OPEN ACCESS

ORIGINAL ARTICLE

Development and Evaluation of Antimicrobial Poly-herbal Gel Formulation for the Treatment of Various Skin Infections

Tehmina Sohail*, Rashid Ali Khan, Hina Imran, Ghulam Fareed, Shazia Yasmeen

Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi, Pakistan.

Authors' Contributions

1Conception & Study Design, Data Analysis and/or Interpretation.
2Critical Review.
3Data Collection & Processing.
4Data Collection & Processing, Drafting of Manuscript.
5Data Analysis and/or Interpretation.

Article info.
Received: August 03, 2022
Accepted: September 30, 2022

Funding Source: *Nil*
Conflict of Interest: *Nil*

Cite this article: *Sohail T, Khan RA, Imran H, Fareed G, Yasmeen S. Development and Evaluation of Antimicrobial Poly-herbal Gel Formulation for the Treatment of Various Skin Infections. RADS J Pharm Pharm Sci. 2022; 10(3):92-99.*

****Address of Correspondence Author:***

tehminasohail23@gmail.com

ABSTRACT

Background: *Azadirachta indica (leaves), Salvadora persica (sticks)* and *Calendula officinalis (flowers)* are being used in various ailments and skin infections individually. It has a range of pharmacological activities like antioxidant, antifungal, antibacterial and wound healing. There is a scarcity of scientific evidence on the formulation of polyherbal gel containing these plants.

Objectives: The current study is aimed at formulating a poly herbal gel using plant extracts having superior antibacterial activity. The prepared formulation will be examined for its physical properties (pH, viscosity, and spreadability) antibacterial study and dermal irritation test.

Methodology: Standard methods were used to conduct studies on ethanolic extracts of *A. indica (leaves)*, *S. persica (sticks)* and *C. officinalis (flowers).* Individual plant extract's antimicrobial activity was studied against *Escherichia coli* (ATCC No. 8739), *Staphylococcus aureus* (ATCC No. 25923), *Pseudomonas aeruginosa* (ATCC No. 9027) and yeast *Candida albicans.* Physicochemical properties of polyherbal gel and skin irritation test were performed according to standard guidelines.

Results: The antimicrobial activity of ethanolic extracts was observed against gram positive, gram negative bacteria and yeast *Candida albican.* On the basis of their antimicrobial activity, 5% w/w polyherbal antimicrobial gel was formulated by incorporating all three extracts in equal ratio, which are then evaluated for physical properties and antimicrobial assay. Acute dermal irritation test of polyherbal gel according to the OECD 404 guideline was conducted on the healthy skin of albino rabbits. The results revealed that animals not showed any signs of irritation, redness, inflammation and oedema. The physical parameters and stability study revealed that the formulated polyherbal gel was found stable at different temperature and compatible to skin. The microbiological studies exhibited significantly strong ($p < 0.05$) activity against *S. aureus, E. coli, Ps. aeruginosa* and *C. albicans.*

Conclusion: The present study concluded that prepared polyherbal gel is safe and can be used effectively as antimicrobial formulation against various skin infections.

Keywords: *A. indica, S. persica, C. officinalis, polyherbal gel, antimicrobial activity, dermal irritation test.*

INTRODUCTION

Green medicines are considered as effective antimicrobial agents for treatment of multiple skin problems due to less side effects commonly associated with synthetic antimicrobials. Globally 88% population relies on natural medicines for their wellbeing [1]. To treat topical lesions plant derived formulation is gaining significant attention these days [2]. Numerous variety of medicinal plants are rich sources of secondary metabolites that contributes to

their wide use as an antibacterial agent to treat skin conditions [3].

Azadirachta indica belongs to Meliaceae family, which is a native to many Asian countries including Pakistan. This plant species have potent phytochemical constituents (nimbin, nimbidin, nimbolide and limonoids) that play an important role in cure of diseases through multiple genetic ways. The earliest antifungal and antibacterial polyphenolic flavonoids compounds isolated from fresh neem leaves were Quercetin and ß-sitosterol [4]. Previous researchers have summarized various therapeutic roles of neem as it has been an excellent anti-inflammatory, anti-arthritic, antipyretic, hypoglycemic, gastric ulcer, antimicrobial and antitumor activities [5].

