Development of Phyto-cosmetic Herbal Sindoor from Rosa indica L. and its Characterization

ABSTRACT

Aim: The objective of this research study was to develop a liquid herbal sindoor containing flower petal extract of *Rosa indica* L., and to evaluate the stability, antioxidant and antibacterial activities of the developed formulation. **Place and duration of study:** The formulation was prepared in the Phytochemistry Division of CSIR- National Botanical Research Institute. The duration of the study period was 90 days.

Methodology: The crude ethanolic extract of *R. indica* flowers was incorporated into a base for the preparation of liquid herbal sindoor. The antioxidant activity of this herbal product was evaluated according to the DPPH radical scavenging method, and the stability study of the formulation was evaluated for 90 days. The antibacterial activity was performed on those strains of bacteria, which are responsible for several skin infections using Disc Diffusion Assay.The HPLC- DAD analysis identified the anthocyanins cyanidin 3,5-diglucoside and pelargonidin 3,5-diglucoside in *R. indica*, flower extract. **Results and Discussions:** The formulation showed antioxidant activity and its stability was assessed in terms of pH values and color stability of the flower extract. It was observed that both these properties did not show any significant changes. **Conclusion:** The prepared herbal liquid sindoor was found safe to use having a potential to substitute the toxic lead tetraoxide (Pb_3O_4) containing sindoor available in the market.

Key words- *Rosa indica*; flower extract; formulation; antioxidant; antibacterial; pH value and color stability; lead tetraoxide

INTRODUCTION

The use of phyto-cosmetics or herbal cosmetics has increased in personal care products. There is a high demand for cosmetics, having additional raw plant-derived ingredients as they are safe, non-toxic, and environment friendly. On the other hand, the over use of products made of synthetic materials has put human health at risk and led to a number of health disorders. Sindoor is a traditional vermilion red or orange-red coloured cosmetic powder, usually worn by married women along the part of their hairline in the Indian subcontinent. Sindoor is a crucial cosmetics item in India for married ladies, worship, and other uses. Long-term usage of synthetic sindoor containing lead tetraoxide (Pb_3O_4) has shown symptoms of skin cancer, edema, hair loss, and greying of the hair (1). Lead is one of the neurotoxins that has been the subject of the most recent and intensive research. Over time, it accumulates like a typical poison (2). In light of the situation mentioned above, a process technique has been developed to produce an alternative, safe, non- toxic, ecofriendly, natural dye

based Sindoor. The process provides an opportunity for the preparation of red color liquid herbal Sindoor using *Rosa indica* as a raw material.

Rosa indica L.is a perennial flower shrub under the Rosaceae family, which contains herbs, shrubs or trees that are thorny, rhizomatous or climbing (3). Rose is used in a wide range of ways. It is traditionally been used in folk medicines, particularly in the field of skincare. It is appropriate for all skin types, although it is particularly beneficial for dry, sensitive, or aging skin. It helps to reduce the redness brought on by swollen capillaries since it has a tonic and astringent impact on the capillaries right below the skin's surface (4). There are many important groups of compounds of rose that play an important role in the antibacterial activity of rose. Among them, the groups of compounds with antibacterial activity are flavonoids, terpenes, anthocyanins. Flavonoids are classified as natural plant compounds, generally secondary metabolites with different phenolic structures (5). Rose flower is one of the best sources for antibacterial activity against microorganisms. Strong antibacterial activity against test pathogens such as *E. coli*, *P. aeruginosa*, and *S. aureus* is shown by ethanolic extracts of rose petals, leaves, and stems (6). In addition to their antibacterial and antifungal properties, flavonoids are also responsible for the aroma and color of flowers (5).

Due to the presence of antibacterial and antioxidant activities *R. indica* extract may be useful for the synthesis of herbal drugs in the future (7). Natural sources with high anthocyanin contents are becoming more and more popular in the food and medical industries for making dietary supplements for therapeutic and nutritional purposes (8).

To find safe cosmetic alternatives in place of toxic traditional products, this work aims to develop an antioxidant formulation containing ethanolic extract of the petals of *R.indica* and, evaluate its stability and antibacterial activity.

MATERIAL AND METHODS

Plant material and preparation of crude ethanolic extract

Flowers of *R. indica* were collected from the temples of Lucknow, Uttar Pradesh, India, in the month of July, 2021. The raw material was authenticated in the Herbarium of CSIR- NBRI, Lucknow (LWG). Petals were separated from the flowers and weighed. The petals were macerated with ethanol (100%) for 24 hours. The ethanolic extract obtained was filtered and concentrated under reduced pressure on rotary evaporator.

