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FORMULATION AND EVALUATION OF HERBAL ANTIBACTERIAL AND ANTIMICROBACTERIAL GEL

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ABSTRACT

The main goal of present research was to formulate and evaluate the antibacterial gel which was prepared from herbal plant. Herbal medicines has become a global important for both medical and economical. The antibacterial gel prepared from herbal plant are more efficacious than synthetic medicines and which shows negligible adverse effects. Although uses of herbal plants instead of systemic medicines which have ability to improve the quality, efficacy and safety of the gel. This study investigated the antimicrobial activity of "Acacia auriculiformis". The plant which are selected for the preparation of wound healing and shows antimicrobial, antibacterial, anti-inflammatory and antiseptic activity.

KEYWORDS:- Antibacterial gel formulation, Evaluation, Acacia auriculiformis, Anti bacterial activity, E. coli.

INTRODUCTION

Herbal products have been used science ancient times in folk medicine for treatment of various disease condition Acacia auriculiformis family – fabaceae and sub family – Mimosoideae is a potent medicinal plant.

The extract of Acacia auriculiformis exhibits various pharmacological effect like antimicrobial, antibacterial, anti-inflamatory, wound healing activity, hypoglycemic, hepatoprotective, antioxidant.

Advantages of herbal drugs[1-8]

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.

Disadvantage[1]

1. Not able to cure
2. Rapid sickness and accidents
3. Risk with self-dosing
4. Complexity in standardizations

The E.coil are bacteria that are commonly found in human and animal diagestive 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 stains is responsible for infant diarreha and gastroentitis.

Fig. 1: Acacia auriculiformis plant.

Fig. 2: Acacia auriculiformis bark.

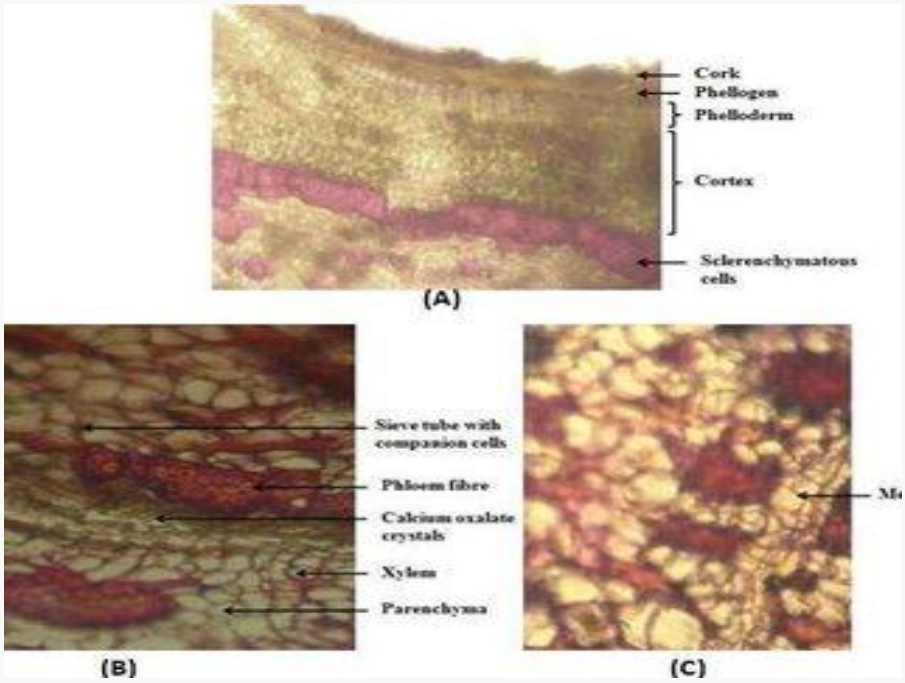

Fig. 3: Trans section of acacia auriculiformis.

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. 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. 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.

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. Aquous extracts of A. auriculiformis show developmental inhibitory effects on Bactrocera cucurbitae.

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.

Skin thickness

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.

