International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 2| ICAM-2019 | 27-34
Proceedings of International Seminar on "Agricultural Microbiome"
Held at Hindustan College of Arts and Science, behind Nava India, Coimbatore, Tamil Nadu 641028, India, 22nd February 2019
| Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Extraction of Natural Dyes from Flowers for Dyeing in Cotton Fabrics as an Effective Waste Management System

M. Ambika[1] and Lali Growther*
[1]Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore.

Received: 20 Feb 2019 / Accepted: 22 Mar 2019 / Published online: 01 Apr 2019
Corresponding Author Email: lalijps@gmail.com

Abstract
The waste flowers of the temple around India can be utilized in a proper manner. This waste can be utilized but unfortunately, is thrown out in water bodies or with community garbage. Such improper disposal of waste causes a serious threat of organic pollution to the environment and also several infectious diseases are likely to occur in epidemic proportion due to contamination of drinking water resources. An ecofriendly method was developed for utilization of these wasted flowers by Extraction of Natural dye which can be used in small and large scale textile Industries. A dye can generally be described as a colored substance that has an affinity to the substrate to which it is being applied. The common waste flowers include Roses, Marigold, Hibiscus, Lotus, Butterfly pea etc. In this study, three flowers (Rose, Marigold, Hibiscus) were used for the extraction of the dye. The flowers were collected and dye was extracted from it by boiling with distilled water. Mango bark powder was selected for natural mordanting. The extracted dye and mordant were tested for its color strength. The dye and the mordant were tested for antibacterial activity. Then the dye and the mordant were applied on the cotton fabrics and dried. The dyed cloths were tested for antibacterial activity and fastness testing's like light fastness, washing fastness and rubbing fastness. The results show that Rose, Marigold, Hibiscus extract has good antimicrobial activity. The use of natural mordant also increases the antimicrobial activity. The bioactive compounds in these extracts were studied using HPLC.

Keywords
Rose, Marigold, Hibiscus, antibacterial dyes, textile dyes, natural dyes.

*****

INTRODUCTION
India has a rich Biodiversity which consist of 4, 90,000 plant species and huge amount of flowers are offered in temples in India, creating a very large waste. In India, West Bengal is in 4th position to promote flowers after Karnataka, Andhra Pradesh and Tamilnadu. Routine disposal of flower waste from thousands of temple become the major issue due to shortage of dumping grounds. Those small flower offering don't remain small anymore, they become a part of a big problem. Sadly, these sacred flowers rot in rivers-killing fishes and creating havoc in the fragile ecosphere of the water body and cause enormous pollution. Various drains and waterways connected to

the water bodies also get clogged, creating civic problems of a great magnitude. We always tend to blame the industrial waste but never give a think to flower pollution. The arsenic that gets into the river doesn't flow down. Iron and oxygen in the water form ferrous and ferric oxide, which in turn bonds with arsenic. Hence, there is need to utilize these flowers for preparation of various ecofriendly products.

Since twentieth century, more interest has been shown in the use of natural dyes [1,2,3,4]. Recently, large and small-scale industries have begun exploring the use of natural colorants as a possible means of producing an ecologically sound product which would also appeal to the "Green -minded" consumer. Nature has gifted us more than 500 dye-yielding plant species. As an effective means of waste management of flowers an ecofriendly method was developed by Extraction of Natural dye which can be used in small and large scale textile Industries. The common waste flowers include Roses, Marigold and Hibiscus. Most of the natural dyes have no substantivity for the fiber and mordants must be used. When metallic salts are used as mordants, only a small amount of these metal salts gets fixed onto the textiles and the rest is discharged as effluent which leads to the contamination of land and water resources. In this study we have extracted natural dyes from waste flowers, applied on cotton fabric with the natural mordant from the bark of the Mango tree (*Mangifera indica*). Antibacterial activity and the colour strength of the dyes and the bioactive compounds were studied using HPLC. This study shows promising results that these flower dyes could be used in textile industries as an ecofriendly alternative.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Rosa (Rose), *Tagetus erecta* (Marigold) and *Hibiscus rosasinensis* (Hibiscus) flowers were collected from the flower market**.**

EXTRACTION OF DYE, MORDANT AND COLOUR STRENGTH

10gms of flowers were collected and thoroughly washed under running tap water. Then the flower petals were crushed. The crushed petals were added in100ml of distilled water and boiled for 1hr. The extract was cooled and was filtered using whatmann no. 1 filter paper. For mordanting, natural extract from the bark of mango tree (*Mangifera indica*) were used. 5gm of the mango bark powder was extracted using 100ml of distilled water for 1hr. The extract of dye and the mordant was measured for its color strength using UV spectrometry. For rose and hibiscus the color strength was measured at 650nm and for marigold at 550nm.

