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FORMULATION, DEVELOPMENT AND EVALUATION OF HERBAL GEL AS EFFECTIVE ANTIBACTERIAL AGENTS

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ABSTRACT

In the recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe with minimal adverse side effect especially when compared with synthetic drugs. The present research aims to formulate and evaluate an herbal gel containing *Terminalia arjuna and Terminalia bellirica* hydroalcoholic extract. The extracts were subjected to various physicochemical evaluations. The gel was prepared by using a suitable gelling agent-carbopol 940. The extract and herbal formulation were screened for the antibacterial activity by agar well diffusion technique against *S. aureus* which are representative types of Gram positive organisms. The formulations were also evaluated for appearance and homogeneity, pH, viscosity, spreadability, drug content uniformity and washability. The extract and herbal formulation was evaluated for its antibacterial activity and it was found that the formulation have elicited promising antibacterial activity which was comparable with that of standard drug (Ciprofloxacin). Results showed that the gels were non-irritant, stable and posses antibacterial activity. The herbal gel formulation exhibits a promising topical gel for bacterial infections. Thus the present study demonstrates an immense scope for development of such herbal gel formulations and also explores the vast potential to further carry out research by exploiting synergistic effect in herbal extracts.

Keywords: *Terminalia arjuna*, *Terminalia bellirica*, Physicochemical evaluations, Herbal gel, Carbopol 940, Antibacterial activity.

INTRODUCTION

Rising antibiotic resistance and the scarcity of new antimicrobials has long been acknowledged [1, 2]. A major challenge in global health care is the need for novel, effective and affordable medicines to treat microbial infections, especially in developing countries of the world, where up to one-half of deaths are due to infectious diseases [3, 4]. Some Staphylococcus spp. and Streptococcus spp. involved in the

pathogenesis of respiratory and skin infections, along with Pseudomonads and members of the Enterobacteriaceae causing gastrointestinal, urogenital diseases and wound contamination are resistant to virtually all of the older antibiotics [5]. Clinical isolates of Staphylococcus aureus, the leading cause of nosocomial infections, are increasingly resistant to an array of antimicrobial agents like penicillin, gentamicin, tobramycin, amikacin, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole and vancomycin [6]. The development of antimicrobial-resistant bacterial species stems from a number of factors which include the prevalent and sometimes inappropriate use of antibiotics, extensive use of these agents as growth enhancers in animal feed, and increased trans boundary passage of antibiotic-resistant bacteria [6]. The problem of antibiotic resistance in humans and animals will continue for a long time [7]. Against this backdrop, the development of alternative drug classes to treat such infectious diseases is urgently required [4]. Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins [8]. These biomolecules are the source of plant-derived antimicrobial substances (PDAms) [4]. Some natural products are highly efficient in the treatment of bacterial infections[9]. Hence, the present investigation was undertaken for preparation of herbal gel formulation of hydroalcoholic extract of Terminalia arjuna, Terminalia bellirica followed by the evaluation of the prepared formulation for its physical appearance, pH, viscosity, spreadability, drug content and antibacterial activity.

MATERIAL AND METHODS

Plant materials

The leaves of the plant of *Terminalia arjuna, Terminalia bellirica* were purchased from local market of Bhopal. Plant material (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study were obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Extraction by maceration process

55.8 gm dried powdered leaves of *Terminalia arjuna* and *Terminalia bellirica* has been extracted with hydroalcoholic solvent (ethanol: water; 80:20) using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

Phytochemical screening

Crude extracts were screened to identify the occurrence of primary and secondary metabolites, viz. carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, saponins, terpenoids, proteins and fixed oils, using standard screening test and phytochemical procedures [10, 11].

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Joshi et al, 2019) [12]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total alkaloids determination

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered [13]. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

Formulating herbal gel

In a beaker, measured amounts of methyl paraben, glycerin, polyethylene glycol, *Terminalia arjuna* and *Terminalia bellirica* hydroalcoholic extract were dissolved in roughly 35 ml of water and swirled at high speed using a mechanical stirrer (or sonicator). Then, while stirring, Carbopol 940 was gently added to the beaker containing the aforementioned liquid. The solution was neutralized by progressively adding triethanolamine solution while stirring constantly until the gel was formed.

