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ORIGINAL ARTICLE

Formulation and Evaluation of Herbal topical Antimicrobial gel

[1*]**Ujwal Havelikar,** [1]**Rohit Singh,** [2]**Akhilesh Patel,** [2]**Vipin Sharma,** [3]**Bhavya Jindal**
[1*,2]Department of Pharmacy, NIMS Institute of Pharmacy, NIMS University Jaipur, RJ, India-303121.
[2]DDR College of Pharmacy, Gurugram, Haryana, India-122504.
[1]Department of NPMC, CSIR Indian Institute of Integrative Medicine Jammu, (J&K) India- 180003.
[3]Department of Pharmacy Practice, NIMS Institute of Pharmacy, NIMS University Jaipur, (RJ), India-303121.

ABSTRACT
Large skin wound infections have high morbidity, which threaten the health of human beings severely. It is essential to develop new wound dressings that can block microbial invasion, eliminate bacteria effectively, adhere to wounds firmly, and have good biocompatibility. In this work, we designed a kind of Acacia auriculiformis dressings with derma-like structure that had good wound care performances. The extraction contents from Acacia auriculiformis, prepared extract from bark and leaves. The topical gel was prepared by solvent emulsification diffusion method and evaluated for Visual examination, pH determination, viscosity, spreadability, extrubility, Irritancy test, in vitro antibacterial studies using agar diffusion method, skin irritation studies and stability studies. Brown colored extract of Acacia auriculiformis bark and leaf was obtained. Brownish red colored dry powdered extract of Acacia auriculiformis was obtained. The topical gel batch was found propre physical properties, pH was found 6.95, viscosity was 7000 Cp, spreadability 2.66 (g/cm/sec) en 90 sec, homogenisity was found after appliying external normal force at 27 +20 C. In vitro antibacterial activity as seen from the zone of inhibition (48 + 0.4 mm). The final batch was stable for 2 months under the room temperature condition. Furthermore, the skin irritation study was performed with selected formulation against E. coli, were the results confirmed the significant antibacterial activity with no skin irritation. Prepared gel can be more therapeutically effective for extract of Acacia auriculiformis which can further be incorporated into topical gel for convenient application.
Keywords; Acacia, auriculiformis antibacterial activity, E. coli, antimicrobial activity

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INTRODUCTION

Antimicrobials are the natural or synthetic agents that hinder the growth of microorganisms or kills microorganisms.[1] The excess use of antibiotics leads to antimicrobial resistance.[2] As synthetic drugs have many disadvantages such as drug resistance and toxicity so, natural products or herbal drugs can be used to overcome the side effects. Plant extracts have gained much attention due to their antimicrobial activity as well as flavoring agent. [3] The efficacy of natural products can be determined by its chemical properties and concentration of the effective component present in it. Components having antimicrobial properties include thiosulfates, glucosinolates, flavonoids, organic acids, saponins, and phenolics.[4, 5] The effectiveness of herbal compounds has regained the interest of academicians and researchers for the fabrication of plant-based medicines as the plant products are without any side effects, no drug resistance, easily degradable, and ecofriendly.[6, 7] Herbal products have been used since ancient times in folk medicine for treatment of various disease condition.

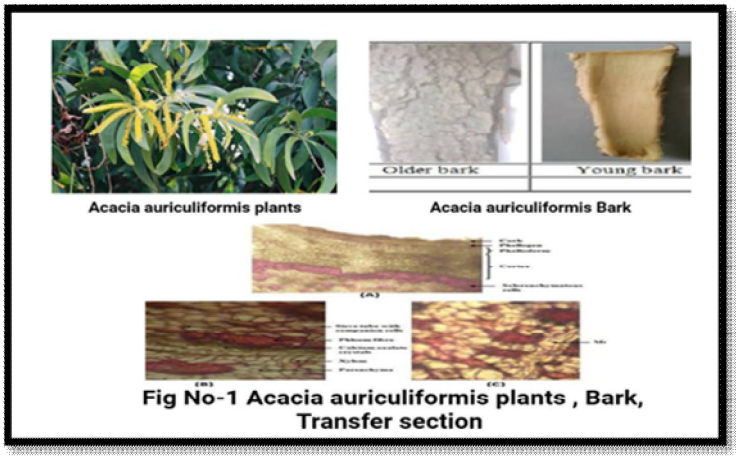

Fig 1: *Acacia auriculiformis* Plants, Bark, Transfer section

Acacia auriculiformis (Family: - Fabaceae and subfamily: Mimosoideae) is a potent medicinal plant. The extract of Acacia auriculiformis exhibits various pharmacological effects like antimalarial, anti-inflammatory, wound healing activity, hypoglycemic, Hepatoprotective, antioxidant, antibacterial and antimicrobial activities.[8]