SCOURING OF COTTON CLOTH AND DYE APPLICATION

Cotton cloths used for dyeing were boiled in10% NaOH solution for 10min to remove starch and other impurities from the cloth. The NaOH treated cotton cloths were then thoroughly washed with cold distilled water. The scoured cotton cloth was soaked in mordant and heated for1hr 30mins at 60^0C. Then the cotton cloth was padded to remove excess water and dried in open air for 24hrs. The mordanted cloth was soaked in extracted dye and heated at 60^0C for 2hrs and dried in open air for 24h [5].

ANTIBACTERIAL ACTIVITY OF DYE

Antibacterial activity of the extracted dye, mordant and the dyed cotton fabric was tested by agar diffusion method. The crushed flower petals(20g) were added in 100ml 0f distilled water and boiled for 1hr. Then the extract was cooled and filtered using whatmann no.1 filter paper. Muller Hinton agar was prepared and organisms like *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus cereus* was swabbed on the media. Holes of 6mm in diameter were made in seeded agar using sterile cork borer. The extracted dye of 10, 20, 30, 40 & 50µL was inoculated onto the plate by in agar wells. The plates were incubated at 37^0C for 24hrs. The resulting inhibition zones were measured in millimeters (mm) [5].

ANTIBACTERIAL ACTIVITY OF MORDANT

The prepared mango bark powder was dissolved in ethanol (bark to solvent ratio was 1:10). The extract was placed in shaker at 150 rpm for 24hrs at room temperature. Then the extract was filtered and re-suspended in Dimethyl Sulfoxide (DMSO) to bring 10mg/ml concentration. The Muller Hinton agar was prepared and organisms like *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus cereus* was swabbed on the media. Holes of 6mm in diameter were made in seeded agar using sterile cork borer. The extracted mordant of 10, 20, 30, 40 & 50µL was inoculated onto the plate in wells. The plates were incubated at 37°C for 24hrs. The resulting inhibition zones were measured in millimeters (mm) [5].

ANTIBACTERIAL ACTIVITY OF DYED COTTON FABRIC

The dyed cotton fabric was cut into 1cm length and was placed using sterile forceps on the MHA agar swabbed with *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus cereus.* The plates were incubated at 37℃ for 24hrs. The resulting inhibition zones were measured in millimeters (mm).

EVALUATION OF FASTNESS

LIGHT, RUBBING AND WASHING FASTNESS

The dyed fabric was exposed to sunlight for 24hrs. The light fastness was evaluated by comparing the color changes of exposed portion to the unexposed original material. The rubbing fastness of the dyed fabric was carried out by rubbing the fabric manually and checking for fading of color. The dyed sample was washed in soap solution and rinsed in cold water [6].

HPLC ANALYSIS

The obtained dried flower petal powders of Rose, Marigold and Hibiscus (10 gms each) was soxhlet extracted with ethanol. The extracts were let to dry, dissolved in DMSO and were analyzed in HPLC.

RESULTS

SAMPLES, EXTRACTION AND COLOUR STRENGTH

Rosa (Rose), *Tagetus erecta* (Marigold), *Hibiscus rosasinensis* (Hibiscus) flowers were collected from the flower market. The collected flowers are shown in Fig 1, 2 and 3. The dye extracts are shown in fig.4.

