WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 17, 757-769. Research Article ISSN 2277– 7105

RESEARCH ON ANTI-FUNGAL ACTIVITY OF ANTHOCYANIN AND FORMULATION OF ANTI-FUNGAL CREAM (SYZYGIUM CUMINI)

Jakhad Aditya Rajesh*, Pawar Karan Sanjay[1], Shejole Sonali Rajendra[2], Khairnar Prachi Rajendra[3] and Sonar Ankita Dipak[4]

B. Pharm Student*, B. Pharm Student[1,2,3] & Guide Assistant Professor[4] (M. Pharm, Dept. Pharmaceutics)

Swami Vivekananda Sanstha's, Institute of Pharmacy (B. Pharm), Mungase, Tal. Malegaon, Dist. Nashik-423201.

Article Received on
14 August 2023,

Revised on 02 Sept. 2023,
Accepted on 24 Sept. 2023

DOI: 10. 20959/wjpr202317-29728

***Corresponding Author**
Jakhad Aditya Rajesh
B. Pharm Student, Swami Vivekananda Sanstha's, Institute of Pharmacy (B. Pharm), Mungase, Tal. Malegaon, Dist. Nashik-423201.

ABSTRACT

The herbal preparation is better with fewer side effects than synthetics, natural treatments are more effective than allopathy in terms of side effects for better human body healing. Herbal products have a growing demand in the world marketed and the plants have been reported in the literature as having various pharmacological action, anti-inflammatory activity, anti-fungal activity and anti-microbial activity. In this study creams were formulated based on the anti-fungal potential of herbal extracts and its evaluation. Jamun(syzygium cumini or syzygium jambolan seeds)were dried and extracted by using ultrasonic method with different solvents such as methanol ,ethanol and acetone. In this study creams were formulated based on the anti-fungal potential of herbal extract and its evaluation. The creams were formulated with almond oil, jamun powder, jamun seeds powder with different concentrations namely A1 to A4. The creams were to be stable during stability studies accordingly ICH guidelines 30± 2°C / 50±5 % RH and 40±2°C / 75±5 % RH for 2 effects having anti-fungal property can be used as provision of barrier to protect the skin and avoid fungal infection of the skin.

KEYWORDS: Herbal Cream, Anti Fungal, Anti Oxidant, Poly Herbal Cream.

INTRODUCTION

The black Jamun (Syzygium cumini L.) is an important indigenous plant of Myrtaceae, commonly known as jamun Or Indian blackberry, originally from Indonesia and India, Which

has anti-oxidant solid antigenotoxic potential. The fruit pulp is sweet, and the seeds are acidic and sour. The presence of oxalic, gallic, tannic acids and other alkaloids creates one to feel such as astringency taste. The pulp and seeds are used for traditional medicine against diabetes, diarrhoea, and ringworm infection also protect against radiation-induced sickness. It is very beneficial as it has anti-fungal, anti-diabetic, cytoprotective, anti-coagulant, analgesic and anti-inflammatory, anti-cancerous, anti-microbial, anti-oxidant, hypo-lipidemic, hepato-protective properties.

JAMUN SEEDS

JAMUN : SCIENTIFIC CLASSIFIATION

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliophyta

Order: Myrtales

Family: Myrtaceae

Genus: Syzygium

Species: S. cumini

Jamun is an evergreen tropical tree native to the Indian subcontinent that has become naturalised in North America, Africa, and Australia. The deep purple to violet rectangular berries with pinkish pulp are often consumed as fruit. Fruits, in addition to their nutritional importance, are utilised in traditional medicine to treat a variety of ailments.