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 rose petals in grams.The yield of the extract obtained was 15.6 %.

Anthocyanin characterization using HPLC- DAD analysis

HPLC analysis of ethanolic extract of *R. indica* was performed on Shimadzu prominence system equipped with an autosampler (SIL-20 AC), LC Solution 1.0 software and DAD detector. The plant

extract was analyzed in triplicate at the concentration of 1mg/ml as described by Huihua Wan et.al (9) under the following conditions: stationary phase: C_{18} column (dimensions 250mm x 4.6mm and particle size of 5 µm); mobile phase: solution A: water + 0.5% (v/v) formic acid and solution B: Acetonitrile (ACN), considering the elution gradient as shown in Table I. The injection volume and the flow rate were set at 10 µl and 0.5ml/min respectively at the constant temperature of 25°C. Before, analysis the solutions were degassed and filtered through 0.22 µm filter membrane. The wavelength was set at 254 nm for analysis. All the reagents used for chromatographic analysis were of HPLC grade.

Table 1: Gradient elution method for HPLC analysis of anthocyanin in *Rosa indica*

Time (min)	**Solution A: water+ 0.5% (v/v) formic acid (% v/v)**	**Solution B: Acetonitrile (ACN) (% v/v)**
0	95	5
5	90	10
30	81	19
50	60	40
50.01- 60	95	5

Development of formulation of liquid herbal sindoor

For the development of the formulation the compatibility between the components and the flower extract has to be studied. The components used were as follows: bees wax, sunflower wax, castor oil, coconut oil and flower extract. These components were mixed together in petridish, kept on a hot plate at the temperature of 50°C.Then the herbal formulation was subjected to stirring on magnetic stirrer for 7 to 8 hours at 45°C.

Stability studies

The stability of the colour of the formulation was analyzed on 0, 30, 60 and 90 days using a colorimeter (High- Quality Spectrophotometer NS800). Using a colorimeter, the colours are expressed numerically in accordance with international standards. Anyone may comprehend what colour is being conveyed in this way. In order to more precisely note the changes in colour, the data from the colorimeter could enable the identification of different samples by any single quality (lightness, chroma, or hue). The informational value of the data is substantially enhanced by the capacity to appropriately define different colours (10). The following parameters—L*, a*b*, C*, and h^{o} —were monitored in order to accurately assess the formulation's colour.

A (previously calibrated) digital pH meter of LABMAN (Model no: LMPH- 12) was used for measuring pH of the sample. Before measurement, the samples were diluted to 10% (w/v) in distilled water (20).

Antioxidant activity

The DPPH radical-scavenging activity of the samples was measured by the method described by Blois (11) with a slight modification using gallic acid and ascorbic acid as reference. The formulation prepared from ethanolic extract of petals of *R. indica* was analyzed for radical-scavenging activities. Briefly, the stock solution of formulation having concentration 1mg/ml was prepared. Various concentrations (20µl, 40µl, 60µl, 80µl, 100µl) of formulation were prepared by adding both methanol (80µl, 60µl, 40µl, 20µl) and 1mL of a DPPH ethanolic solutions. The mixtures were vortexed and incubated for 30 min at room temperature in the dark. The absorbance of mixtures was determined by using a spectrophotometer at 517 nm. UV–Vis absorption spectra were measured on a Perkin Elmer Lambda 35 UV/VIS spectrophotometer (WIN LAB V6 STD Software). The free radical scavenging activity was expressed in percentage using the following formula:

DPPH radical scavenging activity (%) = (absorbance control - absorbance of sample/absorbance control) x 100

Antibacterial activity

Preparation of inoculums of microorganism for testing

Three pathogens *Escherichia coli*, *Bacillus pumilis*, and *Staphylococcus aureus* were used as test subjects to determine the antibacterial activity of rose petals. These three pre-isolated bacterial cultures were obtained from KGMU, Lucknow Microbiology Division. The cultures were subcultured on NA slants and stored at 4^0C until needed. For testing of the sample, the inoculums were prepared from the stock culture and subcultured into the nutrient broth (30ml) using a sterilized wire loop. The inoculate was further incubated overnight at 37^0C in a rotary shaker. These inoculates were further stored at 4^0C until usage.