Fig.1 *Rosa*

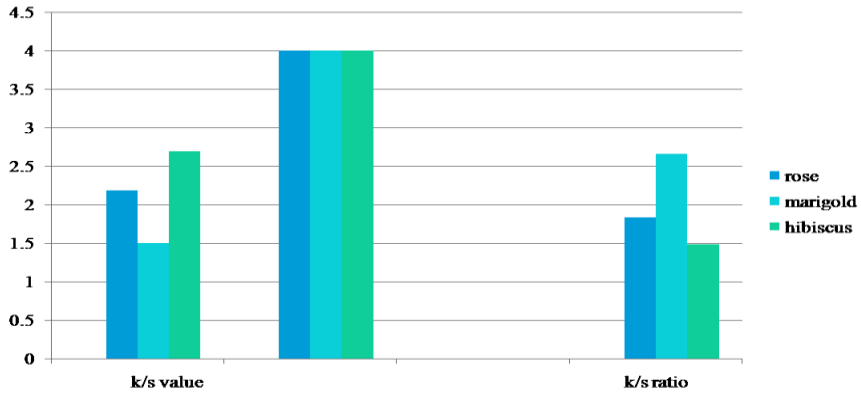
Fig. 2 *Hibiscus rosasinensis*

Fig. 3 *Tagetus erecta*

Fig.4 Extracted dyes from flowers

The colour strength of the dye and the mordant was measured in UV-VIS Spectrophotometer and is shown in fig.5.

Fig 5. UV-VIS Spectrometry Analysis (Color strength (K/S) on dyeing)



APPLICATION OF DYE AND MORDANT IN COTTON FABRIC

The dyed cotton fabric showed good colours. Bright colours were observed in different shades for the three flower dyes and is shown in fig 6.

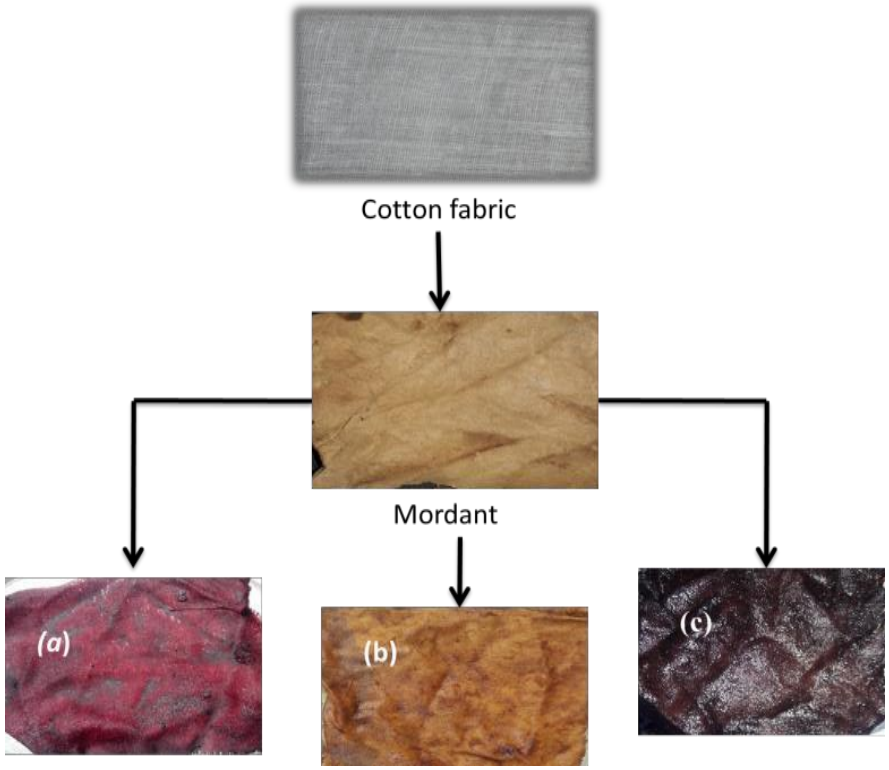


Fig. 6

(a) dyed cotton with rose; (b)dyed cotton with marigold; (c)dyed cotton with hibiscus.

ANTIBACTERIAL ACTIVITY

The antibacterial activities of different concentrations of the dye, dye with the mordant and the cotton fabric is measured as inhibition zones in millimeters (mm) and are shown in table.1, 2 and 3 respectively.