ANTHOCYANIN

Formula : $C_{15}H_{11}O_6N$

IUPAC name : 2-phenyl-1λ^4-chromen-1-ylium

Molar mass : 471.28 g/mol

Density : 0.55 g/cm^3

Melting point : $\leq$ 25°C

Boiling point : Decompose at high temperature

Odour : Rose-like

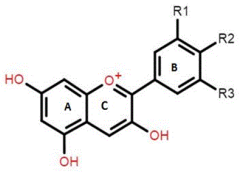
Appearance : White

State : Semi-solid

pH : 4.5 - 6

Solubility : Slightly soluble in water

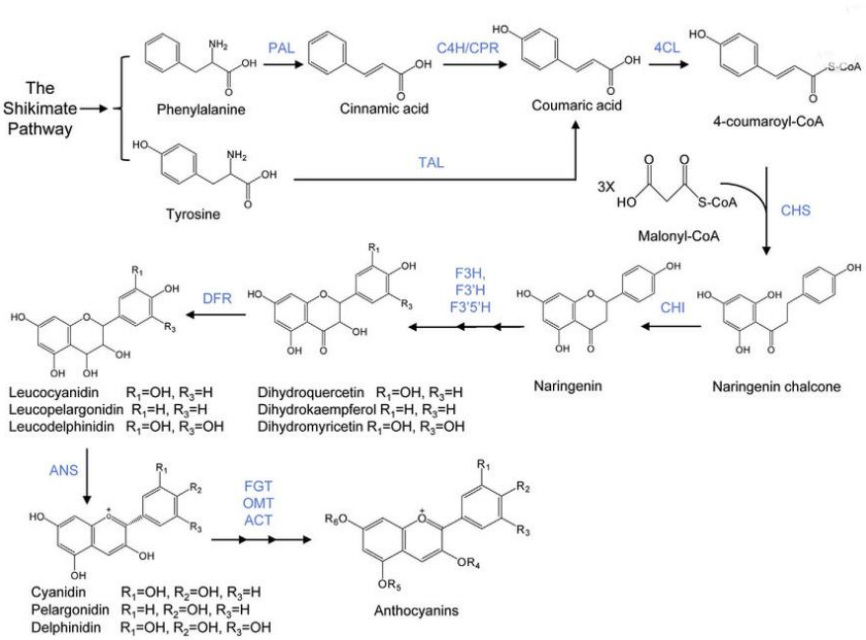
Highly soluble in organic solvent (Ethanol, Chloroform)



STRUCTURE

BIOSYNTHESIS OF ANTHOCYANIN

The phenylpropanoid route or the combined action of TAL and 4CL, respectively, converts the general precursor phenylalanine or tyrosine obtained from the shikimate pathway to 4-coumaroyl-CoA. In order to create one molecule of naringenin chalcone, one molecule of 4-coumaroyl-CoA is condensed with three molecules of malonyl-CoA. This molecule is then transformed to naringenin by CHI. The main intermediate, naringenin, goes through multiple hydroxylation processes to produce different anthocyanidins. The anthocyanidin molecules are further glycosylated and decorated to produce anthocyanins.



DEGRADATION OF ANTHOCYANIN

Metabolic pathway enzymes are understanding the critical to designing and producing anthocyanins in microorganisms by microbial platform. Anthocyanins are commonly assumed to be stable once they gather in the vacuole. More production does not always equal increased yield, at least in the case of anthocyanins. Spontaneous reaction, enzymatic activity could be caused by anthocyanins degradation. It contains some other factors such as light, oxygen, metal ions, pH, ascorbic acid, enzymes that leads to degradation of anthocyanins colour, structure and stability. To retain colour it is essential or to increase stability because anthocyanins degrade quickly due to high reactivity rate. The anthocyanins in grape cells (Vitis vinifera) are easily hydrolysed into anthocyanidins and sugars, by non- enzymatic and enzymatic oxidation. It is easily degraded. Although in plant the degradation does not form in intact, healthy fruit and it only happen when the fruit is damaged by pathogen attack or by physical disturbance. The stability of anthocyanins usually need O-glycosylation, making it possible for anthocyanins to collect in the vacuole without hydrolysis with the help of enzyme anthocynase. The main factors influencing the stability and degradation of anthocyanins and pH, co-factor, enzyme, chelating agent, temperature and light.