Structure of skin

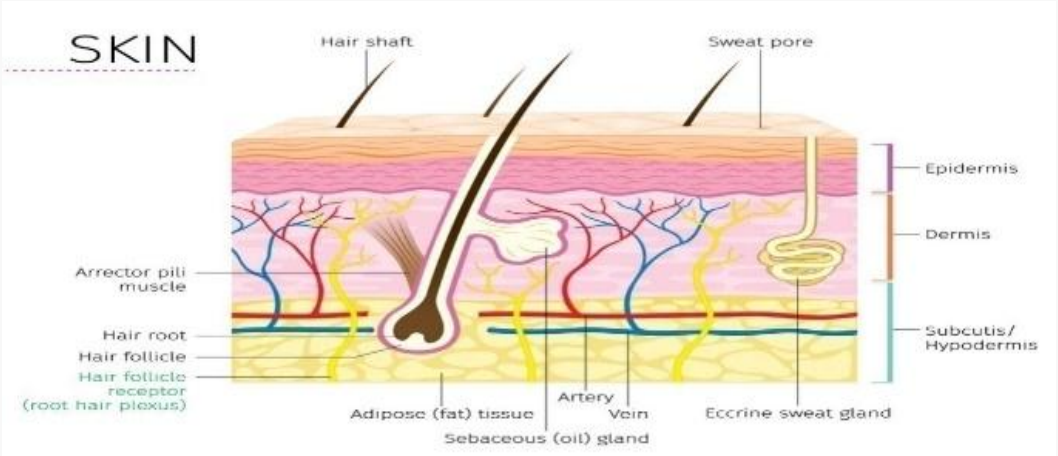

Fig. 4: Human anatomy of Skin and Layer.

Topical formulation

A topical medication is a medication that is applied to particular place on or on the body. Most often topical administration means application to body surface such as the skin or mucous membrane to treat ailment via a large range of classes including creams, foams gels, lotions and ointment. Many topical medication are percutaneous meaning that they are applied directly to the skin. Topical medication may also be inhalation, such as asthma medication, or applied to the surface of the tissue other than the skin ,such as eye drop applied to the conjunctiva, or ear drop placed in the ear, or medication applied to the surface of a tooth.

Objective

- To extract contents from Acacia auriculiformis leaf and bark
- To evaluate antibacterial activity of A*cacia auriculiformis bark* and leaf extract.
- To formulate topical gel using A*cacia auriculiformis* extract

Plan of work

To collect Acacia auriculiformis fresh leaves and bark

⇩

To prepare extract of leaves and bark of Acacia auriculiformis .

⇩

To evaluate antibacterial activity of leaf and bark extract.

⇩

To formulate suitable topical gel using A*cacia auriculiformis* extract.

⇩

To evaluate final formulation for various parameters.

MATERIALS AND METHODS

Collection of plant materials

The bark and leaf of A*cacia auriculiformis* were collected from college campus of Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra.

Chemicals

	Ingredient	**Manufacturer**
1	Carbopol – 940	Geni –chem.
2	HPMC – E15LVprimium	Loba chem.
3	Polyethylene glycol-600	MERCK

4	Methyl paraben	Geni chem.
5	Trimethylamine	Qualigens
6	Ascorbic acid	Oxford laboratory reagent
7	DMSO	Qualigens
8	Amaranth dye	BURGOYNE,BURBIDGES&CO
9.	Nutrient agar	Hi media
10	Ciprofloxacin	Gift sample from Zim Lab, Kalmeshwar.

Extraction of content from A*cacia auriculiformis.*

1.1 Preparation of extract from bark

The fresh bark and leaves were collected from the kamla Nehru college of pharmacy from Butibori, Nagpur. The bark were cleaned with water and were 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 chilled water. After 1 hr of extraction the flask was cooled to room temp and the extract was filtered through whatman 1 filter paper and the filtrate was collected.

1.2 *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 whatman 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.

2. Agar well diffusion method

- Sufficient quantity of nutrient agar was taken and water was added to make up the volume.
- The dispersion was heated to boiling and then transferred to boiling tubes and plugged with cotton.
- The tubes containing agar were sterilized in autoclave at 121°C.
- Then the solution was transferred to petri dish and allowed to cool and solidify.
- Bacterial dispersion was spread on the agar surface.