Organisms	Zone of Inhibition (mm)																			
	Rose					Marigold					Hibiscus					Mango Bark				
Concentration (µl)	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50
E.coli	-	-	8	8	10	-	-	12	13	15	-	-	7	8	12	-	-	-	8	10
S.aureus	-	7	8	12	14	-	10	11	12	14	-	-	8	8	10	-	-	-	-	8
K. pneumoniae	-	8	10	10	12	7	9	10	12	14	-	-	8	8	8	-	-	7	8	10
P. aeruginosa	-	8	10	12	14	-	8	8	10	12	-	-	8	10	12	-	-	-	8	8
B. cereus	-	-	8	8	9	7	8	10	12	14	-	8	10	11	12	-	-	8	8	10

Table 1. Antibacterial activity of dye without the mordant

Organisms	Zone of Inhibition (mm)														
	Rose with mordant					Marigold with mordant					Hibiscus with mordant				
Concentration (µl)	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50
E.coli	-	-	10	12	14	-	-	14	16	18	-	-	10	12	14
S.aureus	-	12	12	14	16	8	10	11	13	15	-	-	11	12	14
K. pneumonia	8	10	11	14	15	10	12	13	14	16	-	10	10	12	14
P. aeruginosa	8	11	12	13	15	-	10	10	12	14	-	-	11	13	15
B. cereus	-	-	8	10	11	10	12	13	14	16	8	11	12	14	15

Table 2. Antibacterial activity of dye with the mordant

Organisms	Rose	Marigold	Hibiscus
E.coli	20	26	20
S.aureus	22	18	18
K. pneumoniae	20	24	16
P. aeruginosa	22	26	20
B. cereus	18	22	18

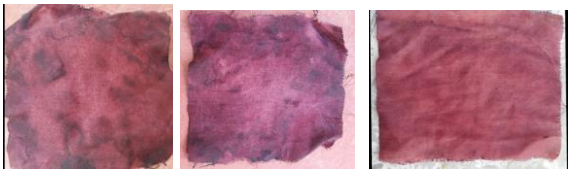
Table 3. Antibacterial activity of cotton fabric(1cm) with the dye(50µl)

EVALUATION OF FASTNESS PROPERTIES

Lightfastness, rubbing fastness and Washing fastness tests (Fig: 7,8,9) showed that the dyes could be retained in the cotton fabric, confirming its ability to be used as an ecofriendly dye.