MECHANISM OF ACTION

By interfering with the integrity of the fungal cells' cell membranes, anthocyanins have antifungal effect. They have the ability to damage the lipid bilayer structure, increasing permeability and allowing intracellular components to flow out. Fungal cell death is the end result of this membrane rupture, which interferes with crucial cellular functions. Anthocyanins can also prevent the functioning of particular enzymes necessary for fungi to produce ergosterol and chitin, which hinders fungi's ability to grow and reproduce. The antifungal activities of anthocyanins are a result of these several processes.

EXTRACTION

Extraction methods used in cosmeceutical involves the separation of active portions of plant tissues from the inactive/inert components by using selective solvents. The purpose of standardizes extraction procedures for crude drugs (plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstruum. The extract thus obtained, after standardization, may be used as cosmeceutical agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any products. This product contains complex mixture of many plant

metabolites, such ad alkaloids, glycosides, terpenoids, flavonoids and lignans. The general techniques of plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), For aromatic plants, hydro-distillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) mat be employed.

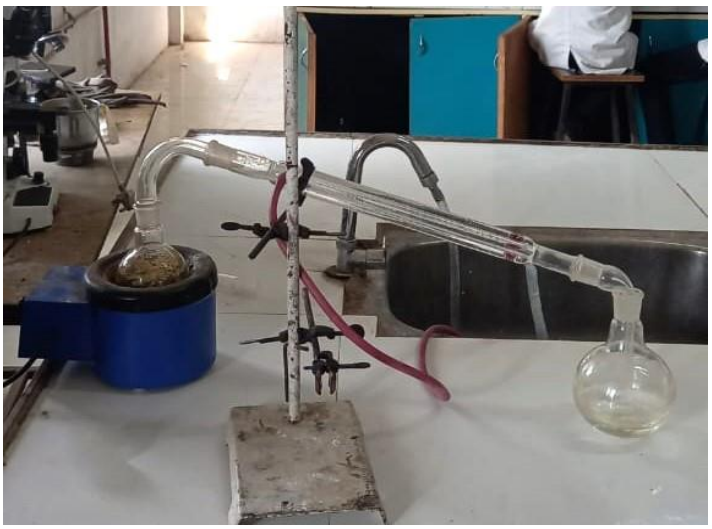
Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase microextraction, protoplast extraction, micro distillation, therm micro distillation and molecular distillation.

Collection of Material – Seeds of Syzygium cumini was collected.

Selection of Solvent – For the extraction of leaves of Syzygium cumini methanol was selected because flavonoids are soluble in methanol which is used in formulation of skin cream and it is very effective.

Selection of Extraction Process – For the extraction of seeds of Syzygium cumini was carried out by distillation process.

Hydro Distillation separates two or more liquid components in a mixture using the principle of relative volatility or boiling points. The greater the difference in relative volatility the greater the non-linearity and the easier it to separate the mixture using distillation. The process involves production of vapour by boiling the liquid mixture in a still and removal of the vapour from the still by condensation. Due to differences in a relative volatility or boiling points, the vapour is rich in light components and the liquid is rich in heavy components.

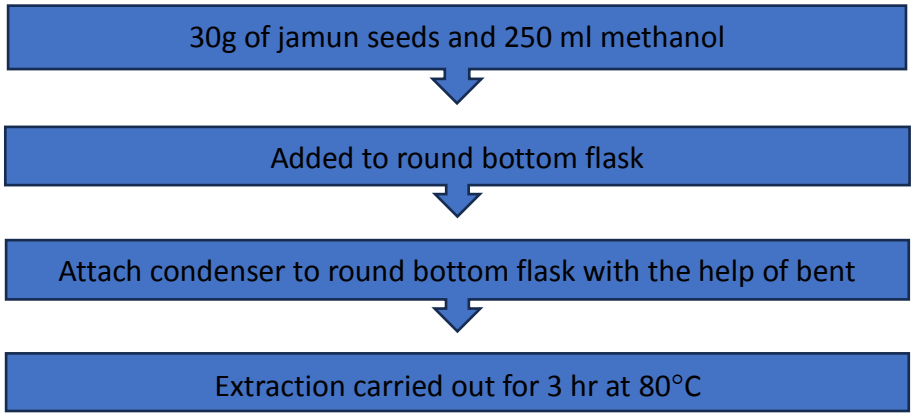


EXTRACTION OF ACTIVE SYZYGIUM CUMINI (JAMUN) LEAVES

Preparation of Material

The plant materials seeds of Syzygium cumini were air dried at room temperature for 1 week. The seed extract prepared by simple-distillation method. 30g of sample with 250 ml methanol transferred in round bottom flask. Round bottom flask attach to condenser with the help of bent tube, extraction was carried out for 3 hr at 100°C.