Calendula Officinalis commonly known as Marigold (yellow to bright orange colored flowers) belongs to the family Asteraceae, the Sunflower Family. The plant is reported for anti-inflammatory, analgesic dermagenic, anti-oxidative and antimicrobial, anthelmintic, carminative, anti-spasmodic, anti-pyretic, antiseptic, anti-emetic and anti-viral properties [6-8]. Salvadora persica L (miswak, toothbrush tree) belongs to Salvadoraceae family and is distributed in Africa, Iran, Pakistan, India, Sri Lanka and Middle Eastern countries [9]. Since long S. persica is used to maintain oral hygiene and treat gum infections [10]. The reported pharmacological activities of S. persica are anti-inflammatory, analgesic, antipyretic, anti-rheumatic anti-microbial, anti-plaque, diuretic and bitter gastric effects. Scientifically reported phytochemical constituents are vitamin C, trimethylamine, salvadourea, salvadorine, tannins, saponins and cyanogenic glycosides [11]. On the basis of reported data, a polyherbal gel containing herbal extracts was formulated due to its proven antimicrobial activity.

Although these plants acquire excellent antibacterial properties, they are not easy to use in their raw state and apply to the skin surface. Therefore, the present study used ethanolic extracts of Azadirachta indica (leaves), Calendula Officinalis (flowers) and Salvadora Persica (sticks) to prepare polyherbal gel to promote their effective use as antimicrobial agents. The formulation thereafter checked for pH, viscosity, spreadability, physical appearance, antibacterial activity, and dermal irritation test.

METHODOLOGY

Collection of plant material

Three plants, A. indica leaves, S. persica sticks and C. officinalis flowers were purchased from marketplace of Karachi and identified by taxonomists. Herbarium identification numbers 134, 135 and 136 respectively were issued and submitted to the PCSIR's Herbarium. After identification and authentication by taxonomists, the plant material was washed and air-dried for seven days then ground into powder.

Extraction of herbal material

Ethanol and distilled water (70:30 ratio) was used to soak the grounded plants material for 7 days. Afterwards, filtration was done by passing each plant material through muslin cloth and then passed through Whatman filter paper. Rotary evaporator (40°C) was used to take out excess solvent to get a solid yield.

Herbal Gel Formulatiom

The herbal gel based on extracts was prepared in two steps. In the 1st step, ethanol extracts of plants were weighed in equal amounts and dissolved in ethanol. In 2nd step, the carbomer 940 polymer was dispersed in an aqueous suspension, followed by the addition of DMDM as a preservative and propylene glycol as a skin moisturizer to prepare a gel. It is infused with polyherbal extracts to improve the spectrum of the gel. The mixture was made viscous and neutralized with triethanolamine [12] Table **1**.

Physical evaluation of the Polyherbal gel

The polyherbal gel formulation was inspected for following physicochemical parameters [13].

pH measurement

The pH meter was used to measure pH. Standard buffer solutions of pH 4, 7, 9 were used to calibrate prior to each use. Electrodes were dipped into the samples at room temperature 10 min prior to reading (Table 2).

Viscosity

Gel's viscosity was studied using Brookfield Viscometer (DV - III ULTRA, USA) at 0.3, 0.6 and 1.5 rpm, using spindle # 7 at 30°C (Table **2**).

Table 1: Gel Composition

S No.	Ingredients	Quantity (%)
1.	Polyherbal Ethanolic extract	5
2.	Ethanol	2.5
3.	Carbomer - 940	1
4.	Propylene glycol	2
5.	Triethanolamine	0.5
6.	Water	q.s to 100 ml
7.	DMDM	0.3
8.	Phenoxyethanol	0.2

Table 2. Physical Parameters of the Gel.

pH	Viscosity			*Spreadability* (gcm/s)	*Extrudability*	Centrifugation Test
	0.3 rpm	0.6 rpm	1.5 rpm			
6.06±0.11	$225x10^3$	92500	36533	27.68	Good	NSL

NSL=No separation of layer

Table 3. Stability Study.

