Annals of Phytomedicine 6(1): 114-120, 2017
DOI: 10.21276/ap.2017.6.1.17 ; Print ISSN : 2278-9839 and Online ISSN : 2393-9885
Ann. Phytomed., 6(1):114-120 (2017)

Original article

Antiacne and wound healing activity of polyherbal topical gel comprising *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Glycyrrhiza glabra* L., *Carica papaya* L. and *Cucurbita pepo* L.

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Received April 24, 2017: Revised May 19, 2017: Accepted May 26, 2017: Published online June 30, 2017

Abstract

Antiacne herbal formulations are used for the treatment of *Acne vulgaris* with the added advantage of not producing adverse effects unlike synthetic drugs. The present study has been designed with the objective for successive solvent extraction of *Andrographis paniculata* (Burm.f.) Wall. ex Nees (aerial parts), *Glycyrrhiza glabra* L. (roots), *Carica papaya* L.(seeds) *and Cucurbita pepo* L. (seeds), followed by development and evaluation of topical herbal gel formulation for antiacne and wound healing activity.

In vitro antiacne activity was performed against *Staphylococcus epidermis* and *Staphylococcus aureus*, a causative organism for *A. vulgaris*, using agar well diffusion method. Three herbal gel formulations were developed comprising 2.5%, 5.0% and 10% of herbal mixture containing methanolic extracts of *A. paniculata, G. glabra, C. papaya* and *C. pepo* using Carbopol 934 as gel base. Formulations were evaluated for pH, viscosity, spreadability, extrudability, stability and acute skin irritancy test. Evaluation of wound healing potential was evaluated by excision wound model in wistar albino rats.

Methanolic extracts exhibited highest zone of inhibition against *S. epidermis* and *S. aureus* than other solvent extracts. Antiacne potential of combined extracts was more than that of individual extracts (19.0±0.089). Developed gel formulations had good stability as per ICH guidelines. Formulations did not produce any skin irritation when observed for about 48 h on animal skin. Significant wound healing activity was observed in combined extracts and developed formulations as compared to standard and control group. The results were statistically significant. Hence, the study was successful in terms of establishing scientific data with regard to safety and effectiveness of selected herbal extracts in promoting antiacne activity in the form of a convenient, stable dosage form formulated using standardized extracts.

Key words: Plant extracts, antiacne, *in vitro, in vivo*, physical paramters, gel formulaion

1. Introduction

Acne vulgaris is a cutaneous disorder of multifactorial origin which manifests in the pilosebaceous follicle. It is characterized by open and closed comedones and inflammatory lesions like papules, pustules and nodules (Lalla *et al.*, 2001). Micro-organisms like *Propionibacterium acnes, S. aureus* and *S. epidermidis* proliferate rapidly, leading to the development of acne. These pathogens play an important role in the pathogenesis of acne. The oxidative stress within the pilosebaceous unit changes the environment from aerobic to anaerobic which is the best suited for this gram positive bacterium. It is implicated in development of inflammatory acne as it activates complement and metabolize sebaceous triglycerides into fatty acids which chemotactically attract neutrophills. *S. epidermidis and S. aureus* are aerobic organisms involved in superficial infection within sebaceous unit. Thus, *P.acne*, S.aureus and *S.epidermidis* are target sites for antiacne drugs (Vats and Sharma, 2012).

Development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatment for many diseases including antiacne properties (Patel *et al.*, 2015).

The present study aims to explore medicinal plants for their antiacne potential so as to avoid side effects and to provide natural essence to the skin. Selection of common herbs, namely; *A. paniculata* (aerial parts), *G. glabra* (roots), *C. papaya* (seeds) and *C. pepo* (seeds) was based on review that they possess many pharmacological attributes such as being antibacterial, antioxidant, antiscar, antiwrinkle and wound healing (Rivera *et al.*, 2013).

Among the skin care formulations, single-phase gel is extensively used for cosmetic products due to its aesthetic appearance.

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Moreover, organic macromolecules are uniformly distributed throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. An ideal formulation for acne should spread easily and leave minimal residue or oiliness (Nand *et al.*, 2012).

