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FORMULATION & DEVELOPMENT OF POLYHERBAL GEL OF *PISTACIA LENTISCUS, ALHAGI CAMELORUM* AND *LITSEA GLUTINOSA* FOR ANTIMICROBIAL ACTIVITY

Saurabh Balpande*, Dr. Shailendra Kumar Lariya, Dr. Shailendra Bindaiya
Radharaman College of Pharmacy, Bhopal (M.P.)

***Correspondence Info:**
Saurabh Balpande
Radharaman College of Pharmacy, Bhopal (M.P.)
Email:
saurabhbalpande21@gmail.com

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ABSTRACT

Skin illnesses are frequent and have a significant impact on the quality of life of sufferers. The use of long-term antibiotics for treatment causes organisms to acquire drug resistance. A wide range of medicinal plants have been identified as valuable sources of natural antibacterial substances as an option that may be effective in the treatment of these troublesome bacterial infections. Thus this study deals with formulation & evaluation of polyherbal gel made of extract of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa*. The plant materials were collected & subjected to extraction. Further qualitative & quantitative studies were also performed. The formulation & evaluation of gel was performed according to standard protocol. Results revealed that hydroalcoholic extract of *Pistacia lentiscus* contains alkaloid, flavonoids, diterpenes & tannin. The *Alhagi camelorum* found to have traces of alkaloid, flavonoid, phenol, protein & diterpene. In case of *Listea glutinosa* only glycosides, phenol, proteins & tannin were found to be present. The total flavonoid content in *Pistacia lentiscus* was estimated to be 0.964 mg/ 100 mg. In case of *Alhagi camelorum* total phenol & flavonoid content was quantified as 1.925 & 1.544 mg/100mg respectively. In case of *Litsea glutinosa in total* 1.600 mg/100mg of phenol was noted. The morphological characteristics shown that the formulated gel is devoid of clogging with good Homogeneity & smooth texture with light brown colour. The washability of all formulations was good while the Extrudability was average for some & good for few formulations. The maximum spreadability ranged from24.24±2 to 32.32±2 gcm/sec. The pH of formulations ranged from 4.76±0.21 to 7.21±0.12. The most viscous formulation was noticed to be PHG3 with viscocity of 3241±32 cps. Further the phenol content & flavonoid content in PHG4 was found to be 82.6±0.4%. & 85.3±0.3 % The polyherbal gel with concentration of 0.5mg/ml, 0.25 mg/ml & 0.125mg/ml estimated to have zone of inhibition of 16±0mm, 15±0.57mm & 12±0.5mm against *E.coli* respectively. While for *Staphylococcus aureus* at same concentration 14±0.47mm, 10±0.5mm & 9±0.74mm respectively. So, it is clear that Polyherbal gel is slightly more potent against *E.coli* in contrast to *S. aureus*.

Keywords: Polyherbal gel, Skin disease, Microbial infection, of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa, E.coli* & *S. aureus*.

INTRODUCTION

Skin illnesses are frequent and have a significant impact on the quality of life of sufferers. According to the ten categories of human disorders in the International Classification of disorders, more than 1000 skin or skin-related diseases are listed above. Surprisingly, skin and subcutaneous diseases were ranked 18th in the global disease burden ranking (GBD2013), while being the fourth major cause of disability globally. Bacterial skin infections, which mostly include cellulitis and pyoderma, account for a large proportion of skin illnesses. Despite the significant influence of bacterial skin illnesses on the worldwide disease burden, there appears to be a global lack of attention (Hollestein and Nijsten, 2014; Hong *et al*., 2008).

The use of long-term antibiotics for treatment causes organisms to acquire drug resistance. This adaptation is multifactorial and is dependent on the organism's susceptibility to the therapy as well as host factors such as hormones, stress levels, and so on (Nikaido, 2009).