Parameters	Storage condition at					
	7th day		15th day		30th day	
	8ºC	40ºC	8ºC	40ºC	8ºC	40ºC
pH	6.0±0.11	6.13±0.057	6.11±0.11	6.21±0.01	6.15±0.05	6.20±0.04
Colour	Slightly brown	Slightly brown	Slightly brown	Slightly brown	Slightly brown	Slightly brown
Consistency	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid	Slight liquid
Centrifugation Test	Not separated	Not separated	Not separated	Not separated	Not separated	Not separated
Extrudibility	Good	Good	Good	Good	Good	Good

Spreadability

For spreadability study, wooden block and glass slide apparatus was used. The spreadability was determined as the function of time in seconds. A rich sample was placed between two slides and unvarying thickness is achieved by putting certain amount of weight to compress it. A 70 g weight was kept and time required for two slides to separate was recorded. Spreadability was calculated by formula given below:

$S = M.L / T$

M = weight kept on upper slide, L = glass slides length, T = time taken to separate the slides.

Extrudability

The prepared gel was filled in a squeezable tube. The gel extrudability was determined in grams and 0.5 cm long strip of polyherbal gel was extruded in 10sec.

Centrifugation Test

For centrifugation test, 10g of gel was added in a tapered test tube. In centrifugation, formulation was circulated for 15 min at 3000 rpm at room temperature.

Stability Study

The short-term stability (30 days) of control (base) and polyherbal gel was checked at 4±0.1 and 40±0.1°C temperature with 75% humidity to observe physical and chemical characteristics like pH, color, appearance, and centrifugation test [14]. Results are mentioned in Table **3**.

Antimicrobial Evaluation

Microbial Culture

Primary cultures of each organism were purchased from reliable source. The bacterial culture used was *Escherichia coli* (ATCC No. 8739), *Staphylococcus aureus* (ATCC No. 25923) and *Pseudomonas aeruginosa* (ATCC No. 9027) and the yeast *Candida albicans.*

Antimicrobial activity

Agar well method was used for antimicrobial activity [15]. For bacterial strains *E. coli*, *S. aureus* and *Ps. aeruginosa* Tryptic Soya agar was used while Sabouraud Dextrose Agar was used as media for the yeast *C. albican.* 0.1ml inoculums (10^6 CFU/ ml) of gram positive and negative bacteria and *C. albican* was thoroughly mixed under sterile condition with 20 ml molten sterile agar and poured into pre-sterilized petri plates. The petri plates left undisturbed for 30-40 min at 4ºC. After settling of media, the holes were made by sterile borer. Holes were filled with 0.1ml of all extracts separately (1mg/ml was prepared by dissolving the extract in DMSO). Chloramphenicol disc was used as standard. Plates having Gram-positive and negative bacteria incubated for 24 hours at 37°C. The plates containing C. albicans incubated for 48 hours at 28°C. The zones of inhibition were measured in millimeters and compared to standard.

Acute Dermal Irritation study

Dermal irritation study was performed according to OECD guideline 404 [16, 17]. White six healthy adult male rabbits with intact skin (1.5-2.0 kg) were carefully selected after a 7days acclimatization period to ensure their suitability for test. The animal's room temperature was 22±3°C with 30-70% relative humidity level followed by twelve hours light and dark cycle. The conventional laboratory diet with an unlimited drinking water was supplied to all animals. The animal's fur on dorsal area of trunk was carefully clipped to expose approximately 6 cm^2 of skin on day before test. 0.5g prepared gel was placed to test area of skin and covered with a gauze patch, with the help of smooth tape while 0.5g gel base was applied as control in the same manner. 4 hours later all samples were removed and skin was examined for any redness and swelling immediately and after 1, 24, 48 and 72 hours according to the skin reaction scoring system (Table **4**).

