methanol	27 (Room temperature)	E6	13.2

Development of formulation of herbal gulal

For the development of the formulation the compatibility between the components and the flower extract has to be studied. Each of the extracts E1, E2, E3, E4, E5, and E6 obtained from *R. indica, T. erecta, H. rosa-sinensis* and *A. marmelos* respectively were added to carbohydrate powder in a definite ratio. The components were thoroughly mixed and allowed to dry on water bath for 2 to3 hours with regular stirring after interval of 30 minutes. The dried product obtained was subjected to grinding after addition of few drops of natural floral essence to it. A fine powder of herbal gulal with smooth texture was obtained.

Evaluation of herbal gulal

The formulation prepared was evaluated with the help of the following parameters:

Solubility: To assess the solubility profile of the prepared formulation, a pre-determined amount of sample was placed in test tube, various solvents were added and test tube was shaken well and observed.

Angle of Repose: To assess the sample's flow behaviour, the angle of repose was calculated using the funnel (10). The following formula was applied to arrive at the result:

$\tan \alpha = H/R$

where, α = angle of repose, H = height of the pile of powder, R = radius of the pile of powder

Sensitivity: To check the irritation effect, a small amount of sample was applied on the forehead.

Color stability: The formulation was under observation for 7 days to note down the change in color of formulated herbal gulal samples.

Water wash ability: Small amount of formulated herbal gulal was applied on hand for a few minutes. Later it was washed with water to observe the water wash ability of the formulation.

pH: A (previously calibrated) digital pH meter of LABMAN (Model no: LMPH- 12) was used for measuring the pH of the sample. Before measurement, the samples were diluted to 10% (w/v) in distilled water (11).

Antibacterial activity

Preparation of inoculums of microorganisms for testing

Three pathogens *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* were used as test subjects to determine the antibacterial activity of *R. indica, T. erecta, H. rosa-sinensis* and *A. marmelos*. These three pre-isolated bacterial cultures were obtained from CSIR-NBRI Lucknow's Microbiology Division. The cultures were sub cultured on NA slants and stored at 4^0C until needed. For testing of the sample, the inoculums were prepared from the stock culture and sub cultured into the nutrient broth (30 ml) using a sterilised wire loop.

The inoculate was further incubated overnight at 37 ^{0}C in a rotary shaker. These inoculates were further stored at 4^{0}C until usage.

Antibacterial activity analysis (Disc Diffusion Assay)

The antibacterial activity of extracts was done by Disc Diffusion Assay (12). The antibacterial activity of extract prepared from *R. indica*, *T. erecta*, *H. rosa-sinensis* and *A. marmelos*, was evaluated against three pathogenic bacterial strains by using the agar well diffusion method under sterilized conditions. For this fifteen NA plates were prepared for extract antibacterial activity. 400 µl inoculums of each selected bacterium was uniformly spread over agar plates with the help of sterilized glass spreader.

After five minutes one well, approximately 7mm in diameter was bored with the help of borer. The equal volume (50 µl) of antibiotic Tetracycline (100 mg/ml), each extract (100 mg/ml) was poured into the wells. The plates were incubated at 37 ^{0}C for 24 hrs in the incubator. The next day, the results were observed and the antibacterial potential was measured in terms of diameter of zone of inhibition.