The main objective of the present study is to prepare and evaluate topical herbal gel formulations loaded with extracts of *A. paniculata*, *G. glabra*, *C. papaya* and *C. pepo* and to assess for *in vitro* antiacne and *in vivo* wound healing activity. The results will be useful in designing specific, novel, safe and effective herbal antiacne formulation for cosmetic and dermatological application without adverse effects which exist with non herbal or synthetic formulation.

2. Materials and Methods

2.1 Authentication of plants

Aerial parts of *A. paniculata*, roots of *G. glabra*, seeds of *C.papaya* and *C. pepo* were collected and were authenticated from National Ayurveda Dietetics Research Institute, Bengaluru (Letter No. SMPU/ RARIMD/BNG/2016-17/831, 21/11/16 and Voucher Specimen No. GCP/PCOG/sh1-4/2016-17).

2.2 Extract preparation

The collected plant material was cleaned, dried under shade at room temperature and powdered. The powdered material was subjected to sequential extraction using pet ether, chloroform, methanol, ethanol and water. Extract filtered and concentrated using rota vapor were stored at – 4°C until use.

2.3 Antiacne activity of extracts by agar well diffusion method

2.3.1 Micro-organisms used

S. epidermis (ATCC 12228) and *S. aureus* (ATCC 29737) were procured from Drug Testing Laboratory, Bengaluru, Karnataka, India.

2.3.2 Preparation of standard

Benzoyl peroxide 100 µg/ml in DMSO solution.

2.3.3 Preparation of extracts

25, 50, 75, 100 mg/ml of chloroform, methanol, ethanol and water extracts of selected drugs were prepared in DMSO solution.

Procedure: Bacterial cultures were grown on nutrient agar at 37±2°C for 18 h. and colonies were suspended in sterile saline (0.85% Nacl) and its turbidity was adjusted to 10^8 CFU/ml. Agar plates were seeded with bacterial cultures aseptically, wells were made on agar plate using cork borer (6.0 mm). 50 µl of different concentrations of different extracts were introduced in each well, respectively DMSO was used as negative control and incubated for 24-48 h. at 37±2°C. Zone of inhibition was measured in mm. These results are shown in Table 2, Graph 1 and Figure 1 (Marie, 2005).

2.4 Development of topical herbal gel formulation

As per the formulation requirements, accurately weighed quantity of Carbopol 934 was dispersed in 50 ml of distilled water, using mechanical stirrer for 30 min at 1200 rpm. Measured quantities of methyl paraben and propyl paraben were dissolved in 5 ml of distilled water with an aid of heating and mixed with glycerine and cooled. This solution was added to Carbopol 934 suspension. Added the measured quantities of herbal mixture (equal proportion of the drugs were combined and from this 2.5, 5 and 10% was taken for formulation) and adjust volume to 100 ml with distilled water. Triethanolamine was added dropwise to get required viscosity/ consistency. pH was adjusted to 6.5-7.0 (Gupta *et al.*, 2008).

Table 1: Different formulations along with ingredients

Ingredients	Formulations		
	F1	F2	F3
Carbopol 934	1.25%	1.25%	1.25%
Propylene glycol	15%	15%	15%
Methyl Paraben	0.2%	0.2%	0.2%
Propyl Paraben	0.1%	0.1%	0.1%
Triethanolamine	q.s	q.s	q.s
Herbal mixture	2.5%	5%	10%
Distilled water	Up to 100 gm	Up to 100 gm	Up to 100 gm

2.5 Physicochemical evaluation of the developed topical herbal gel formulation

2.5.1 Physical appearance

All developed gels were tested for colour, transparency, homogeneity and feel on application and presence of any aggregates recorded by visual inspection.

2.5.2 pH of 1% gel formulation was measured using pH meter

2.5.3 Extrudabilty

Gel formulations were filled in standard caped collapsible lamitubes and sealed. Each of the tube was placed between two glass slides and clamped. A 500 g weight was placed over the glass slide and caps were opened. Extruded gel was observed visually and grades were allotted (++++ excellent, +++ Good, ++ fair, + poor).