Procedure



Test For Flavonoids

Flavonoids were characterized by said reaction to cyanidin. A volume of 2 mL of the plant extract was evaporated to dryness. After cooling, the residue was taken up in 5 mL twice diluted hydrochloric alcohol in a test tube. Then, two or three magnesium turnings were added. The addition of three drops of isoamyl alcohol intensifies a pink-orange or violet, which shows the presence of flavonoids.

Test For Anthocyanins

The presence of anthocyanins has been demonstrated by adding 2 mL of the plant extract with 2 mL of 2 N HCL. The appearance of a pink-red colour that turns purplish blue after addition of ammonia indicates the presence of anthocyanins.

Determination of Total Anthocyanin

Total anthocyanin content (TAC) of freeze-dried extract was determined using the method described by Lima et al. 10 mg of freeze-dried extract was mixed in 5 mL of methanol acidified with trifluoroacetic acid 0.1 % (v/v). Aliquots of the extracts were taken in a 10 mL glass tube and adjust to a volume of 3 mL with methanol acidified with trifluoroacetic acid (TFA) and the absorbance was measured at 530 nm using a Jenway 6705 UV/Vis

spectrophotometer against the blank sample containing the mixture methanol/TFA 0.1 % without the sample extract, TAC was estimated as cyanidin 3-O-glucoside at 530 nm using a molar extinction coefficient of 26,900 (L/mol/cm) and molar mass (449 g/mol) and was expressed as mg cyanidin 3-O-glucoside (mg Cya3G)/g of freeze-dried extract (g FDE).

Anti-Fungal Activity

The different samples were screened for antifungal activity by agar well diffusion method. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02 ml) of inoculum was introduced to molten PDA and poured in to a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of nine different compounds were introduced serially after successful completion of one compound analysis. Incubation period of 24-48 hours at 28°C was maintained for observation of antifungal activity of compounds. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm.

Creams

They are semi-solid emulsions of oil and water. They are divided into two types: oil-in-water (O/W) creams which are composed of small droplets oil dispersed in a continuous water phase, and water-in-oil (W/O) creams which are composed of small droplets of water dispersed in a continuous oily phase. Oil-in-water creams are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off using water.

Procedure

1. All the oil soluble ingredients such as stearic acid, cetyl alcohol, almond oil are taken in first beaker.
2. Then heat it on boiling water bath at 70-80°C (Beaker A Oil Phase).
3. Then at same time all the water soluble ingredients such as methyl paraben, glycerine, propylene glycol, triethanolamine, rose water and distilled water are taken in another beaker and heated at 70-80°C (Beaker B Aqueous Phase)
4. After heating the aqueous phase slowly added in oil phase with continuous stirring until the cream was form.

Sr No.	Ingredients	Role	Batch A	Batch B	Batch C	Batch D
1	Jamun seeds extract	API	2	2	3	3
2	Aloe gel	API	2	3	2	2
3	Steric acid	Emulsifier	12	12.5	11	10.5
4	Cetyl alcohol	Surfactants	4	4.5	6	5
5	Glycerine	Moisturizer	2	-	-	2
6	Propylene glycol	Binder	1	1.5	1	1
7	Almond oil	Base	1	-	2	1
8	Triethanolamine	PH modifier	0.5	0.5	0.5	0.5
9	Rose water	Perfume	Qs	Qs	Qs	Qs
10	Distilled water	Vehicle	Qs	Qs	Qs	Qs
11	Methyl paraben	Preservative	0.029	0.028	0.029	0.28

EVALUATION OF CREAM

Organoleptic Evaluation

The cream thus obtained was evaluated for its organoleptic properties like colour, odour, and taste. The appearance of the cream was judged by its colour and roughness and graded. Results are listed in Table 1.