Table 4. Antimicrobial Activity of 5% herbal extracts and polyherbal gel (Zone in mm).

Microorganisms	Ethanolic extract			Polyherbal gel	Standard Chloramphenicol
	S. persica	***C. officinalis***	***A. indica***		
E. coli	25.16±0.28	28.16±0.28	28.83±0.28	30.4±0.52	31.83±0.28
S. aureus	28.83±0.28	28.33±0.57	29.33±0.57	30.20±0.20	32.06±0.11
Ps. auroginosa	25.13±0.23	27.66±0.50	27.33±0.57	28.40±0.52	30.83±0.76
C. albican	28.73±0.25	27.70±0.26	29.50±0.50	30.23±0.25	31.16±0.76

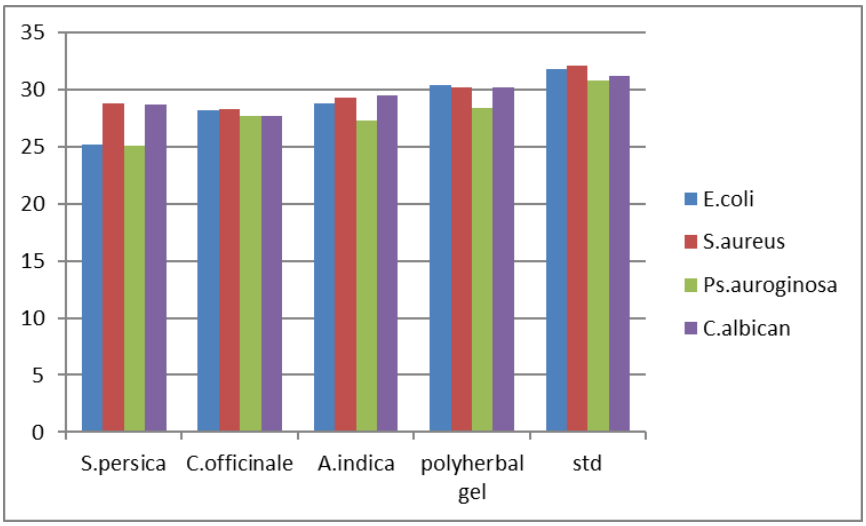

Figure 1. Antimicrobial Activity of 5% herbal extracts and polyherbal gel compare to standard.

RESULTS

Physical evaluations of formulation

The results of pH, spreadability, viscosity and other parameters of polyherbal gel containing *A. indica, S. persica* and *C. officinalis* extracts are shown in Table 2. It was found that the developed formula has a pH of 6.0± that is compatible with skin. In centrifugation test, the phase separation was not observed. The gel viscosities were $225x10^{3,}$ 92500 and 36533 at 0.3, 0.6 and 1.5 rpm respectively while the formulation's spreadability was also good and acceptable.

The prepared polyherbal gel and control base was investigated under different storage conditions to check its stability at 4°C and 40°C. The physical parameters such as color, appearance and odor investigated for the period of 1 month. The pH of formulated gel was found between 6.62- 7.08 (Table **3**). The prepared gel did not show any remarkable change in appearance, odor or color after 30 days. No phase separation was noticed in centrifugation test of base and prepared gel. There was no change in consistency, spreadability and extrudability in formulation and its base at 4°C and 40±1°C during 30 days study.

