WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 5, 779-798. Research Article ISSN 2277–7105

FORMULATION AND EVALUATION OF HERBAL HYDROGEL UNDER EYE PATCH

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Article Received on
07 January 2025,

Revised on 29 Jan. 2025,
Accepted on 18 Feb. 2025

DOI: 10.20959/wjpr20255-35701

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ABSTRACT

This study includes the formulation and evaluation of poly-herbal hydrogel under eye patch. The under eye dark circle is one of the increasing beauty concerns to most of the population. Hydrogel under eye patch has been developed to solve the under-eye problems. The formulation of herbal hydrogel under eye patch includes the herbal materials such as *Tabernaemontana divaricata, Rubia cordifolia,* green tea and honey. The extract of *Tabernaemontana divaricata* helps to reduce itching, imparts anti-inflammatory and cooling effect due to the presence of phenolic and tannin content. Like-wise *Rubia cordifolia* extract consist of Hydroquinone, anthroxyhydroquinones and phenols which provides depigmentation and antioxidant property. Green tea is another herbal ingredient included in the hydrogel patch to provide depigmentation and it also helps to reduce the puffiness under the eye. Moisturizing effect of hydrogel is due to the incorporation of honey in the formulation. Thus, the formulation mainly focuses on reducing the under-eye darkness, give whitening effect, reduce itching and inflammation and provide smooth and nourish under eye area. Formulation is devoid of additives such as colouring agents, opacifying agent, texture modifiers and perfume thus can be considered safe and effective to use around the eye.

KEYWORDS: Under eye dark circles. Under eye patch, Hydrogel, *Tabernaemontana divaricata* (Nandyarvattom), *Rubia cordifolia* (Manjishtha), *Cammellia sinunsis* (Green tea), Honey.

1. INTRODUCTION

The increasing use of digital appliances and rising stress levels have contributed to a common beauty concern: under-eye dark circles. The under-eye area is the most sensitive part of the facial skin and is therefore prone to change. Increased stress or lack of sleep can easily lead to dark circles, a condition also known as periorbital hyperpigmentation. This refers to a colour difference in the area around the eye including under the eyes, eyelids, and corners of the eyes compared to other areas of the face.[1-4]

There are different types of under eye dark circles, primarily four main types.[5]

- Bluish, purple Tone or Vascular Dark Circles.
- Mixed dark circles
- Structural dark circles
- Brownish Tone or Pigmented Dark Circles.

Fig 1: Under eye dark circle.

There are several factors contribute to the appearance of dark circles, including genetics, dermal melanocytosis, post-inflammatory hyperpigmentation, and periorbital edema.

Genetics can be a primary cause of under-eye darkness. The severity of genetically-linked dark circles can vary among individuals. Stress can increase the likelihood of developing dark circles, and managing stress levels may help reduce their occurrence.[6]

Another cause of dark circles is dermal melanocytosis. Melanin, the pigment that gives skin its colour, can contribute to under-eye darkness. This pigmentation can be congenital or

acquired. The process involves an accumulation of melanin in the dermal region, leading to periorbital dark circles.[7-9]

High levels of pigmentation can also result from post-inflammatory hyperpigmentation. Inflammation, which can be triggered by allergic reactions or other dermatological conditions, can contribute to this. Injury or rubbing of the eyes can also lead to inflammation and subsequent hyperpigmentation. Other causes of inflammation in this area include atopic dermatitis, fluid accumulation due to allergies, and contact dermatitis.[10-11]

The accumulation of fluid under the skin is called edema. This is a significant cause of under-eye darkness. Edema can be more pronounced in the morning or after consuming a salty meal.[12]

Under-eye dark circles can be treated with both natural ingredients and medications. Some natural remedies include liquorice, turmeric, manjistha, honey, and coffee. Medical treatments include kojic acid, azelaic acid, hydroquinone, Vitamin C, fillers, and laser therapy.