Table 1: Organoleptic Properties.

Sr. No.	Specification	Limits
1	State	Semisolid
2	Colour	Pinkish white
3	Odour	Characteristic
4	Texture	Smooth

Test For Microbial Growth In Formulated Creams

The formulated creams were inoculated on the plates of Muller Hinton agar media by streak plate method and a control was prepared by omitting the cream. The plates were placed in to the incubator and are incubated at 37°C for 24 hours. After the incubation period, plates were

taken out and check the microbial growth by comparing it with the control. Results are listed in Table 2. The Batch C passed the test.

Table 2: Microbial Test.

Microbial load	Limits	Results
Total microbial count	Not more than 100	65
Limit test: E.coli, S. aureus, Salmonella	No characteristic colonies	Complies

Stability Studies

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability. Stability studies were done according to ICH guidelines. The stability studies were carried out as per ICH guidelines. The cream filled in bottle and kept in humidity chamber maintained at 30 ± 2°C / 65 ± 5 % RH and 40 ± 2°C /75 °± 5 % RH for two months. At the end of studies, samples were analyzed for the physical properties and viscosity. The results are listed in Table 3.

Table 3: Stability After 2 Months.

Formulation	pH	Colour	Viscosity at 20 rpm
F1	5.3	Half white	590
F2	5.5	Yellowish white	610
F3	6.2	Pinkish white	650
F4	6.4	Pinkish white	695

Ph Of The Cream

The pH meter was calibrated using standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.

Spreadability Studies

An important criteria for semisolids is that it posses good spreadability. Spreadability is a term expressed to denote the extent of area to which the cream readily spreads on application to the skin. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of the two, better the spreadability. Two glass slides of standard dimensions were selected. The formulation whose spreadability had to be determined was placed over one of the slides. The other slide was placed on top of the formulations was sandwiched

between the two slides across the length of 5 cm along the slide. 100 g weight was placed up on the upper slide. So that the formulation between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of formulation adhering to the slides was scrapped off. One of the slides was fixed on which the formulation was placed. The second movable slide was placed over it, with one end tied to a string to which load could be applied by the help of a simple pulley and a pan. A 30 g weight was put on the pan and the time taken for the upper slide to travel the distance of 5.0 cm and separate away from lower slide under the direction of the weight was noted.

The spreadability was then calculated from the following formula

$$\text{Spreadability (S)} = \frac{\text{Weight tide to upper slide (W) X Length of glass slide (L)}}{\text{Time taken to separate slide (T)}}$$

Table 4: The results are listed in.

Formulation	Time in seconds	Spreadability [g cm/sec]
F1	11	13.63
F2	11	13.63
F3	10	15
F4	8	18.75

Viscosity

Viscosity of the formulation was determined by Brookfield viscometer. The viscosity measurements were done using Brookfield DV-Ⅱ+ viscometer using LV-4 spindle. The developed formulation was poured into the adaptor of the viscometer and the angular velocity increases gradually from 0.5 to 20rpm.

Homogeneity

The formulations were tested for the homogeneity by visual appearance and by touch.

After Feel

Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

Removal

The ease of removal of the cream applied was examined by washing the applied part with tap water.

Irritancy Test

Mark an area (1 sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythematic, oedema, was checked if any for regular intervals up to 24 hours and reported.

RESULT AND DISCUSSION

The present work on formulation and evaluation of antifungal cream was done. there are various parameters for evaluation of cream such as colour, Odour, consistency, PH of cream, Spreadability, phase separation, microbial growth, washability, skin irritancy test were done.