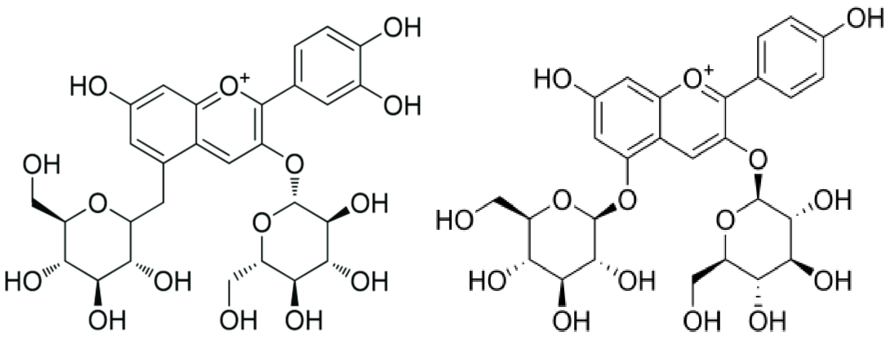
Result and Discussion

The herbal gulal of a variety of colors were formulated using the extracts (E1, E2, E3, E4, E5 and E6) of *R. Indica, T. erecta, H. rosa-sinensis* and *A. marmelos*. These floral extracts were responsible for imparting different colors to the herbal formulations as demonstrated in table 2.

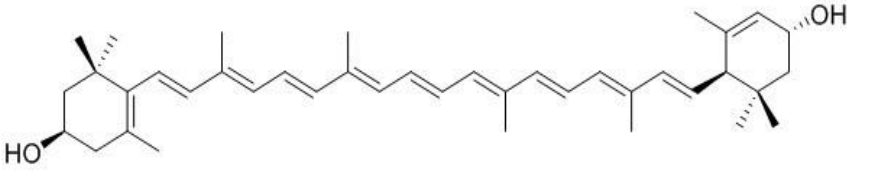
Table 2.Ingredients with prescribed quantity in the formulation of herbal gulal of different colors

S.No.	**Extract used**	**Ratio of carbohydrate powder(R_C): extract used (w/w)**	**Color obtained**
1	E1	11:1	dark pink (F1)
2	E2	15:1	Pink (F2)
3	E3	20:1	Yellow (F3)
4	E4	11:1	Light yellow (F4)
5	E1 and E3	25:1:0.5 (R_C:E1:E3)	Orange (F5)
6	E5	15:1	Light pink (F6)
7	E6	12:1	Light green (F7)

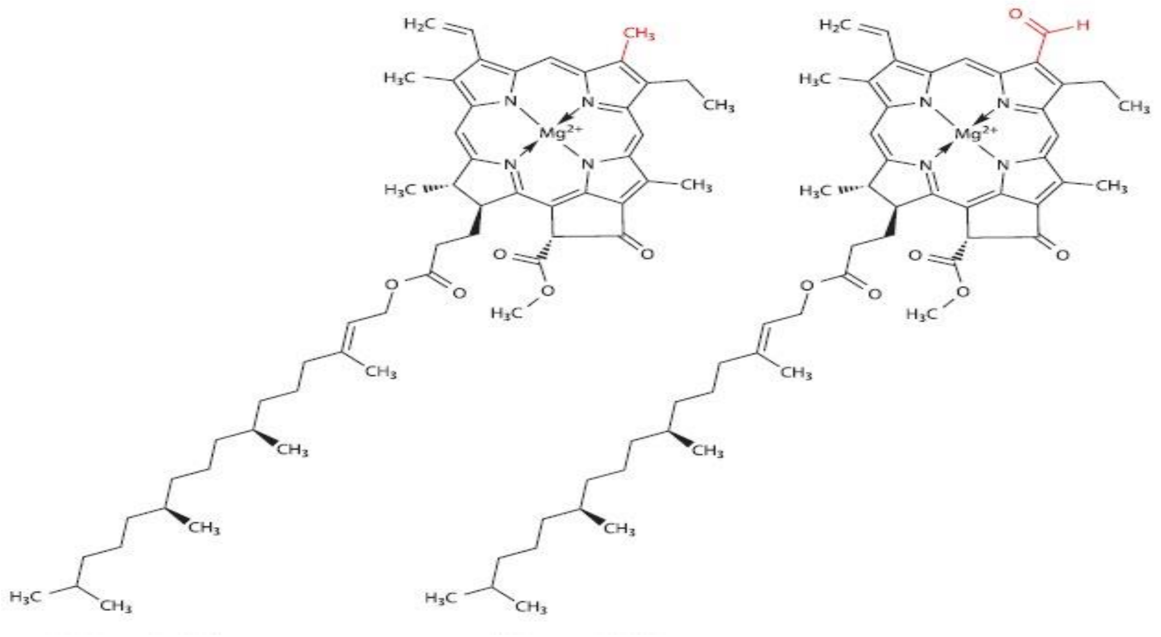
The ingredients were mixed in this fixed ratio so that the brightness and texture of gulal is perfect.