Activity analysis (Disc Diffusion Assay)

The antibacterial activity of formulation and extract was done byDisc Diffusion Assay (12). The antibacterial activity of formulation prepared from *R. indica* petals, was evaluated against three pathogenic bacterial strains by using agar well diffusion method under sterilized conditions. For this three NA plates were prepared for formulation andpetal extract. 400µl inoculum of each selected bacterium was uniformly spread over agar plates with the help of sterilized glass spreader.

After five minutes three wells, approximately 7mm in diameter were bored with the help of borer. The equal volume (50 µl) of antibiotic Tetracycline (100 mg/ml), formulation andpetal extract (200mg/ml) were poured into the wells. The plates were incubated at 37 ^{0}C for 24 hrs in incubator. Next day, the results were observed and the antibacterial potential was measured in terms of diameter of zone of inhibition.

RESULTS AND DISCUSSIONS

With the purpose of obtaining the crude ethanolic extract, the fresh petals of *R.indica* were subjected to maceration with ethanol. The final solution, after being concentrated on a rotary evaporator, was subjected to HPLC analysis thereafter, a formulation was developed for preparation of liquid herbal sindoor. The prepared formulation was evaluated for various physical parameters as described in table 2.

Table 2: Evaluation of physical parameters of herbal formulation

S. No.	Parameters	Observation
1	Appearance	Thick liquid
2	Color	Dark red
3	Texture	Smooth
4	Odour	Pleasant
5	Water wash ability	Washable

The analysis, of the flower extract of *R. indica* using high-performance liquid chromatography coupled to a DAD detector (HPLC–DAD), enabled the identification and quantification of cyanidin 3,5-diglucoside and pelargonidin 3,5-diglucoside, the two analytical markers previously reported in this plant species (13). The retention time (Rt) of the two anthocyanins, 3,5-diglucoside and pelargonidin 3,5-diglucoside were 6.72 and 8.80 min. respectively. The analysis of the peak area percentage quantified the content of these anthocyanins in the crude ethanolic extract, the amount of 3,5-diglucoside and pelargonidin 3,5-diglucoside was 0.0255 and 0.0047 mg/ml, respectively. Figure 1 presents the chromatographic profile of the crude ethanolic extract indicating the identified markers.

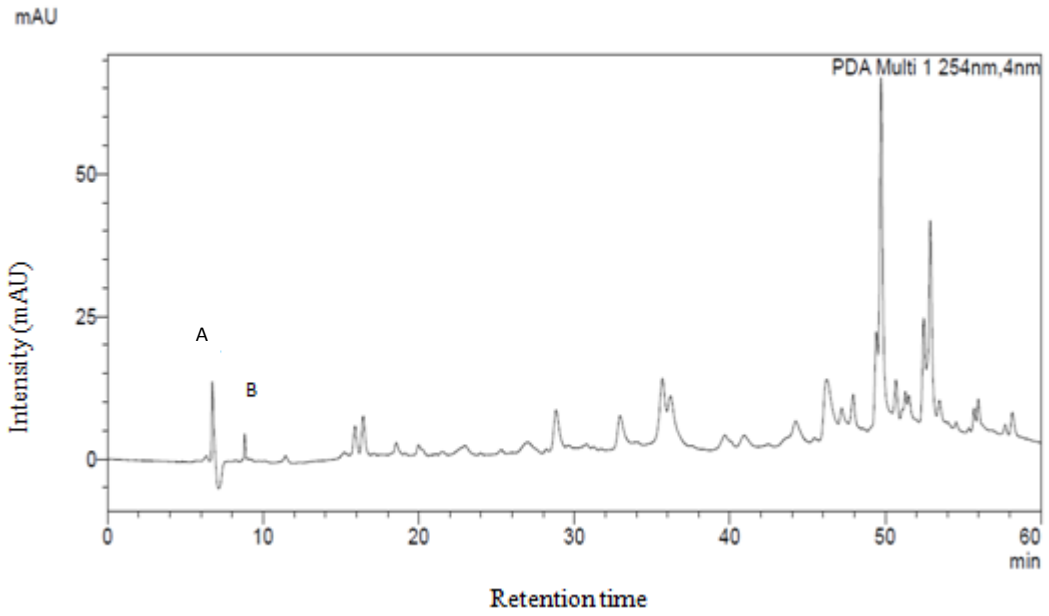

Figure 1: HPLC Chromatogram of ethanolic extract of *R. indica* at the wavelength of 254 nm. (A = cyanidin 3,5-diglucoside, B = pelargonidin 3,5-diglucoside)

On a fresh weight basis the total anthocyanin content ranged from 0.24 to 578.10 mg/100g. The environmental factors such as temperature and light strongly influence the anthocyanin content of the rose petals (14). Lin et al. (2014) reported 0.025 to 18.69 mg/g of total content of anthocyanin in rose petals. Anthocyanins give plants a variety of physiological health benefits and disease preventive actions in addition to giving natural beautiful colors(15).

