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Formulation And Characterization Of Gel Using Honey Bee Propolis

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ABSTRACT

Gels are transparent to opaque semisolid containing gelling agent that merges or entangles to form a three-dimensional colloidal network structure. It is responsible for a gel resistance to deformation and its visco-elastic properties. Gels have better potential as a vehicle to administer drug topically in comparison to ointments because they are non-sticky, require low energy during formulation. Herbal Anti-microbial gel containing Propolis was successfully formulated and characterized. Firstly the Propolis extract was obtained by Soxhlet extraction. Herbal antimicrobial gel were formulated using carbopol-934 as gelling agent is used as preservative. Using Propolis extracts three formulations were formulated based on different formula and concentration. All these formulation were evaluated for visual appearance, pH, viscosity, spreadability, Homogeneity, Washability was determined for prepared anti-microbial gel. Propolis is a resinous substance that honey bees gather from a variety of plants. **1. INTRODUCTION**

Topical drug delivery denotes the application of the drug onto the body employing ophthalmic, rectal, vaginal and skin as the route of administration. The most easily accessible organ of the human body is the skin, which is mostly used for topical drug delivery. For the local treatment of dermatological diseases as well as for cosmetic purposes, several preparations, ranging from solids to semisolids and liquid formulations are available to healthcare practitioners and patients. Within the category of semisolid preparations, transdermal gels offer great potential for use in cosmetics and pharmaceutical fieldsa. The application of gel on the skin has some advantages including the quick release of the drug directly to the target site. It also does not depend on the water solubility of the drugs.

Gels contain more covalent crosslinks than jellies and are hence more rigid than jellies. Gel-forming polymers produce materials that span a range of rigidities, beginning with sol and increasing in rigidity to mucilage, jelly, gel and hydrogel. The gel systems can either be as clear as water (when the ingredients are

not completely dispersed) or be turbid (when the ingredients aggregate and disperse light). The concentration of the gelling agents is mostly less than 10%, usually in the 0.5% to 2.0% range, with a few exceptions. Different kinds of transdermal preparations like lotions, creams, ointments, patches, gels etc. are available, which gel is preferred due to more stability and better application property.

Majority of pharmaceutical gels available in Indian market are used either for analgesic or anti-inflammatory purpose. Others are mostly antimicrobial. For treating bacterial or fungal infections of skin, antiseptic creams, ointments or solutions are commonly used but in near future more number of antimicrobial gels are expected to be launched in Indian market. As a result, India has an opportunity to get an increasing number of gels patented. Transdermal prodrugs could also emerge in Indian market in coming years. Due to ever increasing use of novel penetration enhancement techniques, indications of gels for treatment of systemic diseases are expected to rise up in future. More focus may be directed towards herbal gels in future because of their freedom from all kinds of adverse effects, especially skin irritation reactions. Therefore, the gels hold a great promise as a topical drug delivery system; popularity and use of gels could further increase in future.

2. METHODOLOGY

2.1 Collection of Propolis

Indian propolis is a variety of poplar propolis that is gathered in December from a local beekeeper Madhya Pradesh. Propolis is collected by scraping it off from the top bars of comb frames, where bees deposit it in quite considerable amounts, using a hive tool. When propolis is obtained in this way, it is unprocessed and unfit for use. Before being extracted, the raw materials were sealed in plastic bags and kept at 40 degrees Celsius in a home freezer.

2.2 Extraction Process of Propolis

To get rid of the inert components and keep the active ingredients intact, the right solvent was used. Two distinct propolis extracts—water and ethanolic—were made using the methodology in this investigation. To make 100 mL, 30 g of crude propolis was combined with either 70% ethanol or water. The ingredients were combined at room temperature, with gentle shaking, and away from intense light. The solution was filtered and dried after 8 to 10 days.

The percentage yield ofpropolis was calculated by the formula:

$$\text{Percentage yield (\%)} = \frac{\text{Amount of pure product recovered}}{\text{Amount of crude material used}} \times 100$$

2.3 Phytochemical Screening

To determine the components of propolis, ethanolic, methanolic, and water extracts underwent chemical testing in accordance with established procedures. (Harborne,1973; Trease and Evans, 1989; Misra *et al*., 2011; Vijayalakshmi and Ravindhran, 2012).

