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Research Article

QUANTITATIVE ANALYSIS OF FLORAL LIPIDS: THEIR ROLE IN FLORAL FRESHNESS AND WASTE MANAGEMENT

Vishwanath B. Chachadi*, Hema H. Shalavadi, Divya Nayak, Anusha K. M., Sheeba H. Malekar, Tejashwini V. Teggihalli, Manjula H. Nayak

P.G. Department of Studies in Biochemistry, Karnatak University, Dharwad, India

**Corresponding author: vish2879@gmail.com*

ABSTRACT

Flowers have universal importance due to their traditional, exotic and economic values. However, they are not exploited to the fullest and have become added problem to waste management. The current study describes the analysis of lipid content and composition in selected flowers and usage of this information for further applications. Among different flowers analyzed, Crossandra and Chrysanthemum have exhibited highest lipid content and also similar fatty acid composition. These results directly corelate to their prolonged freshness and anti-bacterial property. These properties are mainly attributed to their differential content and composition of fatty acids. Further, Chrysanthemum flower has high content of oil which can be further exploited in oil industry as most of the temple floral waste in India comprises Chrysanthemum flower. Current study also provides an insight into the fact that lipids of various flowers exhibiting longer freshness can be extracted and produced industrially to be used as artificial moisturizing agents.

Keywords: Flower; Lipids; Fatty acids; Freshness and Moisture

1. INTRODUCTION

Flowers appear in thousands of different varieties, shapes, sizes and color. Freshness of flower plays an important role as it creates visual interest and make flowers commercially very important. Therefore, maintaining flowers in a fresh condition for longer period has better advantage over growing them in a bulk. Flower perishability and their dryness play an important role in floral freshness due to dehydration compared to other parts of the plant [1]. Dehydration of plants is known to influence the production of ethylene which in turn play a major role in flower senescence [2]. Many flowers are affected by this mechanism and notable examples are rose [2-4], flowers of orange [5]. After harvesting, flower continues to ripen and decay which can be delayed slightly with intermittent care using physical and chemical preservation methods. Alternatively, one can modify the chemical composition of flower to hold moisture for longer duration preferably with lipids, as they don't allow water to evaporate from flowers.

Lipid in the form of wax is thought to play a critical role in plant drought tolerance through its ability to retain moisture during transpiration under drought stress [6-8]. Important plant waxes are composed of mainly fatty acids, aldehydes, alkanes, esters, triterpenoids and sterols [9]. Till today, significant research and information on the lipid composition of plants has mainly focused on seed oils [10-14], although other parts of the plants such as leaves, fruits and flowers can synthesize and store lipids [13]. Interestingly, among all these tissues, flowers are least explored for lipid analysis. The lipid composition reported so far in flowers is different among different flowers [15]. Examples include, Marigold (*Tagetes minuta L.*) [16], Basil (*Ocimum basilicum L.*) [17], Yarrow (*Achillea millefolium L.*) [18], and rose [19]. Further, plants like rose (*Rosa hybrida*) soybean (*Glycine max*) and tobacco (*Nicotiana glauca*) have shown increased leaf wax per unit area after being exposed to water deficit [8, 20, 21].

Many studies have reported oils consumed by human have enormous effects on physiology such as lipid metabolism, chronic disease etc., [22]. It is fact that, no single source has been found to be suitable for all purposes as they are extracted from different sources. Therefore, interest in new sources of edible oils has recently grown and, flowers are known to be a good source of oils with natural, nutritional, industrial and pharmaceutical importance. Additionally, many flowers are used as traditional offerings in temples but soon after their use, flowers are considered as a waste and discarded

into wasteland because there is no appropriate method of disposal [23]. As a result, it causes serious health hazard for inhabitants and become environmental pollutant. If appropriate set-up is made to collect such flowers, they can be used for oil extraction industrially. Considering the importance of lipids from flowers, current study was undertaken to screen different flowers for the qualitative and quantitative measure of lipids in a variety of flowers and their applications.