(A) (B)



(C)



(D) (E)

Fig. 1: Chemical structure of cyanidin -3,5-diglucoside (A), pelargonidin -3,5-diglucoside (B), lutein (C), Chlorophyll a (D), Chlorophyll b (E)

The different colors of herbal gulal were obtained due to the presence of a class of compounds like carotenoids and anthocyanins in the floral extracts and chlorophyll (for green color) in leaves extract used for these formulations (F1, F2, F3, F4, F5, F6 and F7). The floral extract of *T. erecta* contains Lutein which is responsible for the yellow color of the flower. It also contains other class of compounds that have antidiabetic, antioxidant and anti-proliferative activity. Nutritional supplements are manufactured by using lutein to prevent the loss of visual acuity caused by age-related macular degeneration or other eye diseases (4).

The floral extract of *R. indica* contains cyanidin-3,5-diglucoside and pelargonidin-3,5-diglucoside, two anthocyanins previously reported in this plant species (13). Anthocyanins give plants a variety of physiological health benefits and disease-preventive actions in addition to giving natural beautiful colors (14). The anthocyanins are the primary colorants among flavonoids; pelargonidin derivatives provide the foundation for orange-red tints, cyanidin derivatives for red tints, and delphinidin derivatives for lilac to blue tints. Anthocyanins are thought to provide a wide range of health benefits, in addition to beautiful natural color to plants and their parts (15).The HPLC chromatogram of *T. erecta* (marigold) extract with standard of lutein (16) and *R. indica* (rose) extract with standards cyanidin-3,5-diglucoside and pelargonidin-3,5-diglucoside (13) are given in fig. 2 and 3 respectively.

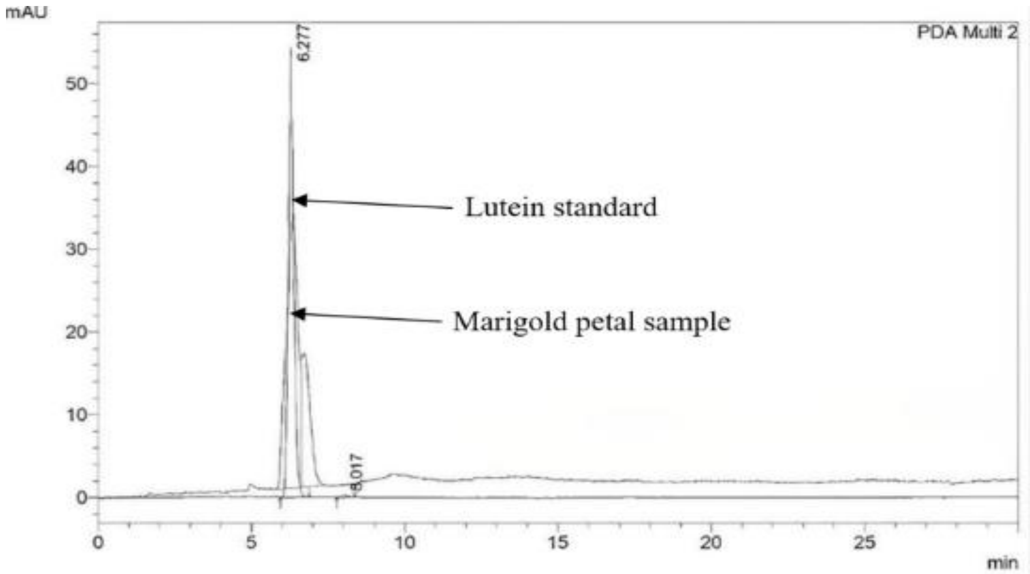


Fig. 2: HPLC Chromatogram of *T.erecta* (marigold) petal extract with lutein as standard