Fig. 7 Fastness properties of Rose dyed fabric

Dyed cotton fabric



Sunlight fastness Rubbing fastness Washing fastness

Fig. 8 Fastness properties of Marigold dyed fabric

Dyed cotton fabric

Sunlight fastness Rubbing fastness Washing fastness

Fig. 9 Fastness properties of Hibiscus dyed fabric

Dyed cotton fabric

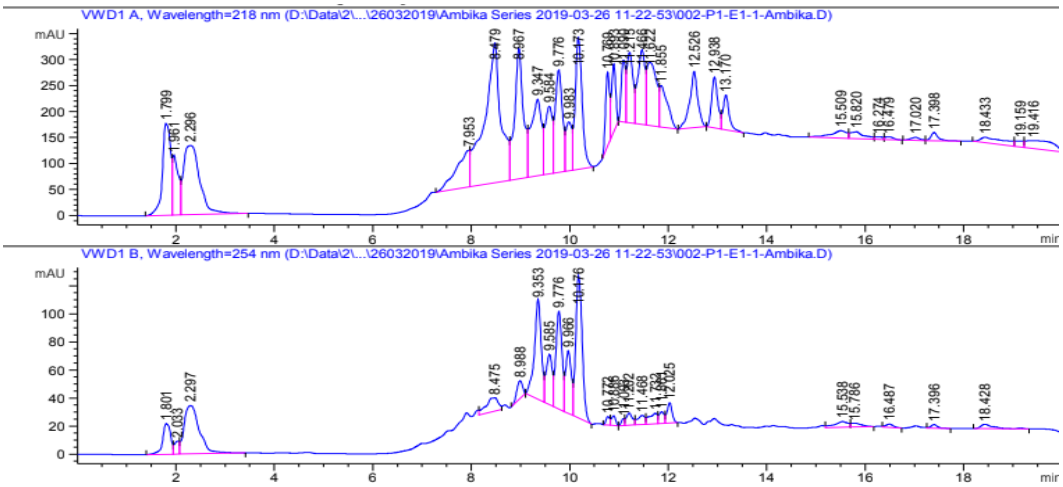
Sunlight fastness

Rubbing fastness

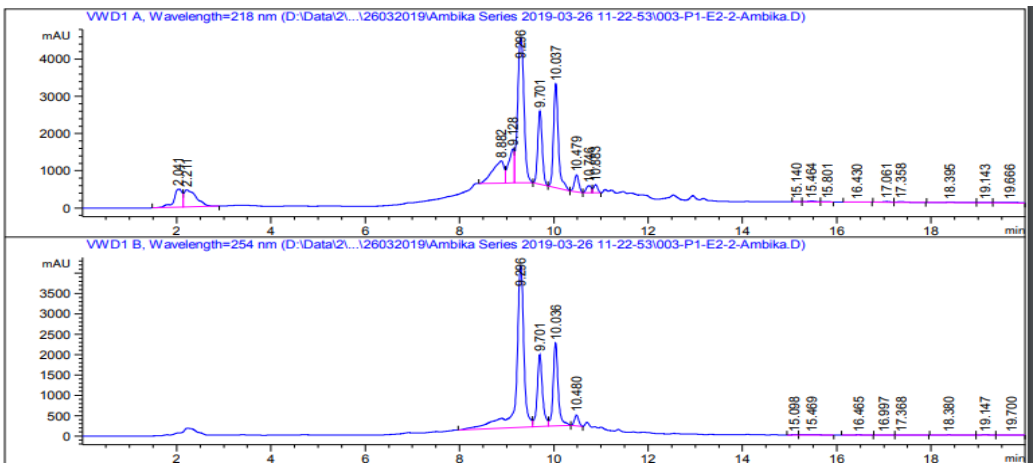
Washing fastness

HPLC ANALYSIS OF ROSE EXTRACT

Peaks showed retention times at 1.9, 2.2, 7.9, 8.9, 9.5, 10.7, 11.4, 12.5, 13.1, 15.8, 16.4, 17.3 and 19.4

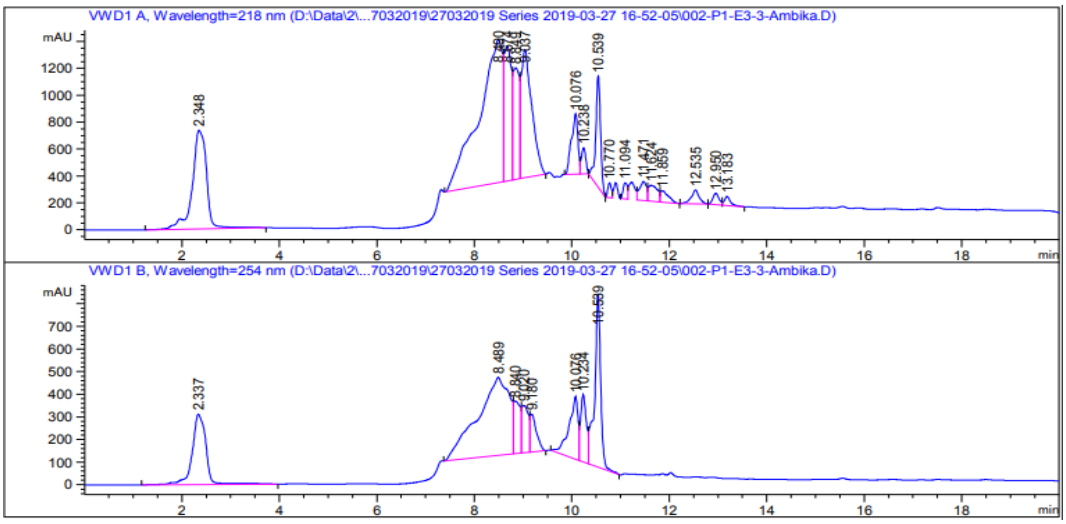


HPLC ANALYSIS OF MARIGOLD EXTRACT



HPLC analysis showed peaks at different retention times. Peaks were observed at the following retention times 2.041, 2.211, 8.882, 9.1, 9.2, 9.7 and 10.037.

HPLC ANALYSIS OF HIBISCUS EXTRACT



Major peaks were seen at retention times of 2.3, 8.4, 8.6, 8.8, 9.03, 10.2, 11.4, 12.5 and 13.1

DISCUSSION

The flowers and the mordant dye were extracted and the color strength was observed. This shows that they are good dye candidates. Fastness test had proved that colour could be retained.

Good antibacterial activities are observed with these flower extracted dyes. Dyes with the mordants showed increased activity. This may be due to synergistic activity. Marigold coated cotton fabric showed maximum zone of inhibition against *E.coli* and *Pseudomonas aeruginosa*. These organisms are common causative agents of diabetic foot ulcers and wound infections. Thus incorporation of these antibacterial dyes will help patients. Rose extracts showed maximum zones of inhibition against *S.aureus* and *Pseudomonas aeruginosa*. Hibiscus was active against *E.coli* and *P.aeruginosa*.