- The wells were bored and solutions of standard antibiotic and extracts were added to the wells.
- The Solutions were allowed to diffuse through agar and then incubated at 37^0C for 24 hrs and zone of inhibition were observed.

3. Preparation of gel

Carbopol 940, HPMC (in 3 different concentrations) was dissolved slowly with stirring in 60ml of demineralised water for 1 h to avoid agglomeration. Then polyethylene glycol solution, methyl paraben, ascorbic acid, amaranth colour were 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. 1 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.

Table no. 1: Formulation of gel base.

Formulation	**F1 (%)**	**F2 (%)**	**F3 (%)**
Carbopol -940	0.5	1	1.5
HPMC	0.5	1	1.5
Polyethylene glycol-600	4ml	4ml	4ml
Methyl paraben	0.2	0.2	0.2
Ascorbic acid	1	1	1
Triethylamine	Q.s	Q.S	Q.S
Amaranth colour	0.2	0.2	0.2
Water	Q.s	Q.S	Q.S

Table no. 2: Formulation of gels using antibacterial extract of acacia *auriculiformis*.

Formulation	F1 (%)	F2 (%)	F3 (%)
Bark extract	0.5	1	1.5
Leaf extract	0.5	1	1.5
Carbopol- 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

4. Evaluation of gel

4.1 Physical examination

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

4.2 Determination of pH

Weighed 50 gm of each gel formulation were transferred in 10 ml of the beaker and measured it by using the digital pH meter. pH of the topical gel formulation should be between 3–9to treat the skin infection.

4.3 Viscosity examination

The viscosity of gel was determined by using a Brookfield viscometer DVII model with a T-Bar spindle in combination with a helipath stand.

a) **Selection of spindle:** Spindle T 96 was used for the measurement of viscosity of all the gels.
b) **Sample container size:** The viscosity was measured using 50 gm of gel filled in a 100 ml beaker.
c) **Spindle immersion:** The T-bar spindle (T96) was lowered perpendicular in the centre taking care that spindle does not touch the bottom of the jar.
d) **Measurement of viscosity:** The T-bar spindle (T96) was used for determining the viscosity of the gels. The factors like temperature, pressure and sample size etc. Which affect the viscosity was maintained during the process. The torque reading was always greater than 10.

4.4 Spreadability test

In this method, slip and drag characteristic of gel involves. Formulated gel (2g) was placed on the ground slide under study. The formulated gel placed (sandwich like) between this slide and another glass slides for 5min to expel air and to provide a uniform film of the gel between slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80g with the help of string attached to the hook and the time (sec) required by the top slide to cover a distance of 7.5cm was noted. A short interval indicated better spreadability.

Formula was used to calculate spreadability.

$S=M \times L /T$

Where,

S= Spreadability

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

L= Length moved by the glass slide

4.5 Extrudability

The gel formulation were filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weight of tubes were 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.[8]

4.6 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.

4.7 Homogenicity

The developed gel were tested for homogeneity by visual inspection they were tested for their appearance with no lump.

5. Evalution of antibacterial properties of gel

- Agar disc diffusion method
- Antibacterial assay of gel was performed by agar well diffusion method. The procedure was same as that used for antibacterial extract.
- The following bacteria were used
- Escherichia coli (Gram -ve)
- The wells were filled with standard antibiotic, antibacterial extract solutions and a single well was filled with gel. The plates were incubated at 37 ℃ for 24 hrs and zone of inhibition was determined.

RESULTS AND DISCUSSIONS

1. **Prepration of extract:** Brown coloured extract of Acacia *auriculiformis* bark and leaf was obtained. Brownish red coloured dry powdered extract of Acacia *auriculiformis* was obtained.
2. **Antibacterial activity of extract:** The antibacterial activity of bark and leaves extract of Acacia *auriculiformis* determine using agar well diffusion method. The result were obtained as follows.