Advantages of Herbal drugs [8]

1. High Low/Minimum cost
2. Complete accessibility
3. Enhanced tolerance
4. More protection
5. Fewer side-effects
6. Potency and efficiency is very high.

The *E. coli* are bacteria that are commonly found in human and animal digestive tract. Most of them live in symbiotic relationship with the human and animals and are part of essential *microflora* within the system. They are first discovered by Theoder *Escherish* also shown that certain bacterium statin is responsible for infant diarreha and gastroentitis.[9]

Acacia auriculiformis is an evergreen tree that grows between to 15–30 m tall, with a trunk up to 12 m long and 50 cm in diameter. The trunk is crooked and the bark vertically fissured.[10] Roots are shallow and spreading. Flowers are 8 cm long and in pairs, creamy yellow and sweet scented. Pods are about 6.5 x 1.5 cm, flat, cartilaginous, glaucous, transversely veined with undulate margins.[11] They are initially straight but on maturity become twisted with irregular spirals. Seeds are transversely held in the pod, broadly ovate to elliptical, about 4-6 x 3–4 mm. At Kozhikode (Kerala, India), flocks of jungle crow (Corvus macrorhynchos), grey-headed myna (*Sturnia malabarica*) and red whiskered bulbul (*Pycnonotus jocosus*) have been observed to feed on the seeds with the aril that are exposed when the pods are split. These birds also probably help in dispersal of seeds.[12]

The generic name acacia comes from the Greek word ‘akis’ meaning a point or a barb and the specific epithet comes from the Latin ‘auricula’- external ear of animals and ‘forma- form, figure or shape, in allusion to the shape of the pod. Extract of *A. auriculiformis* heartwood inhibit fungi that attack wood. Aqueous extracts of *A. auriculiformis* show developmental inhibitory effects on Bactrocera cucurbitae. [12].

Anatomy of skin

Skin is the largest organ in the body. It covers the body entire external surface, serving as a first-order barrier against pathogens, UV light and chemical and provides a mechanical barrier to in injury. It also regulates temperature and amount of water released into the environment. [13]

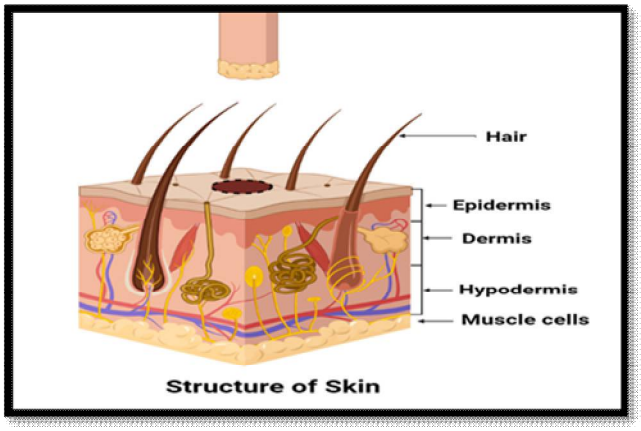

Fig 02: structure of skin

Thickness of skin

Hairless skin of the palms of the hands and soles of the feet is thick skin, referring to thickness of epidermis. The thickness skin based on the thickness of the dermis is on the upper portion of the back. But it is considered thin skin histologically because of epidermal thickness is shown in figure no 2. [13]

MATERIAL AND METHODS

Materials: -

The bark and leaf of A*cacia auriculiformis* were collected from local market of Wardha, Maharashtra. Carbopol-940, HPMC- E15LV Primium, Polyethylene glycol -600, methyl paraben, trimethylamine and Ascorbic acid was purchased from Loba Chemicals Pvt Ltd, Mumbai, India.

Table -1. Composition preparation of gels using antibacterial extract of Acacia *auriculiformis*.