Kojic acid is a naturally occurring product derived from fungi, *Aspergillus and Penicillium species*. It reduces under eye dark circles by inhibiting tyrosinase. Where 1-4% of kojic acid can be applied dermally to treat hyperpigmentation.[13-14]

Azelaic acid, known as 1,7-heptanedicarboxylic acid, was developed to treat acne. However, found to be effective in reducing pigmentation and is now also used to treat melasma. It works by inhibiting DNA synthesis and mitochondrial enzymes in abnormal melanocytes, thus helping to regulate skin colour.[15-16]

Arbutin, a naturally occurring substance derived from plants such as blueberries and. bearberries, inhibits tyrosinase activity. At a concentration of 3%, it helps in reducing under-eye pigmentation. However, higher concentrations can lead to hyperpigmentation.[17]

Hydroquinone, also known as bleaching agent, which is effective in reducing hyperpigmentation. It is mainly used in concentrations of 2-4%. The treatment should be maintained for three and more months to get noticeable results.[18-19]

Topical vitamin C is a natural antioxidant. Ascorbic acid scavenges oxygen radicals that trigger melanogenesis (the production of melanin). It also helps boost collagen production, which can improve the appearance of the skin and help conceal the discoloration caused by blood stains.[20]

Fillers are hyaluronic acid gels, that are incorporated in to affected areas. Patients who have used fillers often experience an immediate reduction in the appearance of dark circles. This makes fillers a relatively quick and efficient method for addressing under-eye discoloration.[21]

Laser therapy is another option for reducing under-eye dark circles. Periorbital hyperpigmentation can be treated with various types and modes of laser therapy.[22]

2. MATERIALS AND METHODS

2.1 Collection of Herbs

Nandyarvattom-*Tabernaemontana divaricate*

The flowers of Nandyarvattom were collected from the surrounding local areas. The collected flowers are then authenticated from the botanical garden. The flowers are then washed properly. It is then dried under the shade and powdered. The powdered flower is then extracted to obtain the crude.

Manjishtha-*Rubia cordifolia*

The dried roots of Manjistha were collected from an Ayurvedic Medical shop. It is then powdered finely by using a mixer. This powder is then subjected to an extraction process to obtain the crude extract of Manjishtha.

Green Tea- *Cammellia sinunsis*

The dried leaves of the green tea were purchased from a vendor. It is then crushed properly to fine powder. The crude extract of the green tea is then extracted using decoction method.

Honey

The pure honey was purchased from a honey bee farm and it is stored in a well closed container.

Fig 2: Nandyarvattom.

Fig 3: Green tea.

Fig 4: Manjishtha.

2.2 Extraction of herbal ingredients

Extraction of Nandyarvattom

The method of extraction of Nandyarvattom is performed by soxhlet extraction. In the conventional extraction method, the dried flower powder is placed in the thimble-holder. The solvent, water and ethanol in the ratio 1:3 is poured into the thimble-holder. When the liquid reaches the overflowing level of the Soxhlet arm it will aspirate to the round bottom flask. This procedure is done for 18 hours at 60-80^{o}C till the complete extract is obtained.

The extract obtained from the whole process is then subjected to steam distillation or rotary evaporation at 40^{0}C to obtain the crude gummy exudate of the resultant flower. The extract was concentrated by dissolving the weighed crude extract (0.3g,0.2g, and0.1g) in 1ml suitable solvent to give concentrations of 0.3g/ml, 0.2gm/ml, 0.1gm/ml respectively.[23-24]

Extraction of Manjishtha

The extraction of Manjistha is done through Soxhlet extraction. In this method the dried powdered root of manjistha is taken in the thimble holder. Then this is extracted successively in ethanol at 65^{0}C. The continuous extraction of Manjishtha is done for 6 hours till the complete extract is strained from the powder.