2.5.4 Spreadability

The parallel plate method was used for determining and quantifying the spreadability. Two glass slide 20×20 cm were taken. Gel formulations were placed over one of the slides. The other slide was placed upon the top of the gel, such that gel was sandwiched between the two slides in an area occupied by a distance of 6 cm. 100 g weight was placed upon the upper slide so that the gel between the two slides gets pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slide was scrapped off. The two slides were fixed to a stand without slightest disturbance and in such a way that only the upper slide slips off freely by the force of weight tied to it. 20 g weight was tied to upper slide carefully. The time taken for the upper slide to travel the distance of 6 cm and separate away from the lower slide under the direction of weight was noted. Experiments are done in triplicate. Spreadability was calculated using the following formula:

$$S = [M \times L] / T$$

where

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide and

T = Time (in sec.) taken to separate the slide completely each other

Table 2: Antiacne activity by Agar well diffusion method of individual and combined extracts of selected plants

		Zone of Inhibition in mm							
		Staphylococcus epidermis				*Staphylococcus aureus*			
		25 mg/ml*	**50 mg/ml***	**75 mg/ml***	**100 mg/ml***	**25 mg/ml***	**50 mg/ml***	**75 mg/ml***	**100 mg/ml***
Andrographis paniculata	**Chloroform**	6.5±0.2887	8.0±0.7638	10.4±0.3055	12.7±0.3930	4.8±0.1667	7.0±0.0667	9.7±0.3512	11.5±0.2906
	Methanol	8.3±0.3330	11.6±0.3300	13.4±0.3055	17.0±0.5812	6.8±0.1667	10.5±0.2906	12.4±0.3712	16.1±0.5859
	Ethanol	6.8±0.1667	8.8±0.1667	10.4±0.2963	11.4±0.2963	5.4±0.2963	7.4±0.3055	9.2±0.3712	11.3±0.3512
	Water	4.5±0.2887	6.5±0.2887	9.5±0.2906	11.3±0.3512	3.6±0.3055	5.4±0.2905	7.9±0.0667	9.9±0.0667
Glycyrrhiza glabra	**Chloroform**	6.9±0.0667	9.0±0.0667	11.5±0.2906	13.5±0.2906	5.5±0.2887	8.4±0.2963	10.5±0.2906	12.4±0.3055
	Methanol	8.5±0.2906	10.50±0.2906	13.0±0.5744	17.1±0.6009	5.9±0.0667	9.4±0.3055	10.2±0.4163	14.1±0.5859
	Ethanol	6.8±0.1667	9.5±0.2963	11.5±0.2887	12.5±0.2887	4.6±0.3180	8.2±0.6110	9.3±0.3180	12.0±0.5783
	Water	5.5±0.2887	8.6±0.3053	10.5±0.2887	12.0±0.2906	4.4±0.3055	7.8±0.1330	9.4±0.2963	12.3±0.3512
Carica papaya	**Chloroform**	5.8±0.4410	8.4±0.2963	10.5±0.2906	11.2±0.3930	3.8±0.1330	7.4±0.3055	10.4±0.2963	11.4±0.2963
	Methanol	8.4±0.2963	10.4±0.2963	12.3±0.3512	15.0±0.5812	6.4±0.2963	8.4±0.2963	10.5±0.2857	12.4±0.3055
	Ethanol	7.8±0.1330	9.9±0.0667	11.2±0.4163	12.8±0.2000	5.9±0.1000	8.5±0.2906	9.7±0.2330	10.9±0.1000
	Water	7.0±0.0667	8.9±0.0667	10.1±0.4933	12.1±0.4660	4.7±0.2330	7.4±0.2963	9.9±0.1000	11.1±0.4933
Cucurbita pepo	**Chloroform**	7.0±0.0667	8.8±0.1333	11.4±0.2963	12.1±0.4933	4.9±0.0667	6.9±0.1000	9.5±0.2963	10.4±0.3055
	Methanol	8.4±0.2963	9.8±0.2000	11.5±0.2906	13.2±0.4163	6.9±0.0667	8.4±0.2906	11.2±0.6110	12.5±0.2906
	Ethanol	5.9±0.1000	8.4±0.2963	10.2±0.3712	11.9±0.1000	4.7±0.2330	8.5±0.2906	9.7±0.2333	11.1±0.2963
	Water	4.9±0.0667	6.9±0.1000	9.4±0.2963	10.9±0.6360	3.9±0.0667	5.8±0.2000	8.5±0.2906	9.9±0.1000
Polyherbal extracts	**Chloroform**	7.0±0.2248	9.5±0.2360	11.5±0.1030	12.5±0.1640	6.3±0.0830	7.2±0.1160	9.5±0.2040	10.5±0.1090
	Methanol	8.66±0.089	12.3±0.0800	14.0±0.0750	19.0±0.0890	7.7±0.0980	10.3±0.1500	13.0±0.0890	16.0±0.0510
	Ethanol	6.0±0.0750	8.5±0.1030	10.5±0.1320	12.0±0.1290	5.0±0.0980	8.5±0.2890	10.0±0.1870	12.5±0.1780
	Water	5.0±0.1410	7.0±0.2160	9.5±0.0750	11.5±0.0510	4.0±0.1670	6.0±0.1640	8.5±0.1320	10.5±0.2190