2. MATERIAL AND METHODS

2.1. Materials

Flowers required for experiments were either directly plucked from respective plants or purchased from florists that were plucked before 12 hours. All the buffer salts and the chemicals were of analytical reagent grade and the reagents were prepared in distilled water unless and otherwise mentioned. Erythromycin and Amoxicillin were purchased from drug house. Solvents used for the oil extraction, fractionation, fatty acid methyl esters (FAMEs) preparation and lipid standards were of analytical and chromatographic grade.

2.2. Methods

2.2.1. Selection of flowers

Flowers were screened and selected for further analysis based on the extent moisture. To assess this, 1 gm of petals were kept at room temperature for 72 hours, then weighed to examine the loss of weight. Finally, flowers which showed highest difference in weight loss were selected for further analysis.

2.2.2. Extraction of lipids from petals

Petals from selected flowers were weighed (5 gm) and homogenized in 100 mL petroleum ether (60-80) for 10 min using a homogenizer. Filtrate was concentrated for 30 min at 70°C using rotary evaporator. The total lipids recovered were transferred to vials with respective solvents and stored in dark at room temperature for further analysis.

2.2.3. GCMS analysis of extracted lipids

The total lipid fractions were derivatized by sodium methoxide catalysis according to the standard protocol. The fatty acid methyl esters (FAMEs) were estimated by both qualitative and quantitative means using gas chromatography-mass spectrometry (GC-MS), with PerkinElmer Clarus 600 T GC-MS (PerkinElmer, Inc., Shelton, U.S.A.) instrument. Briefly, the beginning temperature of the oven was set to 140°C and gradually increased to 220°C at a rate of 2°C/min and maintained at this temperature for 25 min. The injector temperature was 210°C. Flow rate of the carrier gas was set 0.8 ml/min and the split ratio was 1:24. The scans were performed between m/z: 22-395 at a rate of 0.14 scan/s with an intermediate time of 0.02 s between the scans. Identification of FAMEs was achieved by comparing their retention times with those of known standards included in the analysis.

2.2.4. Free fatty acid analysis (Free acidity)

Free acidity was determined by titration of a solution of oil dissolved in petroleum ether with ethanolic solution of potassium hydroxide (0.1 M). The result was expressed as % of free fatty acid. End point is assessed by change in colour from colourless to pink using phenolphthalein as an indicator. Percentage of free fatty acids was determined by using following formula:

% of Free fatty acids = 56.1*N*V/M

Where V is the mL of KOH solution used
N is Normality
M is mass in g of the sample.

2.2.5. Estimation of Chlorophyll

The total chlorophyll content was calculated according to method of Kiritsakis [24]. Absorbance was measured at 630, 670 and 710 nm and petroleum ether was used as blank. The calculation of the total chlorophyll content is as follows:

$$\text{Chlorophyll} = \frac{A_{670}-(A_{630}+A_{710})/2}{(0.091xL)}$$

2.2.6. Estimation of β-Carotene

Beta-carotene was measured according to the method described by Dhibi et al. [25] and the content was expressed using the following equation:

$$\beta\text{- Carotene} = A_{440\text{-}480} \times (10^5)/2.650$$

2.2.7. Antibacterial Property of extracted lipids

Bacillus subtilis and *Escherichia coli* were used in this assay. The strains were maintained and tested on Nutrient agar plates, which were stored at 4°C. The test organisms were cultured overnight at 37°C before being used in the antibacterial assay.

The lipid extracts of flowers were tested for antibacterial activity using the disc diffusion method. Sterile Whatman (#4) blank discs of 7.0 mM diameter, were impregnated with the extracts. Overnight broth cultures were

streaked on to nutrient agar plates, discs (20 µL) were placed on agar plates and incubated at 37°C for 24 hours. PBS was used as a negative control, while erythromycin and amoxicillin discs were used as a positive control. Antibacterial activities were then determined by measuring the clear zone of inhibition to the nearest millimetre.

2.2.8. Application of extracted lipids to flower petals

The fresh rose petals were cut into circles measuring 2 cm diameters. The petals were coated with corresponding lipid extracts from respective flowers. For air control, petals were left alone without any coating and for solvent control petroleum ether was used. After 24, 48 and 72 hours of drying, weight of petals was recorded and % of weight loss in each flower petals was calculated. To calculate the % of weight loss, weight of petals at 0 hour was considered as 100%.