The extract obtained is then subjected to steam distillation or rotary evaporation to remove ethanol from the extract obtained from the above process. The crude extract thus obtained is concentrated using suitable solvent The weighed crude extract is dissolved (2 gm, 3gm, 4gm) in 1 ml suitable solvent to give concentrations of 2g/ml, 3g/ml and 4g/ml respectively.[25]

Extraction of Green tea

The extraction of green tea is done by hot water extraction or decoction method. 0.5g of green tea was weighted and taken in a beaker. To this add 18ml water and boil at 85^{0}c in a water bath. Hot water extraction is proceeding until it reaches half the amount of water.[26]

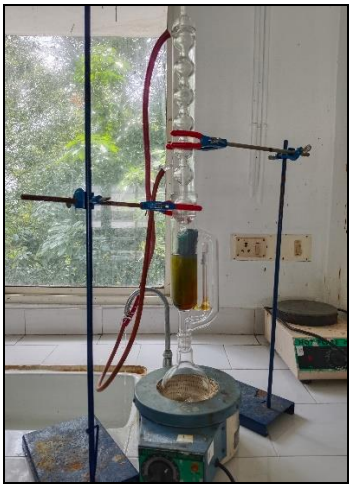

Fig. 5: Soxhlet extraction.

Fig. 6: Steam Distillation.

2.3 Chemical test

Chemical evaluation is performed on the extract obtained via above mentioned method, for all herbal ingredients incorporated into the formulation of the herbal hydrogel under-eye patch. This evaluation identifies the chemical constituents present within the extract that contribute to the desired action. *Tabernaemontana divaricata*, *Rubia cordifolia*, green tea, and honey are subjected to various chemical tests separately to detect the presence of specific chemical compounds. These tests include:

- Carbohydrate
- Alkaloids
- Tannins
- Flavonoids
- Phenols
- Terpenoids

Herbal extracts	Test showing positive results
Tabernaemontana divaricata[27-30]	• Mayer's test • Wagner's test • Hager's test • Keller killiani test • Ferric chloride test • Gelatin test • Shinoda test • Lead acetate test • Salkowski test
Rubia cordifolia[31-35]	• Molisch's test • Benedict test

	• Fehling's test • Hager's test • Dragendroff's test • Wager's test • Shinoda test • Test for tannins • Hydroquinone test • Ammonium hydroxide test • Test for phenol
Honey[36-39]	• Mayer's test • Wagner's test • Hager's test • Test for phenol • Alkaline reagent test • Brontrager's test • Sodium hydroxide test • Hydrochloric acid test • Benedict's test • Test for tannins
Green Tea[40-42]	• Molisch' s test • Barfoed test • Fehling's test • Mayer's test • Wagner's test • Hager's test • Ferric chloride test • Lead acetate test • Liebermann-Burchard test • Catechin test.

2.4 Preparation of herbal hydrogel under eyepatch

- Preparation of polymer solution.

To prepare polymer solution, accurately weigh fine agar powder and keep it separately. The first step is to boil required amount of water until it reaches 90^0C and it shall be confirmed by a thermometer.

Add the weighed amount of agar to the above boiling water as small portions with continuous stirring. Then continue boiling for another 5 minutes without failure of stirring. Ensure the agar dissolves completely. The polymer solution is formed.[43]

Preparation of extract mixture

In a beaker take required amount of methyl paraben (preservative) and dissolve it in required amount of glycerine. By stirring ensure that, it have been completely dissolved. To the

mixture add concentrated extracts of *Tabernaemontana divaricata*, *Rubia cordifolia*, *Camellia sinensis*, and honey with continuous stirring.[44]

- Mixing of two phases.

The extract mixture is added directly to the polymer solution employed with frequent stirring for 2 minutes. Subsequently, the resulting mixture was poured into eye patch moulds. The filled molds were then left to cool at room temperature for five minutes, allowing the hydrogel to set. Once solidified, the under-eye patches were carefully removed from the molds (demolded). A serum was then applied between each individual patch, and the final product was stored in a well-closed container to maintain its integrity and prevent degradation.

- Formulation of herbal hydrogel under eye patch

The herbal hydrogel under eye patch was formulated accordingly. Specifically, three distinct formulations of herbal hydrogel under eye patch, designated F1, F2, and F3 were prepared. These formulations were designed with slight variations in the concentrations of two key components: agar, which provides the gelling structure, and glycerin, which acts as a humectant and plasticizer. This variation in component concentrations was implemented to optimize the patch's properties. Following preparation, each of the three formulations (F1, F2, and F3) undergo a further evaluation process. The purpose of this evaluation was to assess the performance characteristics of each formulation and, ultimately, to choose the best formulation among them. The table indicates the composition of formulations of herbal hydrogel under eye patch.