*Values are expressed in terms of mean ± SEM of values done in triplicate

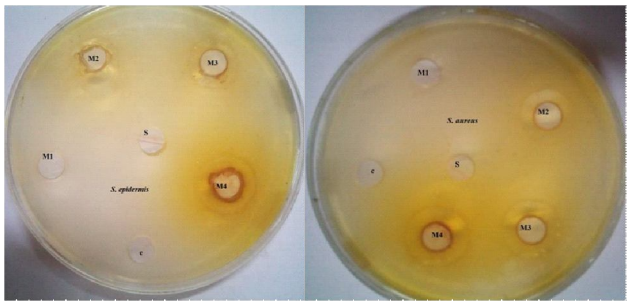
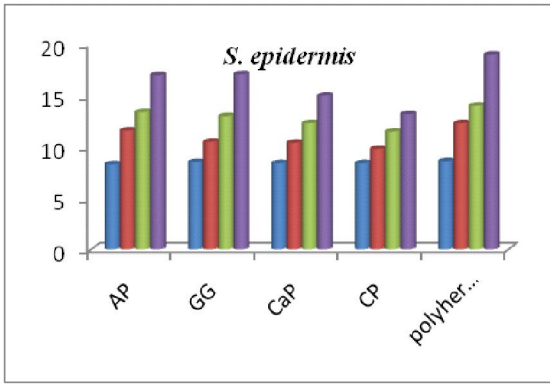
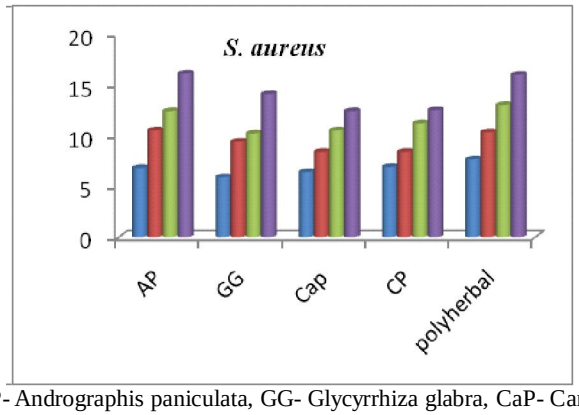


Figure 1: Photographs depicting antiacne activity of polyherbal formulation of methanolic extracts

Graph 1: Graph depicting zone of inhibition (ZOI) of methanolic and polyherbal extracts of selected plants





AP- Andrographis paniculata, GG- Glycyrrhiza glabra, CaP- Carica papaya, CP- Cucurbita pepo

2.5.5 Viscosity

Viscosity of formulated gel bases was measured at 252, using Brook-field viscometer (Model- RTVP) with spindle type-7. Sample holder of the Brookfield viscometer was filled with the gel sample and then spindle was inserted into sample holder. The spindle was rotated at 100 rpm at room temperature. Determinations were carried out in triplicate and the average of three reading is recorded.

2.5.6 Stability studies of herbal gel formulation

Stability studies were performed as per ICH (International Conference on Hormonization) guidelines. The formulated gel was filled in the collapsible tubes and stored at different temperatures

and humidity conditions, *viz*., 25℃ ± 2℃ and 60% ± 5% RH, 30℃ ± 2℃ and 65% ± 5% RH, 40℃ ± 2℃ and 75% ± 5% RH for a period of three months and studied for changes in pH, colour and appearance (Table 3) (Sumeet and Shailesh, 2012).