2.4 Estimation of total polyphenols and Total flavonoids

2.4.1 Total Polyphenolic Content

Using the Folin-Ciocalteu colorimetric technique, the total polyphenolic content of the extracts was measured. In brief, 1 mL of diluted extract and 1 mL of Folin-Ciocalteu's phenol reagent were combined, and 10 mL of sodium carbonate (7% of the total volume) was added after the volume was corrected to 25 mL using distilled water. Using a Jasco V-630 UV-Vis spectrometer at room temperature, absorbance was measured at 760 nm following a 90-minute dark incubation period. The amount of polyphenol present overall was reported as milligrams of gallic acid equivalent (GAE) per gram of extract.

2.4.2 Total flavonoid content

The aluminum chloride colorimetric technique was used to determine the total flavonoid content of all extracts. 0.3 mL of sodium nitrite (5%) and 4 mL of distilled water were added to 1 mL of diluted extract. After five and six minutes, respectively, sodium hydroxide (2 mL, 1M) and aluminum chloride (0.3 mL, 10%) were added. Using water, the volume was adjusted to 10 mL. The Jasco V-630 UV-Vis spectrometer was used to detect the absorbance at 510 nm. Total flavonoid content of the extract was expressed in terms of mg quercetin equivalent (QE)/g of extract.

2.5 Formulation development of gel

Measuring 100 milliliters of water, the mixture was stirred quickly with a mechanical stirrer after measured amounts of methyl paraben, glycerin, polyethylene glycol, and ethanolic extracts of Indian propolis were dissolved. Next, the beaker containing the liquid above was progressively filled with Carbopol 934 while stirring. The solution was neutralized by adding a slow-moving triethanolamine solution and continually stirring until the gel formed.

Carbopol 934 – Gelling Polymer
Triethanolamine - gelling agent, pH Adjusting agent, Neutralizer
Methyl Paraben - Preservative
Distilled Water, Glycerin and Polyethylene Glycol – Solvents

Table 2.1: Formulation of Indian Propalis gel

Ingredients (mg)	F1	F 2	F3	F4
Indian propoli extract	50	50	50	50
Carbopol 934	500	1000	1500	2000
Polyethylene Glycol 600	0.2	0.2	0.2	0.2
Methyl Paraben	0.08	0.08	0.08	0.08
Triethanolamine	1.0	1.0	1.0	1.0
Distilled Water	100 ml	100 ml	100 ml	100 ml

2.6 Evaluation of gel

A. Appearance and consistency:

The texture of the gel formulations containing propolis extracts was examined visually, and the results are shown.

B. Washability

After applying prepared formulations to the skin, the degree and ease of washing with water were manually examined; the results were noted.

C. Extrudability

The gel formulations containing propolis extracts were put into aluminum collapsible tubes and sealed. The material was extruded through the tubes using pressure, and the formulation's extrudability was observed.

D. Determination of Spreadability

The ability of gels to spread readily is one of their key requirements. The ease with which a gel adheres to skin after application is referred to as its spreadability. Two typical glass slides measuring 6 by 2 inches were chosen. The gel composition, the spreadability of which required to be determined, was applied to one of the diapers. The formulation was positioned on the slide, and the second slide was placed on top of it, 6 cm apart. The experiment was repeated and the average of 6 such determinations was calculated for each formulation.

$$\boldsymbol{Spreadability} = \frac{m * l}{t}$$

Where, S=Spreadability (gcm/sec), m = weight tied to the upper slide (20 gram), l= length of glass slide (6cm), t = time taken is seconds.

E. Determination of pH

The pH of the anti-acne gels had been determined using a digital pH meter. After dissolving one gram of gel in twenty-five milliliters of filtered water, the electrode was submerged in the gel solution until a consistent reading was obtained. Two pH measurements were made for every formulation.

F. Drug content

The composition of the drug was measured with 1 gram of gel mixed with methanol in a 10-milliliter volumetric flask. Three milliliters of stock solution and one milliliter of 2% AlCl3 solution have been mixed together. A spectrophotometer was used to measure the absorbance at 420 nm after the mixture was vortexed for 15 seconds and the color creation was left to stand at 40°C for 30 minutes.