A wide range of medicinal plants have been identified as valuable sources of natural antibacterial substances as an option that may be effective in the treatment of these troublesome bacterial infections. The World Health Organisation (WHO) believes that medicinal plants are the best source of a wide range of medications. Many plants have been employed for their antibacterial properties, which are attributed to phytochemicals synthesised in the plant's secondary metabolism. Plants include a wide range of secondary metabolites, including tannins, alkaloids, phenolic compounds, and flavonoids, all of which exhibit antibacterial effects in vitro (Aqil and Ahmad, 2007; Romero *et al*., 2005).

Three such understudied plants namely *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa* is considered for analysing antimicrobial activity in this study.

Since ancient times, *Pistacia lentiscus* Linn. (Family - Anacardiaceae), often known as mastic tree or mastagi, has been utilised in traditional medicine to cure a variety of illnesses. Its many sections include a range of medicinally important chemical compounds, including resin, essential oil, gallic acid, anthocyanins and flavonol glycosides, nortriterpenoids, -tocopherol, and arabino-galactan proteins. It contains antiatherogenic, antibacterial and antimutagenic properties, as well as antioxidant, antifungal, cholesterol lowering, hepatoprotective, anticancer, anthelmintic, wound healing, hypotensive, antiarthritic, and antigout action (Nahida and Siddiqui, 2012; Sehaki *et al*., 2023).

Another plant *Alhagi camelorum* a plant genus of the Fabaceae family, is found in many Asian, Australian, and European countries. Flavonoids, alkaloids (alhacidin and alhacin), steroids, pseudalhagin A, phospholipids, and polysaccharides are among the pharmacologically active secondary metabolites found in Alhagi species. Various portions of Alhagi have been linked to a variety of biological activities including antioxidant, cardiovascular, anti-ulcer, hepatoprotective, antispasmodic, antidiarrheal, antinociceptive, antipyretic, anti-inflammatory, anti-rheumatic,

antibacterial, and antifungal properties (Asghari *et al*., 2016; Verma *et al*., 2021).

Litsea glutinosa (Lour.) C.B. Robinson is a medicinal plant of enormous pharmaceutical significance that belongs to the Lauraceae family. Traditionally, the bark has been used to treat diarrhoea, dysentery, abdominal pain, indigestion, gastroenteritis, edoema, traumatic injuries, colds, arthritis, asthma, diabetes, and as a treatment for pain relief and poignant sexual power. while the leaves have antibacterial and cardiovascular properties, as well as the extreme flow of semen in males. The leaves can also be used topically to cure wounds and bruises, as well as as an emollient to ease the pressures of rheumatic and gouty joints. *L. glutinosa* includes a number of essential oils that have antimicrobial properties (Jamaddar *et al*., 2022). Thus, keeping in mind the advantages of these plants this study delas with formulation & evaluation of polyherbal gel made of extract of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa*.

MATERIALS AND METHODS

Selection and Collection of plant material

Fresh & healthy plant materials, free from diseases of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa* were collected from ruler area of Bhopal (M.P.) in the month of January, 2022.

Extraction

Defatting of plant material

21.74 gram of *Pistacia lentiscus,* 38.14 gram of *Alhagi camelorum* and 33 gram of *Litsea glutinosa* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether in a maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted plant materials of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa* were exhaustively extracted with hydroalcoholic solvent (methanol: aqueous: 80:20v/v) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts

Phytochemical screening

The phytochemical screening was performed according to standard protocol.

Estimation of total phenol content

The total phenolic content was estimated according to the FC method. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added. After mixing, solution was incubated & the absorbance was recorded at 765nm against the reagent blank. Using galllic acid standard curve was prepared. Using the standard curve, the total phenolic content was calculated and expressed as gallic acid equivalent in µg/mg of extract.

Total flavonoid content

Total flavonoid contents of extract were determined and expressed as Quercetin equivalent in mg/100mg of extract. An aliquot (3ml) of extracts or standard solution of Quercetin (5, 10, 15, 20 and 25µg/ml) was added with 1 ml of 2% AlCl 3 solution. The mixture was incubated for 5 min at room

temperature. The solution was mixed well and the absorbance was measured at 4200 nm. Using the standard curve, the total flavonoid content was calculated.