Ingredients	F1 (%)	F2 (%)	F3 (%) Final Batch
Bark extract	0.5	1	1.5
Leaf extract	0.5	1	1.5
Carbapol- 940	0.5	1	1.5
HPMC	0.5	1	1.5
Methyl paraben	0.2	0.2	0.2
Ascorbic acid	1	1	1
Triethylamine	Q.S	Q.S	Q.S
Polyethylene glycol-600	4 ml	4 ml	4 ml
Amaranth colour	0.2	0.2	0.2
Water	Q.S	Q.S	Q.S

Methods: -

Extraction content from *Acacia auriculiformis*

Preparation of extract from bark

The fresh bark and leaves were collected from the local market of Wardha (Maharashtra). The bark was cleaned with water and was dried for a week and into fine powder in a mixer grinder. The powder was passed through 100 mesh sieve and stored in sealed polythene bags. About 2.5 mg of *A. auriculiformis* bark powder was mixed with 10 ml of ethanol in 100 ml round bottom flask attached with graham condenser and heated for 1 hr at 65 C. The condenser was cooled with circulating cilled water. After 1 hr of extraction the flask was cooled to room temp and the extract was filtered through whatmann 1 filter paper and the filtrate was collected. [14]

Preparation of extract from leaves: -

Fresh leaves of *Acacia auriculiformis* were air dried and then crushed by using mechanical blender to obtain a coarse powder. 5mg of the powder sample was used to investigate and established the antibacterial property. 2.5 mg of powder plant was macerated in 10 ml of ethanol for 72 hr at room temperature, and then filtered afterwards into a beaker using funnel and whatmann filter paper No.1 (125mm). The filtrate was concentrated by evaporation in water bath at a temperature of 50° C to obtain the crude extract. [14]

Procedure: -
Carbapol 940, HPMC (in 3 different concentrations) was dissolved slowly with stirring in 60ml of demineralized water for 1 h to avoid agglomeration. Then polyethylene glycol solution, methyl paraben, ascorbic acid, amaranth Color was added and mixed well. Then triethanolamine was added dropwise to adjust pH to 6.5 by stirring the solution until clear consistent gel was formed. Three different gel were prepared using the formulae given in table no. I and the viscosity was determined. Suitable gel base was selected by comparing the viscosity with marketed gel and extracts were added to make final formulation. [15]

Evaluation of prepared antibacterial topical gel: -

Physical appearance
The prepared gel formulae were inspected visually for their color, appearance, texture.

Determination of pH
It was evaluated using a digital pH meter. The pH of the gel was measured by dropping the glass electrode into the formulation. [16]

Viscosity Determination
The viscosity of the prepared gels was determined using Brookfield viscometer by using spindle no. 64 at 10 rpm and temperature of 25o C. [17]

Spreadability:
It was evaluated via using a glass slide and wooden block apparatus. The gel formulation (1 g) was kept on a pre-set glass slide and another movable glass slide was placed over the first glass slide and 50g weight was added to the slide for 5 minutes. The time used for the separation of slides was noted. [16] It can be measured by using the given 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

Extrudability
The gel formulation was filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weight of tubes was recorded and the tubes were placed between two glass slides and were clamped. 500gm was placed over the slides and then the cap was removed. The amount of extruded gel was collected and weighed. The percent of extruded gel calculated as 1. When it is greater than 90% then extrudability is excellent. [2]. When it is greater than 80% then extrudability is good. 3. When it is 70% then extrudability is fair. [18]

Determination of homogeneity:
The homogeneity of formulated gels was evaluated visually. The formulations were evaluated for appearance and existence of aggregates. [16]

Irritancy test
The gel was applied on left hand dorsal side surface of 1sq.cm and observed in equal intervals Upto 24hrs for irritancy, redness and edema.

In vitro antibacterial study of prepared gel [18, 19]

Preparation of agar plates
The agar plates were prepared by dissolving the agar into the water and were autoclaved for 15 minutes at 121o C. Then, the agar medium was allowed to cool at 40-45o C. 25 mL of molted agar was poured into the petri dishes. The agar plates were allowed to solidify under laminar airflow.

Preparation of inoculum
E. coli (ccmb strain- 1687) was used to estimate the antibacterial efficacy of the topical formulations containing *Acacia auriculiformis*. The subculture microorganisms were kept earlier so that the microorganisms must be in their log phase of growth and to ensure the validity of the results.