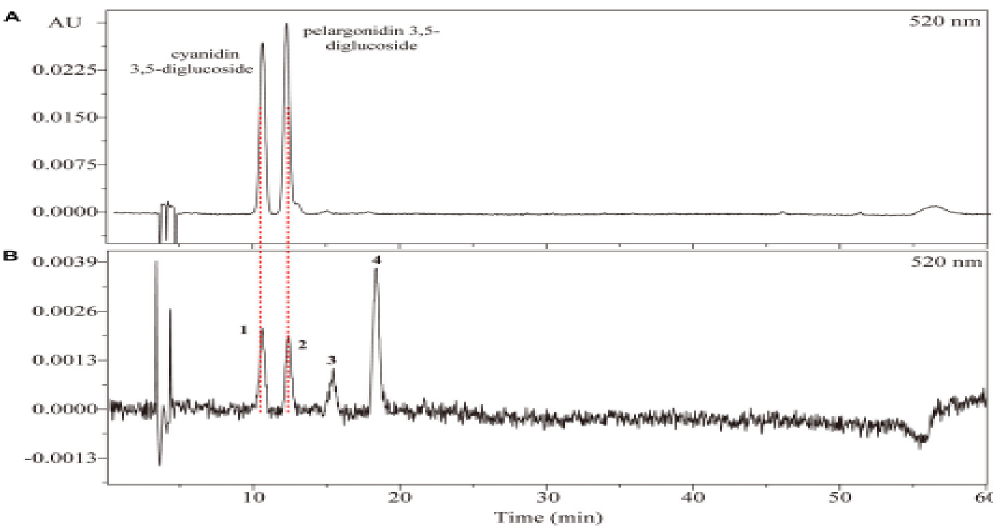


Fig. 3: HPLC Chromatogram of *R. indica* (rose) petal extract (B) and cyanidin-3,5-diglucoside and pelargonidin-3,5-diglucoside standards (A)

Anthocyanins have been shown to have positive effects on oxidative damage, detoxification enzymes, the immune system, and platelet aggregation as well as anti-inflammatory properties. However, structural changes with hydroxyl, methoxyl, glycosyl, and particularly acyl groups, as well as environmental conditions like temperature and light, frequently impact the stability of these anthocyanins (17). On the other hand the chemical compounds used in synthetic dyes are harmful to human health and the environment. Table: 3 gives a comparative analysis of colors and their health effects present in natural and synthetic dye based products.

Table 3. Different chemical and natural coloring agents with their health effects on humans and environment

S.No.	Chemical compound/ Health effects (18)	Natural class of compound/ Health effects (19)
1	Copper sulphate ($CuSO_4$) **Color:** green **Health effects**: Eye allergy, temporary blindness	Carotenoids **Color:** yellow, red and orange **Health effects:** antioxidant, anti-inflammatory activity.
2	Chromium iodide (CrI_2) **Color**: purple **Health effects:** Bronchial asthma &other forms of allergy	Anthocyanin **Color:** purple, red and blue **Health effects:** antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity effects
3	Mercury sulphide (HgS) **Color**: red **Health effects**: Skin cancer and Minamata disease	Flavonoids **Color:** yellow **Health effects:** powerful antioxidant, used in the treatment of heart disease and high blood pressure.
4	Prussian blue [$Fe_7(CN)_{18}$] **Color:** blue **Health effects:** Contact dermatitis	Chlorophyll **Color:** green **Health effects:** anti-inflammatory, antimutagenic, and antioxidative properties.