G. Viscosity

The viscosity of the generated gel was measured using a Brookfield digital viscometer. The viscosity was measured at 10 rpm and 25–30 ^{o}C ambient room temperature using spindle number six. a mouth-fitting, appropriately sized bottle that holds the perfect quantity of gel. It is possible to keep the viscometer spindle within the jar by utilizing a large mouth container. Once the reading was steady, the viscosity was measured. Gel samples were allowed to settle at a constant room temperature for over thirty minutes before to testing.

H.. *In vitro* diffusion profile

For the in vitro diffusion investigations of each formulation, Franz diffusion cells were utilized. built locally as an open-ended, cylindrical tube of 100 mm in height, 3.7 cm2 in size, and a diffusion area of 3.8 cm2. The culture medium used for receptors was phosphate buffer (pH 7.4). Rat belly skin is used to make the dialysis membrane. Because of the skin's connection to the diffusion cell, the stratum corneum side of the skin was near the formulation's release surface on the donor cell. Before being placed on the diffusion cell, a donor compartment was filled with 100 milliliters of isotonic phosphate buffer solution (pH 7.4). After covering the rat skin with a weighed amount of formulation equivalent to 1g of gel made from Propolis extracts, the skin was continuously shaken and gently submerged in 100 ml of receptor media. The overall temperature of the network was kept at 37±1 0C.

I. Skin Irritation Study

0.5 gm of the Propolis gel test component was applied to a 6 cm2 skin area, and the area was covered with a gauze patch. The gauze was removed and a semi-occlusive dressing was applied to hold the patch in place for an hour. After an hour of exposure, the remaining test substance was removed without compromising the integrity or response of the epidermis.

J. Stability Study

The ICH recommendations were followed in conducting the stability research. The prepared Propolis gel was put into the collapsible tubes and kept for six months at various temperatures and humidity levels, including 250 C ± 20C/ 60% ± 5% RH, 300 C ± 20C/ 65% ± 5% RH, and 400 C ± 20C/ 75% ± 5% RH. The appearance, pH, viscosity, and spreadability of the gel were all examined.

3. Result and Discussion

3.1 Results of percentage yield of propolis

Propolis extracts in ethanol and water were weighed, and the percentage yield and weight of the extracted propolis were calculated.

Table 3.1: Results of percentage yield of Indian Propolis

Extract Name	Percentage yield (%)	
	Ethanol	Water
Indian Propolis	38.72%	14.53%

3.2 Results of phytochemical analysis of extracts

The ethanolic and aqueous propolis extracts underwent preliminary phytochemical screening, which revealed the presence of active ingredients such as alkaloids, terpenoids, flavonoids, phenols, tannins, and saponins. Table 8.2 shows the relative effectiveness of the three solvents in extracting different propolis components.

Table 3.2: Phytochemical screening of Indian Propolis extract

S.No	TESTS	EEP	WEP
1.	Alkaloids	++	+
2.	Flavonoids	+++	+
3.	Phenols	++	+
4.	Tannins	++	++
5.	Carbohydrates	+	+
6.	Saponins	++	++

7.	Terpenoids	+	+
8.	Resins	+++	++
9.	Proteins	++	+
10.	Anthraquinones	+	+
11.	Quinones	+++	-
12.	Coumarins	+++	+

3.3Results of estimation of total phenol and flavonoids content

Table 3.3: Estimation of total phenolic and flavonoids content of Indian Propolis extract

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	**Ethanol Extract**	6.50 ± 0.25	10.05 ± 0.45
	Aqueous Extract	3.02± 0.15	4.85± 0.55

3.4 Results of Evaluation of Propolis Extract gel

3.4.1 Physical Characteristics of prepared gel

Table 3.4: Results of Physical Characteristics

Formulation	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
F1	Dark Brown	Absent	Good	Smooth	Good	Good
F2	Dark Brown	Absent	Good	Smooth	Good	Good
F3	Dark Brown	Absent	Good	Smooth	Good	Good
F4	Dark Brown	Absent	Good	Smooth	Good	Good

It has been observed that the gel formulations mentioned above all have smooth textures, good homogeneity, no clogging, and clear colors. The gel compositions mentioned above offer good extrudability and washability.

3.4.2 Results of Spreadability

Table 3.5: Results of spreadability of Propolis Extract gel

Formulation	Spreadability* (gcm/sec)
F1	12.92±0.64
F2	11.52±0.75
F3	10.81±0.21
F4	10.17±0.85

3.4.3 Results of Viscosity

Table 3.5 : Results of Viscosity of Propolis Extract gel

Formulation	Viscosity* (cp)
F1	3847±26
F2	3702±55
F3	3589±42
F4	3403±91

3.4.5 Results of pH

The pH of a topical medication delivery system is crucial, and the findings of a study on herbal formulation's pH indicate that every formulation is appropriate for topical administration.