HPLC analysis of Rose in previous studies had showed the presence of polyphenols, namely Gallic acid, catechin, epicatechin, rutin, *m*-coumaric acid, quercitrin, myricetin, quercetin, apigenin, and kaempferol[7]. Peak areas with similar retention times are seen in our extracts. These extracts are proved to contain antibacterial compounds that can be applied in medical textiles.

The flavonoids, rutin and narcissin, were known as chemical markers of marigold extract [8]. Similar Peak area were seen in our samples.

The findings by Ayyakkannu *et al.* (2016) [9] show that *Hibiscus rosa-sinensis* L. flowers possess significant antioxidant and anti-haemolytic activities. The study further revealed that phenolics and flavonoids may be the main contributors to the antioxidant and anti-haemolytic activities exhibited by the *Hibiscus rosa-sinensis*. In our study various bioactive compounds were observed that needs further characterization. Thus, the flowers have great potential to be used in the development of functional ingredients/foods that are currently in demand for the health benefits associated with their use.

CONCLUSION

This study was conducted to extract the dye from various flowers and to use natural mordant as fixing agent of dye. The extracted dye and mordant was applied on the cotton fabric and the antimicrobial activity of dye and the dye applied cotton fabric was observed. The extraction of natural dye from various flowers used in this research is a method of sustainable waste management which adheres to the concept of Zero waste. It focuses on avoiding and eliminating the volume of waste materials to conserve and recover all resources in the process and can be applied to small as well as in large scale industries. We can get different shades by using different flowers and different natural mordants. This color has no side effects on skin and it has no harmful effect on environment. The natural dye extracts can be used in fields of Food coloration, as pH indicator, in cosmetics and pharmaceuticals, for UV protective clothing's, as deodorizing finishing and for histological staining.

REFERENCES

1) Dixit, S. and Jahan, S., Colourfastness properties of Euphorbia continifolia leaves dye on silk fabric. *Man-made Text India*, *58*(5), pp.252-254: 2005.
2) Sood A Bansal A., Sharma A. and Seema R. Effect of union dyeing of wool with litchi and apricot dye material, Man mad. Text Ind., 58(4), 205-207: 2005.
3) Adeel, S., Ali, S., Bhatti, I.A. and Zsila, F., Dyeing of cotton fabric using pomegranate (Punicagranatum) aqueous extract. *Asian Journal of Chemistry*, *21*(5), p.3493: 2009.
4) Kulkarni, S.S., Bodake, U.M. and Pathode, G.R., Extraction of natural dye from chili (Capsicum annum) for textile colouration. *Universal Journal of Environmental Research and Technology*, *1*(1), pp.58-63: 2011.
5) Kamel, M.M., Helmy, H.M. and El Hawary, N.S., Some studies on dyeing properties of cotton fabrics with crocus sativus (Saffron flowers) using an ultrasonic method. *Journal of Natural Fibers*, *6*(2), pp.151-170: 2009.
6) Samanta, A.K. and Agarwal, P., Application of natural dyes on textiles, 2009.
7) Valentina Schmitzer, Maja Mikulic-Petkovsek and Franci Stampar, Traditional rose liqueur – A pink delight rich in phenolics, *Food Chemistry*, 10.1016/j.foodchem.2018.08.074, 272, (434-440): 2019.
8) Fonseca, Yris Maria, Vicentini, Fabiana T. M. C., Catini, Carolina Dias, & Fonseca, Maria José Vieira. Determination of rutin and narcissin in marigold extract and topical formulations by liquid chromatography: applicability in skin penetration studies. *Química Nova*, *33*(6), 1320-1324:2010.
9) Ayyakkannu Purushothaman1, Packirisamy Meenatchi 1, Saravanan S 1, Ramalingam Sundaram1, Nallappan Saravanan. Quantification of Total Phenolic Content, HPLC Analysis of Flavonoids and Assessment of Antioxidant and Anti-haemolytic Activities of Hibiscus rosa- sinensis L. Flowers in vitro. Int J Pharma Res Health Sci. 4 (5): 1342-1350: 2016.