Table 1 Formulation of herbal gel

Ingredients (%)	HG1	HG2	HG3	HG4	HG5	HG6
Terminalia arjuna extract	1gm	1gm	1gm	1gm	1gm	1gm
Terminalia bellirica extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.5mg	0.75mg	1.0 gm	1.25 gm	1.5 gm	2.0 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

Evaluation of herbal gel

Appearance and consistency

The physical appearance of herbal gel formulations was visually examined for texture, and observations were made [14-17].

Washability

Formulations were applied to the skin, and then the ease and extent of washing with water were physically evaluated.

Extrudability determination of formulations

Herbal gel compositions were placed in collapsible metal tubes or collapsible aluminum tubes. The tubes were pushed to extrude the material, and the formulation's extrudability was tested.

Determination of Spreadability

Two standard-sized glass slides (6 ×2) one of the slides was covered with the herbal gel formulation that was to be tested for spreadability. The second slide was positioned above the first in such a manner that the formulation was sandwiched between them for a total distance of 6 cm down the slide. The herbal gel mixture between the two slides was traced uniformly to produce a thin layer by placing 100 grams of weight on the upper slide.

The excess of the herbal gel formulation clinging to the slides was scraped off and the weight was removed. The bottom slide was attached to the apparatus's board, and one end of the top slide was linked to a string to which a 20-gram force could be imparted using a simple pulley. The time it took for the upper slide to travel 6 cm and separate from the lower slide under the weight's direction was recorded. The experiment was performed six times, with the average of the results determined for each gel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 grams)

l= length of glass slide (6cms).

t = time taken is seconds.

Determination of pH

A digital pH meter was used to determine the pH of the herbal gels. One gram of gel was dissolved in 25 ml of distilled water, and the electrode was dipped in the gel mixture until a steady reading was obtained. It was also reported that she was always reading. Each formulation's pH readings were repeated two times.

Drug content

1 gram of gel was placed in a 10 ml volumetric flask and diluted with methanol to assess the drug concentration. 1 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml stock solution. The mixture was vortexed for 15 seconds before being let to sit for 15 minutes to develop color. A spectrophotometer was used to measure the absorbance at 765 nm.

Antibacterial activity

Hydro-methanolic leave extract of *Terminalia arjuna, Terminalia bellirica* has been incorporated into a gel and studied for its antibacterial properties. The bacterial culture (*S. aureus*) was swabbed over the plate containing Potato dextrose agar media. Different concentration of leave extract and herbal gel (25, 50, and 100 mg/ml) and standard (10 mg/ml Ciprofloxacin) was added to the wells. Then the plate was incubated at room temperature for 2-3 d. The zone of inhibition was measured in mm [18].

RESULTS AND DISCUSSION

The crude extracts so obtained after maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the leaves of *Terminalia arjuna, Terminalia bellirica* using hydroalcohol as solvents was found to be 6.7 and 8.7 % respectively. The results of qualitative phytochemical analysis of the crude powder leaves of *Terminalia arjuna, Terminalia bellirica* are shown in Table 1. hydroalcoholic extracts of leaves sample of *Terminalia arjuna, Terminalia bellirica* showed the presence of alkaloids, phenolics, proteins, saponins and carbohydrate. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of hydroalcoholic extracts of leaves of *Terminalia*

arjuna and *Terminalia bellirica* showed the content values of 0.975 and 0.896 mg/100mg respectively. The determination of the total alkaloid content, expressed as mg atropine equivalents and per 100 mg dry weight of sample. TAC of hydroalcoholic extracts of leaves of *Terminalia arjuna* and *Terminalia bellirica* showed the content values of 0.721 and 0.432 mg/100mg respectively Table 2& 3. The prepared herbal gel is smooth and the color of herbal gel is Green and the intensity of the color increased with the increase in concentration of the extract in the gel. Homogeneity of gel is good Table 4. The pH value of the formulation was found to be in range of 6.9±0.2 to7.2±0.4. The results of the spreadability test was given in Table 5 and observed that HG1 to HG6 with the range of6.17±5to 12.61±10 gcm/s possess a better spreadability. Extrudability study was performed by gel formulations were filled into aluminum collapsible tubes, formulation have average extrudability and good washability of herbal gel. The skin irritation test performed showed no signs of sensitivity, erythema and edema. So the prepared formulations were considered to be non-irritant. The viscosity of formulation was found to be in range of 2265±6 to2784±9 cps Table 5. The efficacy of hydroalcoholic extract and the antibacterial gels from herbal extracts is shown in Table 6 & 7. The antibacterial gels could inhibit the growth of the microorganisms that inhabit *Staphylococcus aureus* and all the formulations exhibited comparatively less efficacy to standard drug but formulation 100mg/ml showed almost equal efficacy to standard. Hence, it is considered best for preparation of antibacterial gels.