Antimicrobial Activity

The antimicrobial activity of the individual herbal extract and polyherbal gel formulation were performed against *E. coli*, *S. aureus*, *Ps. aeruginosa* and yeast *C. albican* (Table **4**, Fig **1**). The ethanolic extracts of *A. indica (leaves) S. persica (sticks)* and *C. officinalis (flowers)* exhibited excellent antimicrobial activity at 5% concentration. Maximum antimicrobial zone of inhibition 29.50±0.50 and 29.33±0.57 were observed by *A. indica* at 5% concentration against *C. albican* and *S. aureus* respectively. Significant antimicrobial activity was also exhibited by *C. officinalis* against all microorganisms in the range of 27.66±0.50 to 28.33±0.57 and maximum activity was achieved against *S. aureus*. *S. persica* also revealed excellent antimicrobial activity against *E. coli*, *S. aureus, Ps. auruginosa* and yeast *C. albican* in the range of 25.13±0.23 to 28.83±0.28 at 5% concentration. Maximum antibacterial activity exhibited by *S. persica* against *S. aureus*. Ethanolic extract of *A. indica* showed maximum activity against *C. albican (yeast)* while *S. persica* and *C. officinalis* extract showed maximum antibacterial activity against *S. aureus.* On the basis of these results a polyherbal gel is formulated by combining these extracts in equal ratio and then antimicrobial activity of formulated gel is again evaluated against microorganisms. As compared to antimicrobial activity exhibited by individual ethanolic extracts, poly herbal gel has greater antimicrobial activity against both gram-negative and gram-positive bacteria and *C. albican (*yeast). Polyherbal gel showed largest zone of inhibition 30.23±0.25 against *C. albican* and also showed significant antibacterial activity in the range of 28.40±0.52 to 30.20±0.20 against all other bacteria. This is may be due to synergistic effect of chemical constituents of herbal extracts. Chloramphenicol 10mg was used as a standard broad spectrum antibiotic. Antimicrobial zones exhibited by polyherbal gel were close to the standard zone of inhibition ranges from 30.83±0.76 to 32.06±0.11mm against all microorganisms.

Table 5. Standard skin reactions scoring system.

Reaction: Erythema	Score	Reaction: Edema	Score
No erythema	0	No edema	0
Very slight erythema	1	Very slight edema	1
Well defined erythema	2	Well defined edema (edges of the area well defined by defined raising)	2
Moderate to severe erythema	3	Moderate edema (raising approximately 1 mm	3
Severe erythema (beet redness) to eschar formation	4	Severe edema (raising more than 1 mm and extended beyond the area of exposure	4

Table 6. Dermal irritation reaction after removing patch.

Rabbit NO.	Polyherbal gel								Gel Base							
	60 min		24 hours		48hours		72hours		60 min		24 hours		48hours		72hours	
	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O
1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
6	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Erythema= E, Oedema=O,PDII=0.00

Acute Dermal Irritation Test

At 72 hours, no sign of erythema, edema or ulceration were observed in test and control sites of all animals (Table **6**). The primary irritation index score of poly-herbal gel and gel base were calculated using the formula given below. It was found to be 0.00 that is non irritant.

Primary Dermal Irritation Index (PDII)

$$= \frac{\text{sum of erythema / oedema}}{\text{No. of test sites x grading interval}}$$

After PDII, scoring was classified according to Draize method of classification (Table **5**).

DISCUSSION

To develop new therapeutic agents, plants are recognized as an essential source of potentially beneficial constituents with little or negligible after effects. In comparison to creams and ointment, topical application of gel enables the rapid absorption of drug directly at the site of action [18, 19]. These days gels are broadly acceptable as choice of vehicles for drug application topically. Herbal extracts with specific medicinal properties are incorporated into dosage forms as an active ingredient to gain extra benefits [20].

S. aureus, *E. coli, Ps. auruginosa, B. subtilis A. niger* and *candida albican* are reported for skin lesions [21, 22]. The antimicrobial properties and wound healing potential of *A. indica* [23, 24], *S. persica* [25, 26] and *C. oficinale* [27] have been investigated earlier on some pathogens separately. However, it is not easy to utilize it on skin surface in raw form. Therefore a poly herbal gel is formulated containing extracts of these plants.