Formulation development of polyherbal gel

Measured quantity of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa* were dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer (or sonicator). Then Carbopol 940 was added slowly to the beaker containing above liquid while stirring. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed

Evaluation of polyherbal gel

pH determination

A pH meter can be used to determine the pH of individual and polyherbal gel formulations.

Appearance and Homogeneity

Visual inspection can be used to assess the physical appearance, clogging, and homogeneity of the generated individual and polyherbal gels.

Viscosity

Individual and polyherbal gel viscosities can be determined using a Brookfield viscometer (Model RVTDV II) at 100 rpm and spindle no. 6.

Spreadability

After one minute, the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) was measured to determine the spreadability of the gel formulations (Dixit *et al.*, 2013).

Extrudability

The gel compositions were placed in conventional capped collapsible aluminium tubes and crimped shut. The weights of the tubes were taken down. The tubes were fastened in place between two glass slides. The cap was removed after 0.5 gram was placed over the slides. The amount of extruded gel was measured and collected. The extruded gel's percentage was calculated.

Drug content

For flavonoid

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 3ml of stock solution was mixed with 1 ml of 2 % AlCl3. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 420 nm using a spectrophotometer.

For phenol

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 2ml of stock solution was mixed with 1 ml FC reagent and 1 ml sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Washability

Formulations were applied on the skin, and the ease and extent of washing with water were manually assessed (Mate *et al*., 2021).

Anti -microbial activity:

The antibacterial activity of the polyherbal gel is determined using the well diffusion method. For antimicrobial experiments, three concentrations were used: 25, 50, and 100 mg/ml. The plates are incubated at 37oC for 24 hours before being checked for clear zones of inhibition surrounding the wells with specific drug concentrations.

In vitro drug release

The drug release experiments were carried out in a Franz diffusion cell (25 ml cell volume). 1 gramme gel was equally placed to the surface of the egg membrane across a set area. Phosphate Buffer (pH5.8) solution was newly made and poured into the receptor chamber. A magnetic stirrer was used to stir the receptor chamber. At appropriate time intervals, the samples (1.0 ml aliquots) were collected and replaced with fresh buffer solution. A UV visible spectrophotometer was used to determine the drug content of the samples. The total amount of medication released through the egg membrane over time was calculated.

Table 1: Formulation of polyherbal gel

Ingredients	PHG1	PHG 2	PHG3	PHG4	PHG5	PHG6
Pistacia lentiscus extract	1gm	1gm	1gm	1gm	1gm	1gm
Alhagi camelorum extract	1gm	1gm	1gm	1gm	1gm	1gm
Litsea glutinosa extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.25mg	0.5mg	0.75mg	1.0 gm	1.25 gm	1.5 gm
Polyethylene glycol	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Methyl paraben	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg
Triethanolamine	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Distilled Water (q.s)	100ml	100ml	100ml	100ml	100ml	100ml

Table 2: Result of phytochemical screening of extract of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa*

S. No.	Constituents	*Pistacia lentiscus* extract	*Alhagi camelorum* extract	*Litsea glutinosa* extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 +Ve -Ve	 +Ve -Ve	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 -Ve	 -Ve	 +Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 -Ve +Ve	 -Ve +Ve	 -Ve -Ve
4.	Saponins A) Froth Test:	 -Ve	 -Ve	 -Ve
5.	Phenol A) Ferric Chloride Test: B) FC reagent test:	 -Ve -Ve	 +Ve	 -Ve +Ve
6.	Proteins A) Xanthoproteic Test:	 -Ve	 +Ve	 +Ve
7.	Carbohydrate A) Fehling's Test: B) Benedict test:	 -Ve -Ve	 -Ve -Ve	 -Ve -Ve
8.	Diterpenes A) Copper acetate Test:	 +Ve	 +Ve	 -Ve
9.	Sterols	-Ve	-Ve	-Ve
10.	Tannins A) Gelatin test:	 +Ve	 -Ve	 +Ve