Table 1 Result of phytochemical screening of hydroalcoholic extract of *Terminalia arjuna* and *Terminalia bellirica*

S. No.	Constituents	*Terminalia arjuna* extract	*Terminalia bellirica* extract
1.	**Alkaloids**		
	Hager's Test:	+ve	+ve
2.	**Glycosides**		
	Legal's Test:	-ve	-ve
3.	**Flavonoids**		
	Lead acetate Test:	-ve	-ve
4.	**Diterpenes**		
	Copper acetate Test:	-ve	+ve
5.	**Phenol**		
	Ferric Chloride Test:	+ve	+ve
6.	**Proteins**		
	Xanthoproteic Test:	+ve	+ve
7.	**Carbohydrate**		
	Fehling's Test:	+ve	+ve
8.	**Saponins**		
	Froth Test:	+ve	+ve

Table 2 Estimation of total alkaloid and phenol content of *Terminalia arjuna*

S. No.	Total alkaloid content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	0.721	0.975

Table 3 Estimation of total alkaloid and phenol content of *Terminalia bellirica*

S. No.	Total alkaloid content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	0.432	0.896

Table 4 Results of physical appearance

Formulation	Colour	Clogging	Homogeneity	Texture
HG1	Green	Absent	Good	Smooth
HG2	Green	Absent	Good	Smooth
HG3	Green	Absent	Good	Smooth
HG4	Green	Absent	Good	Smooth
HG5	Green	Absent	Good	Smooth
HG6	Green	Absent	Good	Smooth

Table 5 Results of washability, extrudability, Spreadability, pH and Viscosity

Formulation	Washability	Extrudability	Spreadability (gcm/sec)	pH	Viscosity (cps)
HG1	Good	Average	12.61±10	6.9±0.2	2753±8
HG2	Good	Average	11.48±9	7.0±0.6	2784±9
HG3	Good	Average	9.82±11	6.8±0.5	2265±6
HG4	Good	Average	7.64±12	7.1±0.3	2638±5
HG5	Good	Average	6.93±8	7.2±0.4	2456±7
HG6	Good	Average	6.17±5	7.2±0.5	2534±3

Table 6 Antimicrobial activity of extract and herbal gel formulation (HG5)

S. No.	Extract /Formulation	Zone of inhibition (mm)		
		100mg/ml	50 mg/ml	25mg/ml
1.	*Terminalia arjuna* extract	10±0.86	8±0.74	7±0.47
2.	*Terminalia bellirica* extract	11±0.57	9±0.5	7±0.86
3.	Herbal gel	23±0.47	18±0.74	13±0.57

Table 7 Antimicrobial activity of standard

Name of drug	Microbes	Zone of Inhibition (mm)		
		10 µg/ml	20 µg/ml	30 µg/ml
Ciprofloxacin	*Staphylococcus aureus*	25±0.47	29±0.47	34±0.47

CONCLUSION

The plant *Terminalia arjuna and Terminalia bellirica* was selected for the study, whose extract was very useful in the treatment of various diseases. On the basis of the study, the data showed that the herbal gels prepared from the dried hydroalcoholic extracts *Terminalia arjuna and Terminalia bellirica* gave the significant antibacterial activity when compared with standard Ciprofloxacin. Phytochemical tests showed the presence of alkaloids, phenolics, proteins, saponins and carbohydrate in the hydroalcoholic extracts responsible for the activity. The studies revealed that the developed single herbal formulation of *Terminalia arjuna and Terminalia bellirica* extract comparatively better than extract but the formulations were non irritant and did not show any skin toxicity when applied daily for 7 days in rats.

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