Table 1: Formulation Table.

Ingredients	F1	F2	F3
Tabernaemontana divaricata	0.5ml	0.5ml	0.5ml
Rubia Cordifolia	0.5ml	0.5ml	0.5ml
Green tea	0.25ml	0.25%	0.25ml
Honey	0.25ml	0.25%	0.25ml
Agar	0.015g	0.02g	0.025g
Glycerine	0.05ml	0.1ml	0.15ml
Methyl paraben	0.08%	0.08%	0.08%
Water	3ml	3ml	3ml

2.5 Evaluation of herbal hydrogel under eye patch

Organoleptic evaluation

The prepared hydrogels are evaluated for their organoleptic properties such as:

- Colour
- Odour
- Texture[45]

Hydrogel acidity

Hydroxyl acidity was evaluated using a pH meter. A sample of the hydrogel under-eye patch of 1 cm × 1cm of length and breadth was immersed in 10 ml of water and allowed to soak for two hours. After this two-hour period, the pH of the water in which the patch had been soaking was measured using the pH meter. This measurement provided an indication of the hydroxyl acidity of the hydrogel patch.[46]

Hydrogel weight

The weight of each individually prepared under-eye patch was precisely determined using an analytical balance. To ensure accurate measurement and avoid contamination, each patch was carefully placed in a clean and dry Petri dish before weighing. The weight of each individual patch, thus obtained, was the recorded.[44]

Hydrogel thickness

The thickness of the under-eye patch was precisely measured using a screw gauge, a tool designed for accurate measurement of small thicknesses. To ensure a representative measurement, thickness readings were taken at several different points across the surface of the patch. Each individual thickness measurement obtained was carefully recorded. The average thickness of the patch was then calculated from these recorded values.[44]

Swelling Index

The swelling of the hydrogel was done by using water as a solvent. Cut out a piece with uniform centimetre as prescribed and is weighed, where this weight is taken as initial weight (W_d). Then this piece is soaked in the 30ml of water. Keep it undisturbed for 2 hours.[44] After 2 hours weight of the swollen patch is taken which is the final weight and denoted as (W_s). The swelling index can be calculated by using the following equation.[47]

$$\text{Swelling index} = W_d - W_s / W_d \times 100$$

Shrinkage of hydrogel

The hydrogel patch was cut into small square pieces with an area of 2.25cm^2 and weighed. The test was carried out at room temperature for 4 hours. The shrinkage of hydrogel patch was determined by comparing both area (initial and final) and the weight (initial and final).[48]

Equilibrium water content Percentage measurements (EWC%)

It is the maximum percentage of water absorbed by hydrogel to reach saturated hydration point. The procedure involves that, cut off a hydrogel disc having 1 cm in diameter from hydrogel patch and weighed (W_1). Three such disc where cut off from different patches, were then placed in vacuum oven at 70^{0}C until they reach a constant weight (W_2).[45]

$$EWC\ (\%) = (W_1\text{-}W_2/W_1) \times 100$$

Antimicrobial study

Antimicrobial property was analysed by well diffusion method. S. aureus bacterial strain was cultured by using nutrient broth. 1.3g nutrient broth was dissolved in 100ml distilled water and subjected to autoclave at 121^{0}C for 20 minutes. After that it was incubated at 37^{0}C for 24 hours. By mixing 2.28gm nutrient agar in 60 ml distilled water and autoclaved, produces agar.

Pour the agar in to sterile petri dishes and allow them to solidify. Label the plates with date and other relevant information (such as F1, F2). For inoculating the plates, evenly swab the surface of each agar plate with the bacterial culture using a sterile cotton swab. This produces a uniform lawn of bacteria on the plate. Sterilize a Cork borer by dipping it in alcohol and flaming it. Cut out small wells of diameter about 6-8mm carefully in the agar plate at equal intervals, with the help of sterilized cork borer and cut off 6-8 mm diameter circles from hydrogel patch, it is considered as the antimicrobial substance and place carefully in the desired well created and label. Incubate the plates at the appropriate temperature (usually 37°c) for 18-24 hours. Inversely place the plates to prevent condensation from accumulating on the agar surface.