The pH analysis of the extract, base (castor oil, coconut oil, sunflower and bees wax) and formulation was performed during the study period for 0, 30, 60 and 90 days and no significant variation was observed .The observed pH values were around 4.2 showing biocompatibility with the skin, being potentially non- irritating. The pH gradient for the skin surface ranges from 4 to 6 (16). The pH value of the used base ranged from 7.4 to 7.6. Finally, the pH value of the formulation ranged from 4.0 to 4.3 over the period of study (90 days).

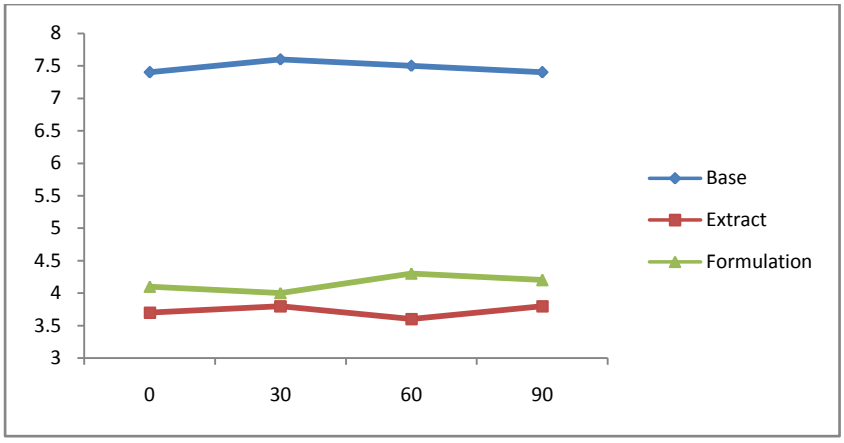

Figure 2: pH values of base, extract and formulation during the study period (0, 30, 60 and 90 days)

The color of the formulation was evaluated at the time interval of 0, 30, 60 and 90 days to observe any significant change in color as described in table 3. The color parameters such as L* (indicating lightness), a*(redness), b*(yellowness), C*(chroma), h^o (hue) were measured using colorimeter. While the parameter b* is positive for yellowish colors and negative for bluish hues, the parameter a* is positive for reddish colors and negative for greenish colors. Each hue can be thought of as being equivalent to a member of the greyscale that is in between black and white according to L*, an approximation of brightness. Thus, each scale's L value represents the degree of lightness or darkness. The delta values (ΔL, Δa, and Δb) reflect the degree of variation between a standard and sample for L, a, and b respectively, and ΔE is the total color difference. L*, a*, and b* all have delta values i.e. ΔL, Δa, and Δb respectively, that can be either positive (+) or negative (-). The sign of the delta value indicates whether the sample is redder or greener than the standard. Delta E (ΔE), is always positive (17). The value of a*and ΔE were always found to be positive (+) in each measurement. No significant change in color of the formulation was observed during the study period.

Table 3: The values of color parameters L* (indicating lightness), a*(redness), b*(yellowness), C*(chroma), h^o(hue) measured using colorimeter

ΔT*	Standard					Formulation					Difference between standard and formulation					
	L*	a*	b*	C*	h^o	L*	a*	b*	C*	h^o	**ΔL**	Δa	Δb	ΔC	Δh	ΔE
0	0.24	0.08	0.07	0.11	42.42	0.23	0.07	-0.05	0.09	324.51	-0.01	-0.01	-0.12	-0.02	-0.12	6.12
30	0.60	0.27	0.32	0.42	50.34	0.52	0.12	0.54	0.55	77.82	-0.08	-0.15	0.22	0.14	0.23	0.28
60	0.33	0.39	-0.95	1.03	292.3	0.31	0.35	-0.44	0.56	308.07	-0.01	-0.04	0.51	-0.47	0.21	0.51
90	0.29	0.09	-0.03	0.03	268.9	0.60	0.99	-1.18	1.54	509.89	0.31	0.90	-1.15	1.15	0.15	1.55

ΔT* = Time interval in days

The antioxidant potential, of the formulation was tested according to the DDPH radical scavenging assay. The concentration of the sample necessary to scavenge 50% of the DPPH free radicals, or the IC_{50} value, was computed from the graph. The term "IC_{50}" refers to the quantity or concentration of extracts required to neutralize 50% of free radicals (8). Polyphenols present in the extract have a major role, in the antioxidant activity. The antioxidant potential of the formulation according to DPPH

assay has an IC_{50} value of 27 ± 05 µg/ml in case where gallic acid was used as reference standard as described in figure 3.