Table 3: Stability studies of topical herbal gel formulations at different temperatures and humidity conditions

Formulation	Parameters	Initial day	Values after 30 days at different temperatures and humidity conditions		
			$25^0C \pm 2^0C$ and 60% ± 5% RH	$30^0C \pm 2^0C$ and 65% ± 5% RH	$40^0C \pm 2^0C$ and 75% ± 5% RH
F1	Colour	Light green	No change	No change	No change
	Appearance	Homogenous	Homogenous	Homogenous	Homogenous
	Spreadability (g.cm/sec)*	19.33±0.1432	19.23±0.1438	19.23±0.1438	19.23±0.1438
	Viscosity (cps)*	17853±23	17863±34	17863±34	17863±34
	pH*	6.5±0.02	6.5±0.02	6.5±0.02	6.5±0.02
	Extrudability*	+++	+++	+++	+++
F2	Colour	Green	No change	No change	No change
	Appearance	Homogenous	Homogenous	Homogenous	Homogenous
	Spreadability (g.cm/sec)*	23.14±0.1672	23.04±0.1652	23.04±0.1652	23.04±0.1652
	Viscosity (cps)*	20880±23	20870±23	20870±23	20870±23
	pH*	6.8±0.01	6.8±0.01	6.8±0.01	6.8±0.01
	Extrudability*	++	++	++	++
F3	Colour	Dark green	No change	No change	No change
	Appearance	Homogenous	Homogenous	Homogenous	Homogenous
	Spreadability (g.cm/sec)*	27.03±0.3905	27.13±0.3915	27.13±0.3915	27.13±0.3915
	Viscosity(cps)*	22080±23	22065±22	22065±22	22065±22
	pH*	7.1±0.02	7.1±0.02	7.1±0.02	7.1±0.02
	Extrudability*	+++	+++	+++	+++

'*' Values are expressed in ± SEM (n = 3), '++' indicates Good, '+++' indicates Excellent

2.6 Evaluation of wound healing activity of combined extracts and topical herbal gel formulation in animals

The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment (Letter No. DCD/GCP/20/E.C/ADM/ 2015-16).

2.6.1 Treatment protocols

The animals were numbered, weighed and then divided into seven groups with six animals in each group.

Group I : Control

Group II : Standard

Group III : Combined extracts 5%

Group IV : Combined extracts 10%

Group V : Formulation 1

Group VI : Formulation 2

Group VII : Formulation 3

2.6.2 Excision wound model

Rats were depilated on the back after being anaesthetized with combination of 0.1ml of ketamine and 0.2 ml of xylazine injection. Cutaneous circular wound of 2 to 3 cm diameter was made on the pre-shaved sterile dorsal surface of the animal by excision and wound was left open to the environment. One wound was made on each animal of all the groups. Animals were housed individually in metallic cages. Animals in each group were topically applied with respective formulations, standard and control daily thrice until the formation of complete epithelial layer, starting from the first day. All the animals were monitored daily and observed for any fluid, evidence of infection and any other abnormalities.The wound area of each animal was measured from the first day of wounding till complete healing on alternative days. Wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelization time. The percentage of wound contraction was determined using the following formula and shown in (Table 4) (Mageswari *et.al.*, 2015).

$$\% \text{ Wound contraction} = \frac{\text{Initial wound size} - \text{specific day wound size} \times 100}{\text{Initial wound size}}$$

2.7 Statistical analysis

Mean values were determined for each parameter. The results were calculated for the test and the control group animals using Graph pad prism version 5.0. A level of probability 0.05 and 0.01 was taken as statistically significant.

3. Results and Discussion

3.1 Antiacne activity of individual and combined herbal extracts

Table 4: Evaluation of topical herbal gel on wound healing by excision wound method in rats