3. RESULTS AND DISCUSSION

3.1. Variation in moisture retaining property of flowers.

To determine the flowers that remain fresh for longer duration of time, total 10 flowers were analysed based on their local availability and abundance. These flowers include Jasmine, Vinca rosea, Plumeria, Crape jasmine, Chrysanthemum, Sugandhi, Marigold, Crossandra, Sunflower and Round jasmine. Results were obtained after determining the weight of the flowers at different time intervals up to 72 hours. As summarized in table 1, Crossandra flowers exhibited maximum moisture retaining capacity as it showed maximum weight per gm of initial weight (0.23 g). Followed by Crossandra, Round jasmine (0.19 g) and Chrysanthemum (0.17 g) showed highest moisture retaining property while Crape jasmine 0.14 g) and Sugandhi (0.1 g) showed lowest moisture retaining capacity. These results suggest that Crossandra flowers were more tolerant to dryness while Sugandhi were least tolerant to dryness.

Table 1: Weight of petals (in gm) after different time point incubated at room temperature and 40°C

	Room temperature						40 °C					
Time (hour)	0	15	24	40	48	72	0	15	24	40	48	72
Flowers												
Jasmine	1	0.65	0.43	0.20	0.18	0.16	1	0.22	0.21	0.22	0.20	0.20
Vinca rosea	1	0.47	0.33	0.19	0.16	0.14	1	0.15	0.16	0.17	0.17	0.16
Plumeria	1	0.69	0.61	0.45	0.39	0.20	1	0.19	0.17	0.17	0.17	0.16
Crape jasmine	1	0.21	0.14	0.12	0.11	0.10	1	0.15	0.16	0.17	0.15	0.14
Chrysanthemum	1	0.75	0.66	0.46	0.38	0.20	1	0.29	0.20	0.19	0.18	0.17
Sugandhi	1	0.39	0.26	0.13	0.11	0.09	1	0.10	0.13	0.13	0.11	0.10
Marigold	1	0.58	0.50	0.33	027	0.16	1	0.18	0.17	0.18	0.17	0.17
Crossandra	1	0.73	0.63	0.48	0.43	0.29	1	0.44	0.34	0.25	0.24	0.23
Sunflower	1	0.24	0.21	0.21	0.21	0.20	1	0.15	0.19	0.20	0.21	0.20
Round jasmine	1	0.52	0.36	0.17	0.15	0.14	1	0.16	0.21	0.21	0.21	0.19

3.2. Quantification of lipids from selected flowers

Based on the above results and to examine whether lipid content has any role in moisture retaining property of flowers, we selected 6 flowers, viz., Crossandra, Round jasmine, Chrysanthemum, Sugandhi, Vinca rosea and Crape jasmine. Total lipids were extracted from selected flowers by solvent extraction method and quantified. As presented in table 2, Chrysanthemum and Crossandra exhibited maximum quantity of lipid content in their flowers with 9.44% and 3.04% respectively.

These results were consistent with the earlier results that these two flowers retained moisture for longer duration relating to their lipid content.

Table 2: Quantification of lipids present in selected flowers

Flower	Total weight of lipids in 5 gm flower	% of lipid present
Sugandhi	0.007	0.14
Round jasmine	0.013	0.26
Chrysanthemum	0.472	9.44
Vinca rosea	0.016	0.32
Crape jasmine	0.017	0.34
Crossandra	0.152	3.04