Inoculation of agar plates
The solidified agar plates were taken and the prepared inoculum was applied to it by streaking method. The plates were kept to dry for 5 min at room temperature.

Preparations of agar well diffusion assay
Agar well diffusion assay is done by above-dried plates. Wells were prepared by using a sterile corn borer by making holes on the inoculated agar plates. Each well was 5 mm in diameter. A weighed amount of the formulation final batch was placed into each well. The plate was incubated at 37o C for 72 hours and observed for inhibition zones. The area of the inhibition zones was measured by using a ruler to the nearest millimeter.[18, 19]

Stability studies

The stability of final topical gel batch was evaluated for 3 months, under accelerated conditions of temperature and humidity (i.e.40±2°C/75±5%RH) and at room temperature conditions. The formulations were tested for physical appearance and the drug content at intervals of 15, 30, 45, 60, and 90 days.[20]

RESULT AND DISCUSSION

Physical properties: -

The prepared topical gel was examined for various physicochemical parameters & results are depicted in Table 2.

Table 2: physicochemical characteristics of topical gel

Formulation code	Appearance	Colour	pH	Viscosity (CPS)	Spreadability (g cm/sec)	Homogeneity
Final batch	Viscous	Pink	6.95	7000	2.66	27±20C
Marketed	Viscous	Pink	6.8	7500	2.76	26 +20C

In vitro antibacterial studies

The in vitro antibacterial study was performed by measuring and comparing the diameter of the zone of inhibition (mm) for the prepared final formulation of gel. A weighted amount of gel was used as the control in the study. The antibacterial activity of *Acacia auriculiformis* against E. coli was significant and is seen in Fig no 03. Table no 3. The topical gel showed remarkable antibacterial activity as seen from the zone of inhibitions while showed a small zone of inhibition.

Table 3: Antibacterial activity of topical gel

BACTERIA	DIAMETER OF ZONE OF INHIBITION IN MM			
Escherichia-coli	Bark extract	leaves extract	Standard (ciprofloxacin)	Gel (bark leaves extract)
	7mm	15mm	22mm	20mm

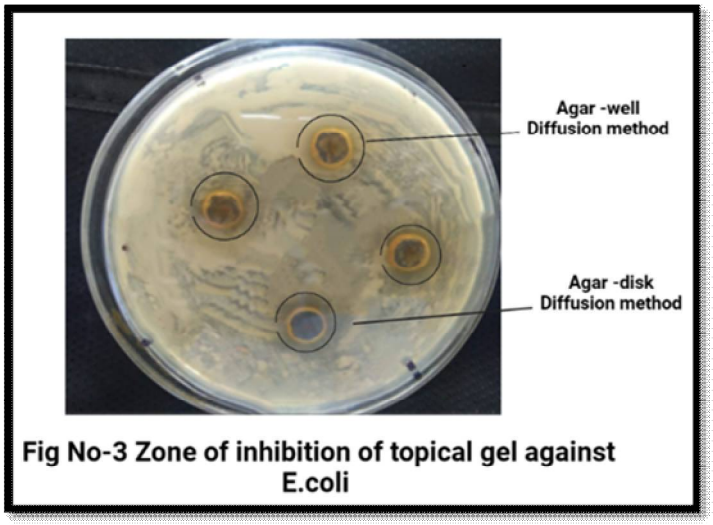

Fig No 3: Zone of inhibition of tropical gel against E. coli

Stability studies

The stability studies of topical gel formulation were carried out in the stability chamber (Remi SC-10 [plus]) under accelerated conditions of temperature (40±2o C) and relative humidity (75%±5) and room temperature conditions for three months. At accelerated conditions, topical gel (final) remained stable as observed by physical appearance (no viscosity change and color change) for 3 months.

CONCLUSION

Pharmaceutical studies can serve as a basis for proper identification, collection and investigation of the plant. The present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations. These parameters, which are being reported, could be useful in

the preparation of the herbal monograph for its proper identification. The present study suggests that the antibacterial activity of different parts of A*cacia auriculiformis* can be useful against various topical infections. The gel of A*cacia auriculiformis* bark and leave extract was evaluated for various properties. The gel containing leaf and bark extract was found to show better antibacterial property. Thus it can be concluded that herbal gel containing natural antibacterial agent can be the more popular source for prevention of topical bacterial infection.

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