Table 3: Estimation of total phenolic and flavonoids content of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa*

S. No.	Hydroalcoholic extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	*Pistacia lentiscus*	-	0.964
2.	*Alhagi camelorum*	1.925	1.544
3.	*Litsea glutinosa*	1.600	-

Table 4: Results of physical appearance

Formulation	Colour	Clogging	Homogeneity	Texture
PHG1	Light Brown	Absent	Good	Smooth
PHG2	Light Brown	Absent	Good	Smooth
PHG3	Light Brown	Absent	Good	Smooth
PHG4	Light Brown	Absent	Good	Smooth
PHG5	Light Brown	Absent	Good	Smooth
PHG6	Light Brown	Absent	Good	Smooth

Table 5: Results of Washability and Extrudability

Formulation	Washability	Extrudability
PHG1	Good	Average
PHG2	Good	Average
PHG3	Good	Average
PHG4	Good	Good
PHG5	Good	Good
PHG6	Good	Average

Table 6: Results of pH, Viscosity and spreadability

Formulation	Determination of pH	Viscosity (cps)	Spreadability (gcm/sec)
PHG1	4.76±0.21	2341±18	24.24±2
PHG2	6.24±0.16	2638±27	25.14±1
PHG3	7.21±0.12	3241±32	27.56±3
PHG4	6.85±0.13	2796±15	30.23±2
PHG5	6.90±0.14	2024±20	31.24±1
PHG6	6.70±0.11	3174±23	32.32±2

***Mean±S.D., Average of three determinations**

Table 7: Results of Flavonoids and phenol content

Formulation	Flavonoids content (%)	Phenol content (%)
PHG1	69.8±0.2	70.5±0.3
PHG2	70.6±0.3	68.7±0.5
PHG3	74.9±0.4	72.3±0.2
PHG4	**85.3±0.3**	**82.6±0.4**
PHG5	66.2±0.6	65.8±0.7
PHG6	72.8±0.4	75.4±0.5

(n=3)

Table 8: Antimicrobial activity of standard drug against selected microbes

S. No.	*Standard*	Microbes	Zone of inhibition (mm)		
			10µg/ml	20µg/ml	30µg/ml
1.	Ciprofloxacin	*Escherichia coli*	22 ± 0	27 ± 0	31± 0.5
		Staphylococcus aureus	20 ± 0	24 ± 0	27 ± 0.5

Table 9: Antimicrobial activity of polyherbal gel formulation (PHG4) against selected microbes

S. No.	Microbes	Zone of inhibition (mm)		
		0.5mg/ml	0.25 mg/ml	0.125mg/ml
1.	*Escherichia coli*	16±0	15±0.57	12±0.5
2.	*Staphylococcus aureus*	14±0.47	10±0.5	9±0.74

Table 10: Results of *In-vitro* drug release study of polyherbal gel formulation (PHG4)