3.3. Qualitative and quantitative analysis of lipids in selected flowers.

In order to determine the fatty acid composition and to relate their presence with moisture retaining property in flower, lipids extracted from selected flowers were subjected to GC analysis. Results of GC analysis are presented in figure 1 and summarized in table 3. It is observed that, lipids extracted from Crossandra and Chrysanthemum exhibited almost similar type of fatty acid composition except lauric acid which is exclusively detected in Crossandra with very high quantity. Interestingly, among these two flowers, quantity of stearic acid and linoleic acid is more in Chrysanthemum whereas oleic acid is more in Crossandra lipids. Linoleic acid is moderately found in other three flowers. Lauric acid is detected in all flowers except Chrysanthemum lipids. Myristic acid is completely undetected in all flower's lipids except very slight quantity in Vinca rosea flower. Palmitoleic acid is reasonably found in all flower lipids except in vinca rosea. Margaric and Ginkgolic acids were completely undetectable in all the flowers. Among two important linoleic acids α and γ, α-linoleic acid is commonly detected in all the flowers. Among long chain fatty acids, Arachidonic acid is moderately detected in Chrysanthemum but not in other flowers. None of the other long chain as well as very long chain fatty acids was undetectable in any of the flowers.

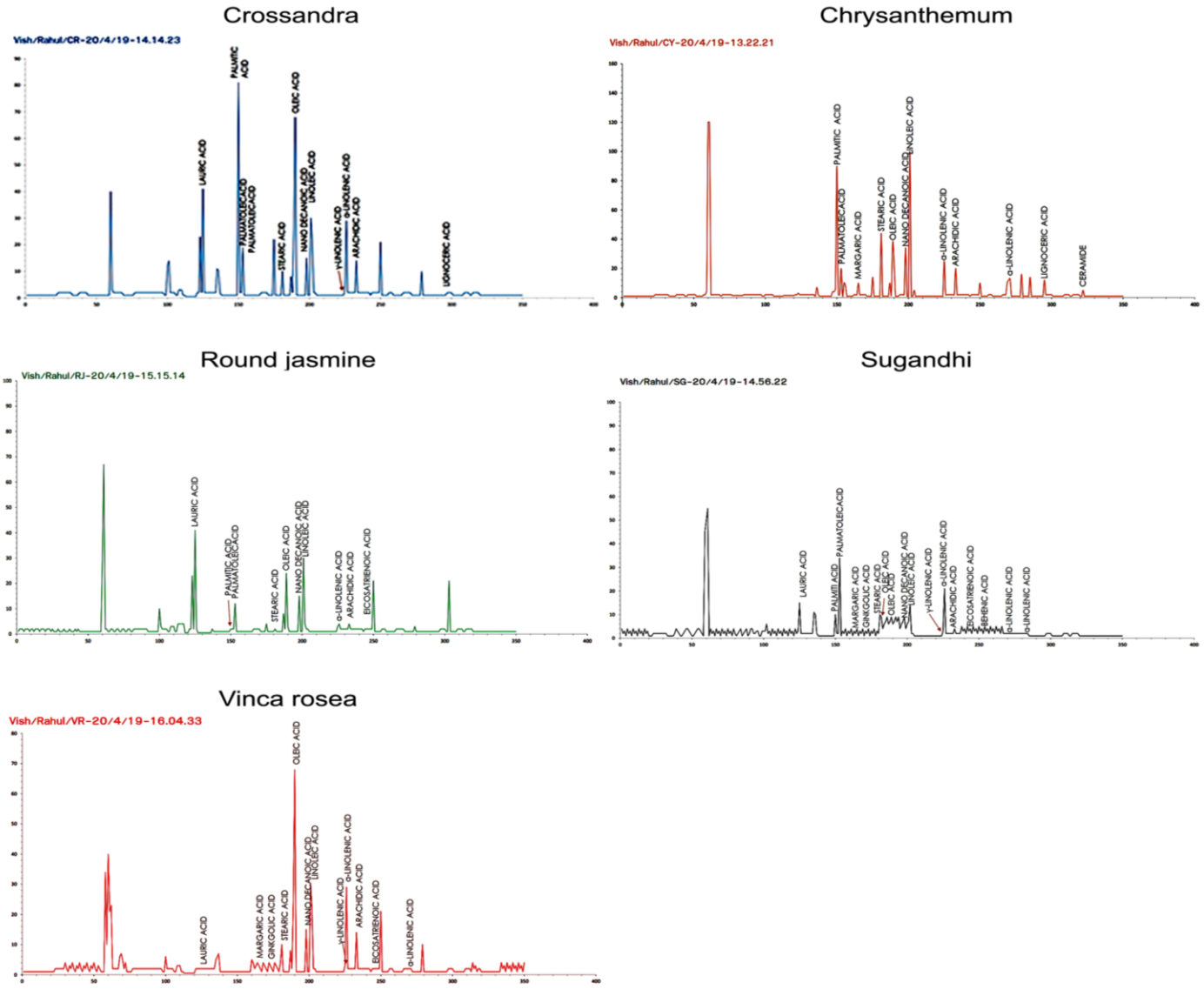


Fig. 1: GC-Chromatograms showing qualitative and quantitative composition of fatty acids detected in lipid extracts of selected flowers. Along X-axis, retention time and Y-axis quantity measured.

Table 3: Quantitative and qualitative composition of fatty acids in selected flowers

Retention time	Std value	Fatty acid	Modification	Crossandra	Chrysanthemum	Round jasmine	Sugandhi	Vinca rosea
12.5	C12:0	Lauric acid	NONE	42	ND	41	16	2
14.5	C14:0	Myristic acid	NONE	ND	ND	ND	ND	2
15.0	C16:0	Palmitic acid	NONE	82	85	2	10	ND
15.3	C16:1	Palmitoleic acid	1n9	19	20	12	34	ND
16.5	C17:0	Margaric acid	NONE	ND	8	ND	2	4