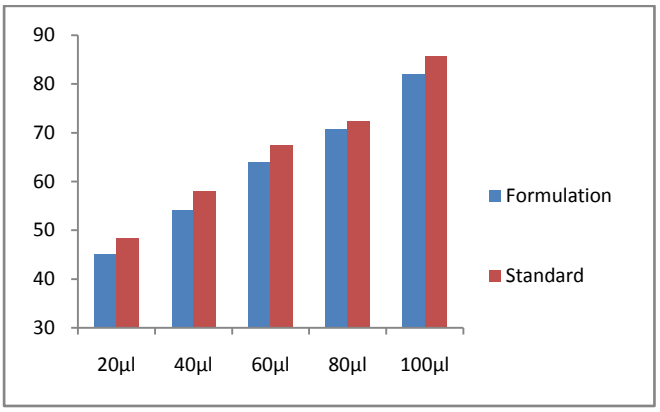

Figure 3: Analysis of Free Radical Scavenging Activity of herbal formulation at different concentration using Gallic acid as standard

The antioxidant activity of formulation was also determined using ascorbic acid as reference standared (figure 4) and the IC_{50} value was 32.82 ± 03µg/ml.

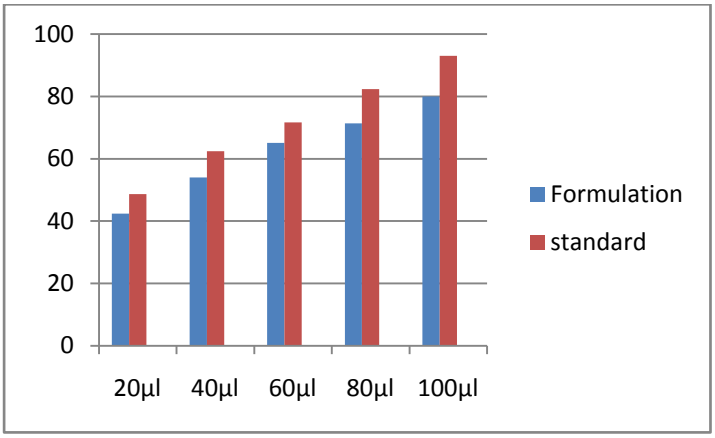

Figure 4: Analysis of Free Radical Scavenging Activity of herbal formulation at different concentration using Ascorbic acid as standard

Results of the test for antibiotic susceptibility were expressed as the diameter of the inhibition zone (in mm) of ethanolic extract and formulation 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 ethanolic extract and formulation are demonstrated in Table 4:

Table 4: Antibacterial susceptibility assay of *R. indica* petals extracts and herbal formulation

	Zone of Inhibition (in mm)		
Tested Bacteria	EthanolicExtract	Herbal formulation	Tetracycline
E. coli	22.0 ± 1.0	23.0 ± 1.0	31.0 ± 2.0
S. aureus	18 ± 1.0	17 ± 0.0	30.0 ± 1.0
B. pumilis	20 ± 0.0	21.0 ± 1.0	34.0 ± 1.0

CONCLUSION

In this research efforts were made to illustrate the compatibility between the base used and the crude ethanolic extract of petals of *R. indica*. The stability study of the formulation at room temperature and pressure showed no significant change in the pH and colour of the sample. The formulation also showed antioxidant activities carried out by DPPH radical scavenging method using gallic acid and ascorbic acid as standard . The anti-bacterial activity on various strains of bacteria (*S. aureus*, *B. pumilis*, and *E. coli*) indicated the efficacy of the formulation against such bacterial strains and demonstrated the antibacterial stability of the formulation. All these studies confirmed the anti-irritant, anti- allergic and antibacterial properties of the prepared formulation (liquid herbal sindoor). Thus this herbal product with no side effects may be used as substitute of lead tetraoxide (Pb_3O_4) containing sindoor available in the market. . However, in future, further studies are still required to develop such herbal based products using *R.indica* and to discover their beneficial health effects.

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