S. No.	Time (Min.)	Percentage drug release
1.	15	19.98
2.	30	39.95
3.	60	65.58
4.	90	79.98
5.	120	98.95

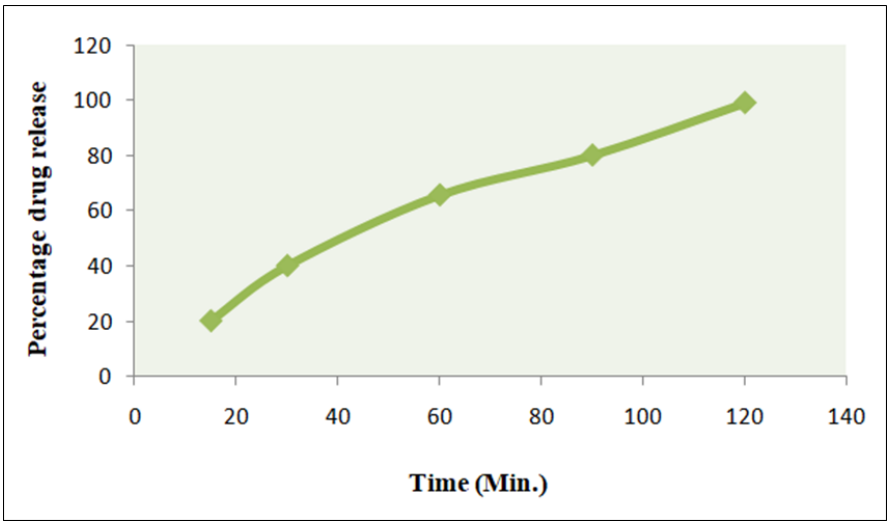

Figure 1: Graph of *in-vitro* dug release Study of polyherbal gel formulation (PHG4)

RESULTS AND DISCUSSION

The qualitative studies were performed at first place for all the three plants. Phytochemical screening suggested that hydroalcoholic extract of *Pistacia lentiscus* contains alkaloid, flavonoids, diterpenes & tannin. The *Alhagi camelorum* found to have traces of alkaloid, flavonoid, phenol, protein & diterpene. In case of *Listea glutinosa* only glycosides, phenol, proteins & tannin were found to be present. Further quantitative studies were performed to know the exact quantity of phenol & flavonoids. The total flavonoid content in *Pistacia lentiscus* was estimated to be 0.964 mg/ 100 mg. In case of *Alhagi camelorum* total phenol & flavonoid content was quantified as 1.925 & 1.544 mg/100mg respectively. In case of *Litsea glutinosa in total* 1.600 mg/100mg of phenol was noted.

The next procedures deals with evaluation of polyherbal gel. The washability of all formulations was good while the Extrudability was average for some & good for few formulations. The maximum spreadability of 32.32±2 gcm/sec was associated with formulation PHG6. The pH of formulations ranged from 4.76±0.21 to 7.21±0.12. The most viscous formulation was noticed to be PHG3 with viscocity of 3241±32 cps.

Further the phenol content was noted to be maximum for PHG4 formulations with value as 82.6±0.4%. Also, in flavonoid the content the PHG4 formulations stands superior with % flavonoid content of 85.3±0.3.

The antimicrobial activity of Polyherbal gel was then analysed with well diffusion method against *Escherichia coli* & *Staphylococcus aureus*. To compare the efficacy of Polyherbal gel, the antimicrobial assay of standard drug ciprofloxacin was also performed. The zone of inhibition of ciprofloxacin against *E. coli* at concentration of 10µg/ml, 20µg/ml and 30µg/ml was observed to be 22 ± 0 mm, 27 ± 0 mm & 31± 0.5mm respectively. While for *S. aureus* with the same drug concentration zone of inhibition was noted to be 20 ± 0mm, 24 ± 0mm and 27 ± 0.5mm. The polyherbal gel with concentration of 0.5mg/ml 0.25 mg/ml & 0.125mg/ml estimated to have zone of inhibition of 16±0mm, 15±0.57 mm & 12±0.5mm against *E.coli* respectively. While for *Staphylococcus aureus* at same concentration 14±0.47mm, 10±0.5mm & 9±0.74mm respectively. So, it is clear that Polyherbal gel is slightly more potent against *E. coli* in contrast to *S. aureus*. The *in-vitro* drug release study of polyherbal gel formulation (PHG4) revealed that in about 120 minutes 98.95% drug is released.

CONCLUSION

The data from the study revealed that polyherbal gels made from extracts of *Pistacia lentiscus, Alhagi camelorum* and *Litsea glutinosa* (L.) Pers.f had significant anti-microbial effect. Because phytochemical studies revealed the presence of phenol, flavonoids, steroids, Diterpenes and protein in the extracts, it is possible that they suppress the growth of bacteria. When compared to individual gels, the polyherbal gels demonstrated a synergistic effect that may be effective for the treatment of skin diseases.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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