17.2	C17:1	Ginkgolic acid	1w8	ND	ND	ND	2	4
18.1	C18:0	Stearic acid	NONE	11	44	2	10	10
18.3	C18:1	Oleic acid	1n9	ND	ND	ND	8	ND
18.9	C18:1	Oleic acid	1n7	69	36	24	8	68
19.9	C19:0	Nonadecanoic acid	NONE	14	30	16	4	15
20.1	C18:2	Linoleic acid	2w6 (c9, c12)	30	104	30	16	30
22.4	C18:3	γ- Linolenic acid	3w6	4	ND	ND	4	2
22.5	C18:3	α- Linolenic acid	3w3	29	28	6	22	28
23.2	C20:0	Arachidic acid	NONE	14	20	6	4	14
24.5	C20:3	Eicosatrienoic acid	3w6	ND	ND	2	2	2
27.1	C18:3	α- Linolenic acid	3-c9, t11, c13	ND	14	ND	2	2
28.2	C18:3	α- Linolenic acid	T9, t11, c13	ND	ND	ND	2	ND
29.5	C24:0	Lignoceric acid	NONE	2	9	ND	ND	ND

3.4. Free fatty acids analysis of floral lipids.

Free acidity is an important quality factor and has been extensively used as a traditional criterion for classifying oil in various commercial grades. In order to determine the quality of oil/lipids extracted from selected flowers, lipids were subjected to free fatty acid analysis. Amount of free fatty acids were determined by acid neutralization with strong base (NaOH). As evidenced by table 4, lipids of Round jasmine were of high quality as they contain minimum amount of free fatty acids while Crossandra lipids were of poor quality.

Table 4: Amount of free fatty acids present in selected flower extracts

Flowers	KOH required	Free fatty acids
Crossandra	2.71	25.34
Chrysanthemum	0.60	5.61
Round Jasmine	0.04	0.37
Sugandhi	0.06	0.56
Vinca Rosea	0.10	0.94
Crape Jasmine	0.12	1.12

3.5. Quantification of chlorophyll and β-carotene

The level of both chlorophyll and β-carotene is directly proportional to the intensity of the color of the oil. As shown in the table 5, Crape jasmine exhibited least chlorophyll content followed by Chrysanthemum and Crossandra lipids. Round jasmine and Sugandhi showed similar chlorophyll content while *Vinca rosea* exhibited maximum chlorophyll content among all the five flowers tested. Similarly, Round jasmine, Crossandra and *Vinca rosea* exhibited very low content of β-carotene, Crape jasmine showed moderate amount of β-carotene whereas Chrysanthemum and Sugandhi showed much higher values of β-carotene (Table 5).