Table 3.6: Results of pH of Propolis Extract gel

Formulation	pH
F1	6.97±0.01
F2	7.01±0.01
F3	7.05±0.02
F4	7.03±0.02

3.4.5 Results of *In Vitro* Drug Release Study

Table 3.7: *In vitro* drug release study of prepared Propolis Extract gel formulation

S. No.	Time (hr)	% Cumulative Drug Release			
		F1	F2	F3	F4
1	0.25	8.13	7.52	7.20	6.8
2	0.5	15.27	17.35	14.29	13.84
3	1	23.81	28.21	22.22	21.53
4	1.5	42.08	45.21	39.84	36.52
5	2	58.83	62.37	54.53	51.67
6	2.5	79.71	75.49	71.81	70.18
7	3	90.37	87.76	85.59	79.23
8	4	99.13	99.38	96.47	94.61

3.4.6 Results of Skin irritation study results

Table No 3.8: Results of Skin irritation study results

Treatment	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII
Control	A	A	A	A	A	A	A
Propolis Extract gel Formulation (F2)	A	A	A	A	A	A	A

Grade: A-No Reaction, B-Slight patchy erythema, C-Slight but confluent or moderate but patchy erythema, D-Moderate erythema, E-Severe Erythema with or without edema.

3.4.7 Results of Stability study of optimized formulation

Table 3.9: Results of Stability study of optimized Propolis Extract gel formulation

Formulation Code	Months	Spreadability	pH
Polyherbal Formulation (F2)	**I**	11.52±0.75	7.01±0.02
	II	11.02±0.75	6.99±0.2
	III	10.87±0.36	6.98±0.1

CONCLUSION

Propolis exhibits broad range antibacterial action against a variety of microbes and is non-poisonous. Our thorough screening's results convinced us that propolis has more benefits than just those listed above, and it also shows promise for the future discovery of novel bioactive compounds. The outcomes have demonstrated that propolis extracts have a safe place in the pharmaceutical and other industries. Therefore, more funding and study are required to screen traditional Propolis's bioactive components, which may offer useful therapeutic applications.

The extract from Indian propolis was enhanced with flavonoids and polyphenols. Total polyphenolic content (TPC) and total flavonoid content (TFC). Ethanol and water had total phenolic contents of 7.80 mg/100 mg and 3.82 mg/100 mg, respectively (corresponding to quercetin). Quantitative analysis of the ethanol and aqueous extract indicated total flavonoid contents of 12.72 mg/100 mg and 5.37 mg/100 mg, respectively, which are comparable to gallic acid. Ethanolic extract Indian propolis has a greater value of total flavonoid and phenolic content based on estimation of total flavonoid and phenolic content. It has been observed that the gel formulations mentioned above all have smooth textures, good homogeneity, no clogging, and clear colors. The gel compositions mentioned above offer good extrudability and washability. The Spreadability was measured on the basis of slip and drug characteristics of the gels and was in the range of 12.92±0.64, to 10.17±0.85 gms/cm sec. Gel viscosity was measured with a Brookfield viscometer model DV-II. Since a higher temperature reduces gel viscosity and vice versa, the temperature that affects viscosity was kept at 25°C. The viscosities of F1, F2, and F3 were determined to be 3847±26 cps, 3702±55 cps, 3589±42 cps, and 3403±91 cps, in that order. The formulae mentioned above were used to measure the viscosity of various gel samples. The viscosity of formulation F2 is good. As the concentration of polymer increases, the gels become more viscous. Increased links between the polymer molecules cause a compact, rigid, and dense mass to develop, which explains why viscosity increases with polymer concentration. In gels with high polymer concentrations, there is less liquid than in gels with low polymer concentrations, which accounts for the hardness. Put another way, the higher the polymer concentration, the more shear stress is needed to achieve a certain rate of shear. The pH values of the gels made with propolis extract fell between 6.97 and 7.05, which is considered acceptable. The formulations' pH values fall between 6.97 and 7.05, which means that they are safe to apply to the skin to won't cause irritation or other negative consequences.

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