The synthetic colors contain heavy metals responsible for soil and water pollution. The heavy metals in environment get absorbed and lead to bioaccumulation in living system. Environmental pollution caused by heavy metals is persistent, covert, and long-term. Metals are non-biodegradable having lengthy half-life, therefore biological species are unable to decompose them, and they remain in their body parts and surroundings for long time, posing health risks (18).The synthetic colors are also used in food color. Mixtures of food colors (and other chemicals) are found in many foods and diets, and these mixtures may have additive or synergistic effects. Combinations of dyes can lead to hyperactivity and other behavioral issues in certain children, in addition to issues with organ damage, cancer, birth defects, and allergic responses (20).

The nutritional hazards of synthetic food colors have been detected in the liver and kidney (21). Keeping in mind the consequences of the use of synthetic dye-based product, a natural dye-based product 'herbal gulal' of different shades of color was formulated as shown in fig 4. Further the evaluation of different parameters of the formulated herbal gulal samples (F1, F2, F3, F4, F5, F6 and F7) was done as described in table 4.

Table 4. Evaluation of formulated herbal gulal

S.No	Evaluation Parameters	F1	F2	F3	F4	F5	F6	F7
1	Appearance	Dark pink powder	Pink powder	Yellow powder	Light yellow powder	Orange powder	Light pink	Light green
2	Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
3	Odour	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
4	Solubility: Water Ethanol	 Insoluble Insoluble	 Insoluble Insoluble	 Insoluble Insoluble	 Insoluble Insoluble	 Insoluble Insoluble	 Insoluble Insoluble	 Insoluble Insoluble
5	Angle of Repose	56^o	48^o	58^o	45^o	52^o	47^o	42^o
6	Sensitivity to skin	Not sensitive	Not sensitive	Not sensitive	Not sensitive	Not sensitive	Not sensitive	Not sensitive
7	Color stability	Stable	Stable	Stable	Less stable	Stable	Stable	Less stable
8	Water washability	Washable	Washable	Washable	Washable	Washable	Washable	Washable
9	pH	6.5	6.3	7	7	6.8	6.4	6.8

Fig.4: Different colors of formulated herbal gulal (F1 Dark pink; F2 Pink; F3 Yellow; F4 Light yellow; F5 Orange; F6 Light pink; F7 Light green)

Results of the test for antibiotic susceptibility were expressed as the diameter of the inhibition zone (in mm) of different extract (E1, E2, E3, E4, E5, and E6) against each bacterial strain employed. The zone of inhibition for the standard antibiotic (Tetracycline) against each strain was also recorded. Data of antibacterial activity of extracts are demonstrated in Table5:

Table 5. Antibacterial susceptibility assay of extracts (E1, E2, E3, E4, E5, E6)

	Zone of Inhibition (in mm.)		
Extract	***E. coli***	***S. aureus***	***P. aeruginosa***
E1	22 ± 0.4	18 ± 0.2	20 ± 0.4
E2	12.7 ± 0.2	9.5 ± 0.0	11 ± 0.3
E3	22.7 ± 0.6	15 ± 0.2	19 ± 0.5
E4	9.5 ± 0.3	12.7 ± 0.4	10 ± 0.0
E5	23 ± 0.8	22 ± 1.0	29 ± 0.6
E6	29 ± 0.7	17 ± 1.0	31 ± 0.4
Tetracycline	31 ± 0.5	30 ± 0.0	34 ± 0.4

Conclusion

In this research work efforts were made for the formulation of natural dye-based herbal gulal. A cost effective and eco-friendly technology was developed for the formulation of herbal gulal. The different parameters such as solubility, angle of repose, sensitivity to skin, color stability, water wash ability, pH, and antibacterial activity of the formulations were evaluated. After analysing the result of different aforementioned parameters it was concluded that the formulated herbal gulal had no side effects and is safe to use. It may be used as a good substitute to synthetic dye-based gulal available in the market. Further, this technology may promote people to open small scale industries for the production of herbal gulal and serve as a source of employment to large number of rural population.

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