Table 5: Chlorophyll content in selected flowers (mg /kg of oil/lipids)

Flower	Chlorophyll (mg/kg)	β-Carotene (mg/kg)
Crossandra	0.011	2.177
Chrysanthemum	0.009	6.301
Round Jasmine	0.013	1.815
Sugandhi	0.013	5.320
Vinca Rosea	0.024	2.905
Crape Jasmine	0.008	3.686

Although there is variation in their chlorophyll as well as β-carotene content, but the values are far less than that of standard value signifying the good quality of lipids that are extracted from these flowers. Absorbance values used for calculation of chlorophyll and β-carotene content were obtained after scanning the absorbance at 630, 670, and 710 nm for chlorophyll and 460 nm for β-carotene.

3.6. Anti-bacterial potential of extracted lipids

After determining the quantity, composition and quality of lipids that are extracted from selected flowers, next we determined whether these lipids have any potential of anti-bacterial property. *Bacillus subtilis* and *Escherichia coli* (*E. coli*) were used for the analysis and antibiotic tests were performed using McFarland standards. Results of these experiments were presented in Fig. 2 and summarized in table 6. Results indicate that only Crossandra, Chrysanthemum and Round jasmine showed antibacterial effect on Gram negative *E. coli*. Interestingly, none of the floral extracts exhibited anti-bacterial effect on Gram positive *Bacillus subtilis*. From these results it could be broadly concluded that, these lipids were only effective against Gram negative but not Gram-positive bacteria. Crossandra, Chrysanthemum has shown much higher anti-bacterial potential than Round jasmine extracts.

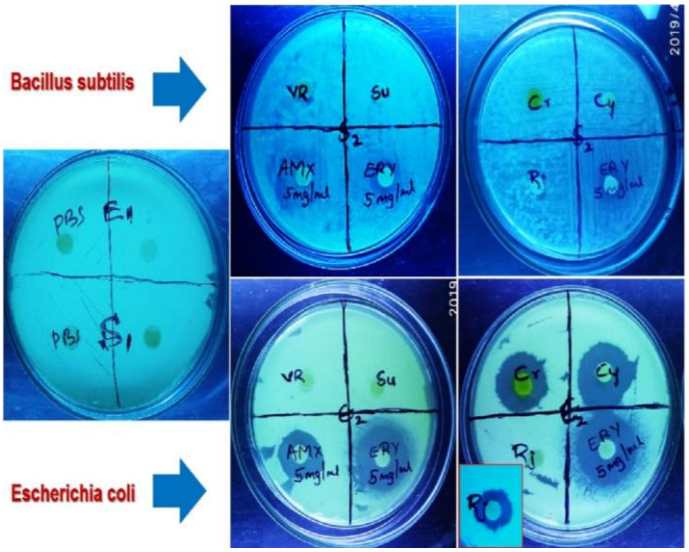


Fig. 2: Anti-bacterial effect of lipid extracts of various flower extracts on Gram positive and negative bacteria as evidenced by zone of growth inhibition measured in cm (Inset: Positive antibacterial activity of Round jasmine from duplicate plate).

Table 6: Antibiotic effect of various flower extracts on Gram Positive and negative bacteria