Post wounding days	Wound area (cm^2) (mean ± SEM) and percentage wound contraction						
	Control	**Vaseline**	**CE 5%**	**CE10%**	**F1(2.5%)**	**F2(5%)**	**F3(10%)**
0	2.35±0.070	2.36±0.0422	2.40±0.0366	2.38±0.054	2.25±0.0721	2.33±0.034	2.31±0.040
3	2.2±0.0897(6.38)	13±0.0422(9.74)^{ns}	2.12±0.033(11.66)^{ns}	2.01±0.054(15.54)^{ns}	1.98±0.079(12.00)^{ns}	1.86±0.0211(20.17)*	1.83±0.068(20.77)*
5	1.91±0.0875(18.72)	1.86±0.0422(21.180^{ns}	1.76±0.0425(26.63)*	1.54±0.057(35.59)*	1.56±0.123(30.66)*	1.35±0.034(44.20)*	1.13±0.061(52.11)**
7	1.51±0.0705(35.74)	1.42±0.0564(39.83)^{ns}	1.12±0.0308(53.33)*	0.76±0.088(63.86)*	1.18±0.068(47.55)^{ns}	0.86±0.047(66.52)*	0.62±0.036(73.16)*
9	1.20±0.0766(48.93)	1.06±0.0705(55.08)^{ns}	0.72±0.0366(70)*	0.42±0.030(82.35)**	0.76±0.065(66.22)*	0.5±0.0449(78.54)*	0.24±0.0211(89.61)**
11	1.08±0.0732(54.04)	0.85±0.0559(63.98)*	0.41±0.0308(82.91)**	0.06±0.0231(97.47)***	0.54±0.047(76.00)*	0.34±0.083(85.40)**	0.03±0.016(98.70)***
13	0.80±0.0685(65.95)	0.48±0.0673(75.48)*	0.13±0.0168(96.91)***	-	0.32±0.067(84.64)**	0.11±0.017(97.26)**	-
15	0.65±0.0673(72.34)	0.12±0.0289(96.28)**	-	-	0.12±0.0148(94.22)**	-	-
17	0.32±0.0583(93.26)	-	-	-	-	-	-

Values are presented as mean ± SEM; (ns- not significant, * $p<0.05$: less significant; ** $p<0.01$: significant; *** $p<0.001$: highly significant versus Control) by one way ANOVA.

Among various solvent extracts of *A. paniculata*, *G. glabra*, *C. papaya* and *C. pepo*, methanolic extracts had better antiacne potentials which was evident by highest zone of inhibition exhibited with these extracts. Among the selected drugs, *G. glabra* had greater antiacne activity with 17.10.6009 mm, followed by *A. paniculata* (17.00.5812 mm), *C. papaya* (15.00.5812 mm) and *C. pepo* (13.20.4163).

Improvement in antiacne activity was observed in combined herbal mixture as compared to individual extracts (Table 2, Figure 1 and Graph 1).

3.2 Stability studies of developed gel formulations

Stability studies of developed gel formulations revealed that none of the parameters had any major changes either in colour, appearance, pH, extrudability, spreadability and viscosity. Hence, formulation was considered stable (Table 3).

3.3 Evaluation skin irritation for herbal formulation

At the end of the study period, none of the animals topically applied with any of the three gel formulations exhibited any irritation in terms of swelling, redness, itching, erythema and edema. Hence, formulations were considered to be non-irritant upon topical application on skin. Hence, developed formulations can be considered safe for topical use.

3.4 Evaluation of wound healing activity of topical herbal gel formulation in animals

Topical application of combined extracts (5% and 10%) and herbal gel (2.5%, 5% and 10%) elicited a significant reduction in the wound area**.** There was no significant differences among all groups upto 2nd day, but from 3rd day, there was significant differences in healing process among all groups. On 5th day, wound contraction was better in treated groups when compared to Control and Vaseline group. On 11th day, wound was completely healed with Combined extract (10%) and Formulation 3(F3) while in other groups, healing was not complete. On 13th day, Combined extract (5%) and Formulation 2(F2) completely healed wounds, while Control and Vaseline group animals exhibited 65.95% and 75.48% healing, respectively. It took 15 days for complete healing in animals treated with F1 and Vaseline groups (Table 4 and Figure 2). All the tested extracts and polyherbal formulations were effective in healing wounds.

Results indicate that F3 with 10% of herbal mixture had best wound healing, as the rate of epithelization and more contraction occured at rapid phase and healed completely in less duration as compared to other extracts. This could be due to the fact that higher concentration of extracts are required for fast healing process.

4. Conclusion

We were successful in developing a stable and safe topical polyherbal gel, comprising *A. paniculata, G. glabra, C. papaya* and *C. pepo* and demonstrates antiacne and wound healing efficacy by simple *in vitro* and *in vivo* models. Our study supports the traditional claims of these drugs as healing and anti-infective agents. Study is also in support of the traditional claim of using herbs in combination for safe and better therapeutic effects than using them individually.