Chemical constituents of *A. indica, S. persica* and *C. oficinale* are considered to be having antioxidant, anti-inflammatory, analgesic and antiseptic and wound healing properties [28]. Although herbal products contain natural preservatives but their quality must be maintained for ensuring the efficacy and safety of the gel. The Physico-chemical testing and stability studies are well-known methods for proving the efficacy of herbal products [13]. According to ICH guidelines short-term stability studies demonstrated that the polyherbal gel were stable at 8°C and 40°C with no change in pH, color, viscosity or spreadability. On the basis viscosity and spreadability results it can be conclude that polyherbal gel had good applicability. A literature search revealed that all extracts were individually known to have potential antimicrobial effects but no literature is available on antimicrobial activity of herbal based gel containing *A. indica, S. persica* and *C. oficinale.*

During skin irritation study, no signs of irritation, redness, and inflammation were observed. The gel prepared was smoothly spreadable and readily absorbed on the skin and was homogeneous without any lumps observed. This study clearly indicated that polyherbal gel formulation was very potent against pathogenic bacteria and safe for skin. The likely reason for its effectiveness is presence of active photochemical constituents that demonstrate antimicrobial activity and showed synergistic effect in this formulation.

CONCLUSION

Studies have shown that polyherbal gels prepared with ethanolic extracts of Azadirachta indica, Salvodora persica, and Calendula officinal at a concentration of 5% did not cause skin irritation after performing a patch test. Physical analysis, stability studies and antimicrobial activity of formulated polyherbal gel confirms its potency and effectiveness.

Therefore, this formulation is safe for use on human skin. Antimicrobial activity of polyherbal gel was found to be significant as broad spectrum antibiotic standard chloramphenicol. The effect of polyherbal gel is considered to be synergistic effect of all the plant constituents. Plant extract at a concentration of 5% showed significant antimicrobial activity of the formulation.

REFERENCES

1. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens, World Journal of Agricultural Science.2008; 4(5):839-843.
2. Pandey S, Seth A, Tiwari R, Singh S, Behl HM, Singh S. Development and evaluation of antimicrobial herbal cosmetic preparation. African Journal of Pharmacy and Pharmacology. 2014; 8(20): 514-51.
3. Pooja C, Kuppast IJ, Virupaksha JH, Ravi MC. A review on Sida acuta. International Journal of Universal Pharmacy and Bio Sciences.2015; 4(1): 36-48.
4. Vinoth B, Manivasagaperumal R, Rajaravindran M. Phytochemical analysis and antibacterial activity of Azadirachta indica a juss, International Journal of Research in Plant Sciences.2012; 2(3):50-55.
5. Biswas k, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). Current Science.2002;82(11):1336– 1345.
6. Arora D, Rani A, Sharma A .A review on phytochemistry and ethnopharmacological aspects of genus Calendula. Pharmacognosy Rev.2013; (7):179-87.
7. Martins FS, EC da Conceição, Bandeira ES, JO Silva Junior, Costa RM. The effects of extraction method on recovery rutin from Calendula officinalis L. (Asteraceae). Pharmacognosy Magazine .2014; 10:S569-73.
8. Butnariu M, Coradini CZ . Evaluation of Biologically Active Compounds from Calendula offi cinalis Flowers using Spectrophotometry. Chemistry Central Journal.2012; 6:35.
9. Halawany HS. A review on miswak (Salvadora persica) and its effect on various aspects of oral health," De Saudi Dental Journal.2012; 24(2):63–69.
10. Hassan S, Mohammed NAY, Leonard W. Ethnobotanical and antibacterial potential of Salvadora persica l: a well known medicinal plant in Arab and Unani system of medicine. Journal of Medicinal Plants Research.2011; 5(7): 1224-29.
11. Emira N, Mejdi S, Najla T, Hafedh H, Riadh K, Eulogio V, Amina B. Antibacterial, anticandidal and antioxidant activities of Salvadora persica and Juglans regia extracts. Journal of Medicinal Plants Research, 2011; 5(17): 4138-46.
12. Panda P, Ghosh A. Formulation and Evaluation of topical dosage form of Eupatorium odoratum linn. And their wound healing activity. The International Journal of Pharma and Bio Sciences. 2010; 2:1-10.
13. Bhinge SD, Bhutkar MA, Randive DS, Wadkar GH, Kamble SY, Kalel PD. Formulation and evaluation of polyherbal gel containing extracts of Azadirachta indica, adhatoda vasica, piper betle, Ocimum tenuiflorum and Pongamia pinnata. Marmara Pharmaceutical Journal . 2019;23(1):44–54.
14. Pandey S, Seth A, Tiwari R, Singh S, Behl HM, Singh S. Development and evaluation of antimicrobial herbal cosmetic preparation. African journal of pharmacy and pharmacology. 2014; 8(20): 514-518.
15. Habamu Y, Eguale T, Wubete A, Sori, T. In vitro antimicrobial activity of some selected Ethiopian medicinal plants against some bacteria of veterinary importance, African Journal of Microbiology Research.2010; 4(12): 1230-1234.
16. OECD test guidelines 404 acute dermal irritation and corrosion:2002 Paris,France
17. Gatne MM, Tambe K, Adarsh, Ravikanth K. Acute Dermal Irritation study of polyherbal gel Mastilep in rabbits. International journal of pharmaceutical sciences research 2015; 6(8):3473–3476.
18. Avinash S, Gowda DV, Suresh J, Aravind RAS, Srivastava A, Osmani RAM. Formulation and evaluation of topical gel using Eupatorium glandulosum michx. for wound healing activity. Der Pharmacia Lettre. 2016; 8(9): 255-266.
19. Patel H, Panchal MS, Shah S, Vadalia KR. Formulation and evaluation of transdermal gel of sildenafil citrate. International Journal of Pharmaceutical Research And Allied Sciences. 2012; 1(3): 103-118.
20. Kharwade RS, Mahajan NM. Formulation and Evaluation of Nanostructured Lipid Carriers Based Anti-Inflammatory Gel for Topical Drug Delivery System. Asian Journal of Pharmaceutical and clinical research. 2019;12(4):286–291
21. Giudice PD. Skin Infections caused by Staphylococcus aureus. A review article Acta Derm Venereol. 2020; 100:208-216.