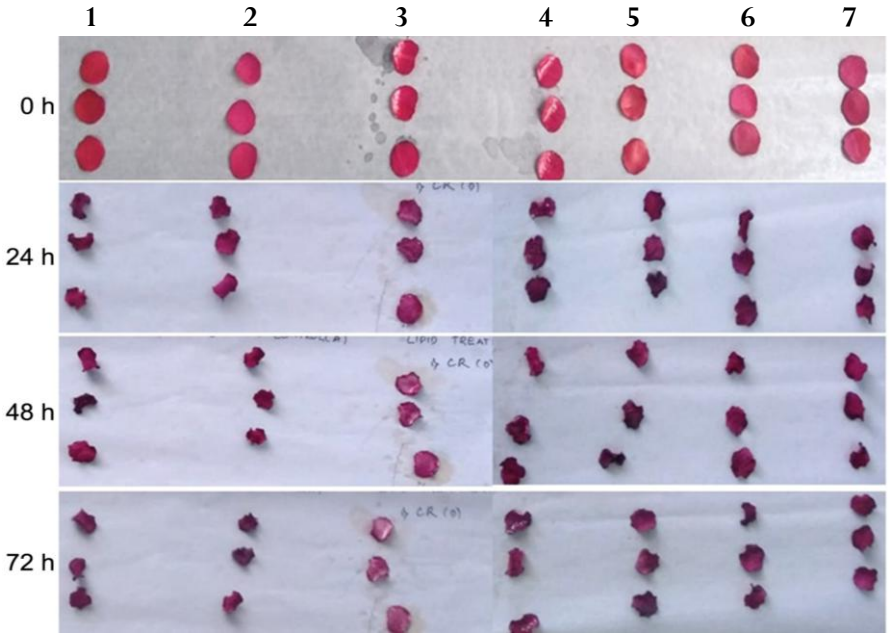
Lipid extracts	*Bacillus subtilis*	*Escherichia. coli*
PBS	--	--
Crossandra	--	1.5 cm
Chrysanthemum	--	1.5 cm
Vinca rosea	--	--
Sugandhi	--	--
Round jasmine	--	1.2 cm
Erythromycin	1.5 cm	2.0 cm
Amoxicillin	2.0 cm	1.5 cm

3.7. Flower lipids exhibited artificial moisturizing property

Since lipids extracted from selected flowers showed variation in fatty acid content, free fatty acids and pigmentation correlating with duration of floral dryness, we believed that they can be used as artificial moisturizing agents. To test this idea, rose petals (2 cm diameter) were coated with known quantity of extracted lipids for defined time intervals and then weight of the petals were determined before and after application of lipids. Results of these experiments were summarized in table 7 and presented in figure 3. Interestingly, petals coated with Crossandra and Chrysanthemum lipids retained moisture for longer duration as evidenced by their lesser weight loss after 24, 48 and 72 hours of incubation at room temperature. On the contrary, it is intriguing to note that, the lipids extracted from both Crossandra and Chrysanthemum flower were very viscous and the lesser weight loss in these petals due to added weight of lipids could not be ruled out. To confirm these results, detailed studies are need with systematic analysis.

Table 7: Weight of rose petals after 24, 48 and 72 hr of incubation with and without lipid coat

Treatment	0 h		24 h		48 h		72 h	
	Weight (gms)	Moisture %	Weight (gms)	Moisture %	Weight (gms)	Moisture %	Weight (gms)	Moisture %
Control	0.043	100	0.008	18.60	0.0073	16.98	0.0080	18.60
Solvent control	0.042	100	0.007	16.67	0.0070	16.67	0.0080	19.05
Crossandra	0.051	100	0.028	54.90	0.0270	52.94	0.0253	49.61
Chrysanthemum	0.051	100	0.015	29.41	0.0177	34.71	0.0170	33.33
Round jasmine	0.043	100	0.006	13.95	0.0080	18.60	0.0077	17.91
Sugandhi	0.047	100	0.007	14.89	0.0097	20.64	0.0080	17.02
Vinca rosea	0.042	100	0.008	19.05	0.0087	20.71	0.0073	17.38



1-Air Control; 2-Solvent Control, 3-Crossandra, 4-Chrysenthemum, 5-Vinca rosea, 6- Round jasmine, 7-Sugandhi

Fig. 3: Texture and quality of rose petals after 24, 48 and 72 hours of drying at room temperature with and without application of lipid extracts of test flowers

4. CONCLUSION

Flowers are exotically, traditionally and economically very important, they can be better exploited for many purposes than existing uses. In this context, current study describes the analysis of lipid content both qualitatively and quantitatively in flowers and their application in many ways. Among many flowers screened, Crossandra and Chrysanthemum have exhibited highest moisture retaining property as well as anti-bacterial properties. These properties are mainly attributed to their differential content and composition of fatty acids. Another important outcome of this study is that flowers can be exploited in oil industry as most of the temple waste in India comprises floral waste, especially Chrysanthemum flower. Study also provides an insight into the fact that lipids of various flowers exhibiting longer freshness due to moisture retaining capacity, can be extracted and produced industrially to use as artificial moisturizing agents as these are natural in origin.

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