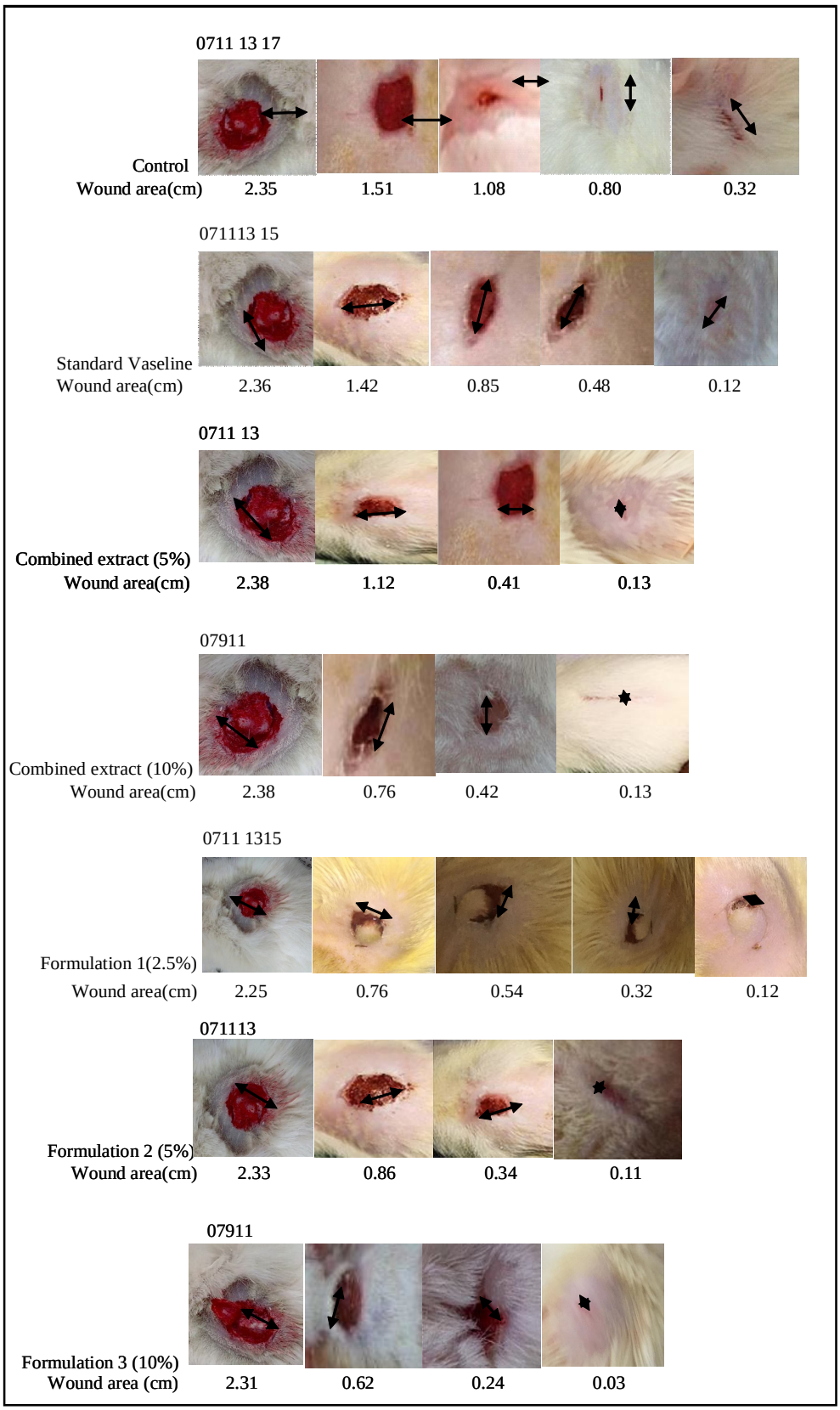


Figure 2: Photographs of day-wise progress in wound healing with control, standard, combined extracts and formulations.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgements

We are thankful to the American Association of Government College of Pharmacy (Bengaluru) Alumini (AAGCPA) for providing financial assistance for research work.

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