22. Kumar CP, Chandra DS, Kumar DS. Antimicrobial activity of Ficus racemosa L. and Cissampelos pareira L. var. Hirsuta (DC) froman, against E. coli, P. aeruginosa, S. aureus and B. subtilis. International Research Journal of Pharmacy. 2012; 3(12): 151-153.
23. Nayak A, Nayak RN, Soumya GB, Kishore B, Mithun K. Evaluation of antibacterial and antimicrobial efficacy of aqueous and alcoholic extracts of neem (Azadiractha indica) an in vitro study. International Journal of Advanced Research and Publications. 2011; 2(1): 230-235.
24. Susmitha S, Vidyamol KK, Ranganayaki P, Vijayaragavan R. Phytochemical extraction and antimicrobial properties of Azadirachta indica (Neem). Global Journal of Pharmacology. 2013; 7(3): 316-320
25. Hassan S, Mohammed NAY, Leonard W. Ethnobotanical and antibacterial potential of Salvadora persica l: a well known medicinal plant in Arab and Unani system of medicine. Journal of Medicinal Plants Research. 2011; 5(7): 1224-29.
26. Emira N, Mejdi S, Najla T, Hafedh H, Riadh K, Eulogio V, Amina B. Antibacterial, anticandidal and antioxidant activities of Salvadora persica and Juglans regia extracts. Journal of Medicinal Plants Research. 2011; 5(17): 4138-46.
27. Roopashree, T.S. Raman Dang, R.H. Shobha Rani and C. Narendra, Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. International Journal of Applied Research in Natural Products.2008; 1(3): 20-28.
28. Imran H, Rehman A, Sohail T, Shaukat S,Khokher A.Wound healing potentialactivity of polyherbal ointment containing Salvadora persica, Azadirachta indica and Calendula officinalis Extracts :An experimental study. Pakistan Journal of Scientific and Industrial Research. 2022; 65B (1):55-61.