Result for F3 formulation

Sr. No.	Evaluation Parameter	Result
1	Colour	White
2	Odour	Rose like
3	Consistency	Smooth
4	State	Semi-solid
5	pH	4.7
6	Spreadability	Easily Spreadable
7	Phase separation	No Phase Separation
8	Melting point	21
9	Skin irritancy	No Irritancy
10	Washability	Easily Washable
11	Test for microbial growth	Inhibits Microbial Growth

The present research was done for the formulation of antifungal cream from jamun seed extract. The antifungal activity of jamun seed is mimic by the addition of almond oil which provides soothing effect as well as antifungal activity against four type of fungal strain. These cream formulation was o/w type of emulsion. The formulation can be easily wash with water. The formulation F3 considered as the most stable formulation.

CONCLUSION

The cream was formulated from jamun seed extract which shows antifungal activity. The cream is for topical application which shows good result without causing any adverse reaction at the site of application. The fungal growth is inhibited and controlled by these formulation and other evaluation parameters are also passed by the formulation and gives good result.

REFERENCES

1. Bhakti J. Chhangani Mahatma Gandhi Ayurveda College, Hospital and Research Center, Salod, Wardha, Maharashtra.
2. Rignall A. ICHQ1A (R2) stability testing of new drug substance and product and ICHQ1C stability testing of new dosage forms. ICH quality guidelines: An implementation guide, 2017.
3. Gowri SS, Vasantha K. Phytochemical screening and antibacterial activity of Syzygium cumini (L.) (Myrtaceae) leaves extracts. Int J Pharm Tech Res., 2010; 2(2): 1569–1573.
4. Tayade Jayshri A and A V Patil, October 26-28, 2015, Polyherbal formulation, 3rd International Conference and Exhibition on, Pharmacognosy, Phytochemistry & Natural Products Volume 3, Issue 6 ISSN: 2329-6836 NPCR, an open access journal, 137.
5. https://en.m.wikipedia.org/wiki/Cream_(pharmacy)
6. Ageorges A, Fernandez L, Vialet S, Merdinoglu D, Terrier N, Romieu C. Four specifc isogenes of the anthocyanin metabolic pathway are systematically co-expressed with the red colour of grape berries. Plant Sci., 2006; 170: 372–383.
7. Ajikumar PK, Xiao W-H, Tyo KE, Wang Y, Simeon F, Leonard E, Mucha O, Phon TH, Pfeifer B, Stephanopoulos G. Isoprenoid pathway optimization for Taxol precursor overproduction in Escherichia coli. Science, 2010; 330: 70–74.
8. Bueno JM, Saez-Plaza P, Ramos-Escudero F, Jimenez AM, Fett R, Asuero AG Analysis and antioxidant capacity of anthocyanin pigments. Part II: chemical structure, colour, and intake of anthocyanins. Crit Rev Anal Chem., 2012; 42: 126–151.
9. Parmar P.B; Baria A.H; Faldu S.D; Tank H.M; Design And Evaluation Of Herbal Formulation In Semisolid Dosage Form Journal Of Pharmacy Research, 2009; 1095-1097.
10. Bag, A., Bhattacharyya, S.K., Pal, N.K., Chattopadhyay, R.R. In vitro antibacterial potential of Eugenia jambolana seed extracts against multidrug-resistant human bacterial pathogens. Microbiological Research, 2012; 167(6): 352-357.
11. Winkel-Shirley, B. Flavonoid Biosynthesis. A Colourful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. Plant Physiology, 2001; 126(2): 485–493.
12. Grotewold, E. (2006). The Science of Flavonoids. Springer.
13. Holton, T. A., & Cornish, E. C. Genetics and Biochemistry of Anthocyanin Biosynthesis. The Plant Cell, 1995; 7(7): 1071–1083.

14. M. B Mapunya, R. V. Nikolova, and N. L; Melanogenesis and Anti-tyrosinase Activity of Selected South African Plants, 2012.
15. Lily Talakoub, M.D., and Naissan Wesley, M.D; Dark circles under the eyes; Dermatology News, 2015.
16. Dr. Mahesh Prasad, Dr. Antesh Kumar jha, shiv Kumar Shrivastava, "Practical book of industrial pharmacy Nirali Prakashan", 54-55.
17. Dr. Sweta Srivastava, Ms. Supriya Shidhaye, "A Practical book of herbal drug technology", PV Publication, Edition, 2020; 24-26.