After incubation for 18 hrs, observe the plates for a clear zone of inhibition around the wells. Measure the diameter of each zone of inhibition using a ruler or calliper, this indicates the area where bacterial growth has been inhibited by the antimicrobial substance.[49]

3. RESULT AND DISCUSSION

Evaluation of herbal hydrogel under eye patch

The herbal hydrogel under eye patch is a small patch that can be applied in the under eye area for reducing the under eye dark circles. These patches include herbal products that can reduce the darkness, inflammation, puffiness and helps in giving a cooling effect. These products are free from any harmful chemical ingredients.

Fig. 7: Hydrogel under eye patch.

Organoleptic Evaluation

The prepared formulation was evaluated for their organoleptic properties such as colour, odour, texture and hardness. The colour & texture was evaluated by vision and touch and the odour was evaluated by forming a group of odour sensitive people and sampling was performed. All three formulations have same observation and the result was depicted in the table.

SL. NO	Evaluation parameter	F1	F2	F3
1.	Color	Brown	Brown	Brown
2.	Odor	Characteristic smell	Characteristic smell	Characteristic smell
3.	Texture	Smooth	Smooth	Smooth
4.	Nature	Soft jelly like	Soft jelly like	Soft jelly like

Hydrogel Acidity

Determining the pH is a crucial parameter. As the pH of the formulation may affect the skin, thus variations in pH will interfere with the natural pH of the skin and may cause harmful effects such as scaling, rashness and skin damage. So it is important to formulate the patch in the appropriate pH to that of the skin. And the pH of all three formulations was determined by using a PH meter and the result are shown in the table.

Fig. 8: pH determination.

SL.NO	Evaluation parameters	F1	F2	F3
1.	Hydrogel acidity	6.27	6.3	6.22

Hydrogel weight

The weight of the hydrogel under eye patch was determined by using a weighing balance. Each of the three formulations was weighed separately. It is noted that the weight of the hydrogel increases with the increase in the amount of agar used, which is shown in the table.

Fig. 9: Weight Determination.

SL.NO	Evaluation parameters	F1	F2	F3
1.	Hydrogel weight	2.65g	2.73g	2.82g

Hydrogel thickness

The thickness is another parameter to be taken into consideration. The formulation should have a uniform thickness all over the area. The thickness was determined by using a screw gauge, which is depicted in the table.

Fig. 10: Hardness Determination.

SL. NO	Evaluation Parameters	F1	F2	F3
1.	Thickness	2.07mm	2.00mm	1.8mm

Swelling index

Swelling index was performed to determine the swelling ability of the patch in accordance with the variation of concentration of agar. As the concentration of agar increases, swelling also increases.

Fig. 11: Swelling Index.

SL.NO	Evaluation parameters	F1	F2	F3
1.	Swelling Index	9.03%	9.16%	9.6%

Shrinkage of hydrogel

Shrinking is the process in which the water in the patch gets evaporated and it results in the decrease of area along with slight change in the weight. The result indicate that water evaporation occur in the resultant product in the open atmosphere there for it is stored in the serum of the extract to maintain the moisture content and it is stored in a well closed container. The result is shown in the table.

Fig. 12: Shrinkage Determination.

SL.NO	Evaluation parameters	F1	F2	F3
1.	Shrinkage • Initial weight • Initial Area	 0.37g 2.25 cm^2	 0.36g 2.25cm^2	 0.40g 2.25cm^2
	• Final weight • Final area	0.12g 1.44cm^2	0.2g 1.69cm^2	0.23g 1.96cm^2

Equilibrium water content Percentage measurements (EWC%)

Equilibrium water content refers to maximum percentage of water absorbed by hydrogel to reach full hydration. As the concentration of the humectant in the formulation increases, the percentage of equilibrium water content increases accordingly. The result of the above study is depicted in the table no:

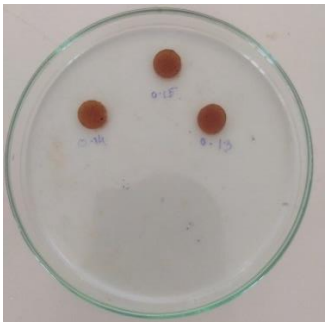

Fig. 13: Water content determination.

SL. NO	Evaluation parameters	F1	F2	F3
1.	Equilibrium water content % measurement	79.2%	84.3%	87.9%

Antimicrobial study

Antimicrobial study test was conducted to determine the degree of efficacy of extracts used in under eye hydrogel patch against bacterial species for both formulations. Antimicrobial study for under eye hydrogel patch was performed by well diffusion method. And the zone of inhibition indicates how effectively the formulation shows action against bacterial strains.

Fig 14: Zone of inhibition.

DISCUSSION

The results suggest that the herbal hydrogel under-eye patches, particularly formulation F2, has the promising characteristic of an eye patch. The combination of herbal ingredients, including *Tabernaemontana divaricata*, *Rubia cordifolia*, Green tea, and honey, provides a

range of potentially beneficial properties, including anti-inflammatory, antimicrobial, cooling, depigmenting, and moisturizing effects. The presence of these diverse phytochemicals in the extracts suggests the potential use of these ingredients in the herbal hydrogel under-eye patch. The alkaloids, flavonoids, phenolic, and terpenoids identified are known for their antioxidant, anti-inflammatory, and potentially other beneficial properties. These properties could contribute to the efficacy of the under-eye patch in addressing issues like dark circles, puffiness, and wrinkles. As looking into the different types of evaluation that we had done with the all three formulations, optimum characteristic and stability was more for F2 formulation than F1 and F3. The study was finalized that F2 have optimum weight, thickness, water content and antimicrobial property, which is usually needed for a better under eye patch formulation.

The future evaluation of hydrogel includes the study of antioxidant property of the under-eye patch. This study doesn't contribute to the evaluation of drug release from the patch and the rate of drug release. So determination of release rate of the drug and the concentration of the drug that will be releasing from the hydrogel can be remarked as the future aspects.

CONCLUSION

Under eye hydrogel is considered as one of the best emerging cosmetic products in the market due to their customer satisfaction and the efficiency it provides. In this generation, the increase in the screen time may cause an increase in the under eye darkness, puffiness and inflammation. To tackle this problem, we have introduced a formulation mainly using the herbal ingredients.

The herbal ingredients that we mainly used in the preparation of the under eye patch are the Nandyarvattom, manjistha, green tea and honey. As these herbal ingredients contain chemical constituents such as alkaloids, tannins, phenols, terpenoids flavonoids etc.it can provide a wide range of activity such as cooling effect, whitening, antimicrobial, anti-inflammatory and moisturising effect. Nandyarvattom is a commonly occurring flower in local area which have been traditionally used for providing cooling effect and antimicrobial effect. Manjistha is also a traditionally used herb that have the efficiency in skin whitening and reducing marks in the skin. Green tea as an agent that reduce the puffiness and honey as commonly used to provide a moisturising effect and depigmentation effect.

The main purpose of this investigation was to provide a stable and efficient under eye patch denying the complete use of artificial and synthetic chemical ingredients that are commonly used in the marketed products. To evaluate the efficiency of the formulation, many evaluation tests has been carried out and it has come to meet the quality.

The herbal ingredients along with glycerine is mixed with the polymer agar solution and mixed well. The mixture is moulded in a mould shape resembling the under eye region so that it will be easy in the application and provide action on the desired area under the eye. Then the evaluation tests are done by using certain parameters and the formulation F2 is selected as the best formula compared to the formulation F1 and F3. The formulations are then subjected to evaluation including the microbial test and find it as an efficient formulation. For the further conclusion regarding the activity and effect of the formulated herbal hydrogel under eye patch needed to undergo future evaluation studies.

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