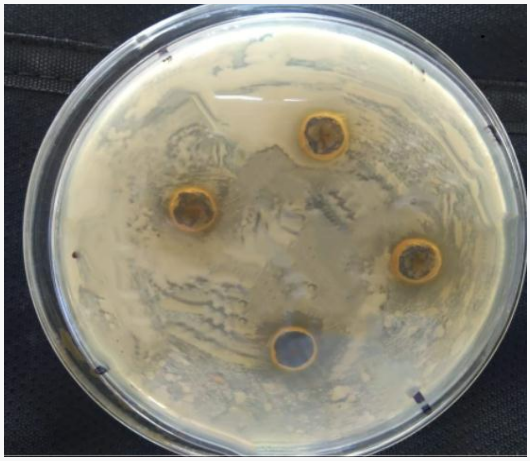

Figure 5: Result of antibacterial activity of acacia *auriculiformis* leaves.

Plates showing the results of antibacterial activity of extract of A*cacia auriculiformis bark and* leaves against the test Gram negative bacteria *Escherichia coli* on Nutrient Agar plates using Agar well diffusion method with Ciproflaxacin solution (10µg) in one well which served as a positive control.

In this in vitro study it was revealed that the extract at different concentrations exhibited antibacterial activity against the bacterial strain tested. The ethanolic leaf and bark extract of Acacia *auriculiformis* exhibited a high degree of activity against the organism tested.

3. Formulation of gel using various formulae

Carbopol and HPMC gel were prepared by using various contents and the consistency was determined. suitable gel was then selected and the formula was used for formulation of final gel containing extract. The viscosity result of gel are as follows.

Formulation	Viscosity(cp)
F1	2700cp
F2	8250cp
F3	15000cp
Marketed gel	7500cp

The formula F2 containing 1% carbopol and 1% HPMC was found to show suitable consistency when compared with marketed gel.

4. Herbal antibacterial gel was then prepared and was evaluated for various evaluation parameters. The result are as follows.

4.1 Physical properties

Fig. 6: Final herbal gel.

Table no. 4: Physical properties.

Sr. no.	Properties	Observation
1	Colour	Pink
2	Odour	Characteristics fruity
3	Appearance	Viscous
4	Texture	Smooth

4.2. pH of gel formulation

Fig. 7: Final herbal gel pH measurement.

Table no. 5: pH of gel.

Formulation	Ph
Final gel	7.04

4.3 Viscosity

Table no. 5: Viscosity of gel formulation.

Formulation	Viscosity
Final gel	7000cp

4.4 Spredabilty study

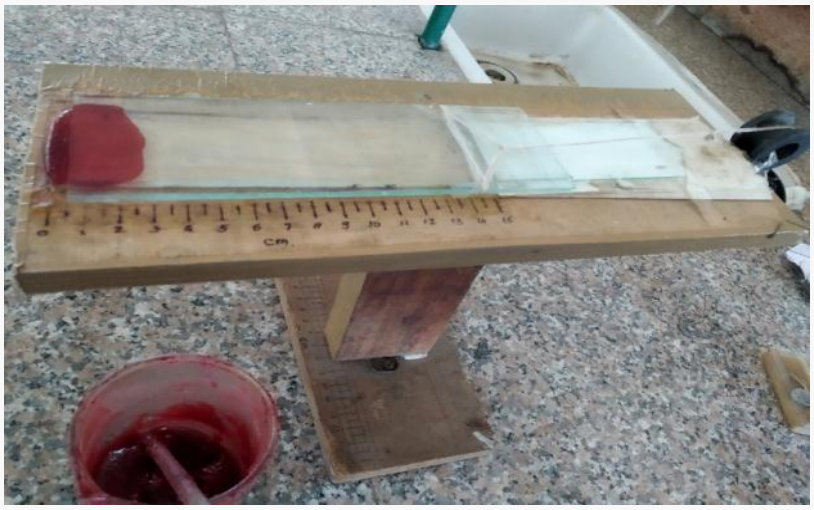

Fig. 8: spreadability measurement.

Formulation	Time (sec)	Spredability(g/cm /sec)
Final gel	90	2.66

4.5 Homogenicity

Containe by applying normal force at 27+20C. In addition, bulk of content shall extrude from the crimp of container and then rolled it gradually.

5. Antimicrobial activity of gel

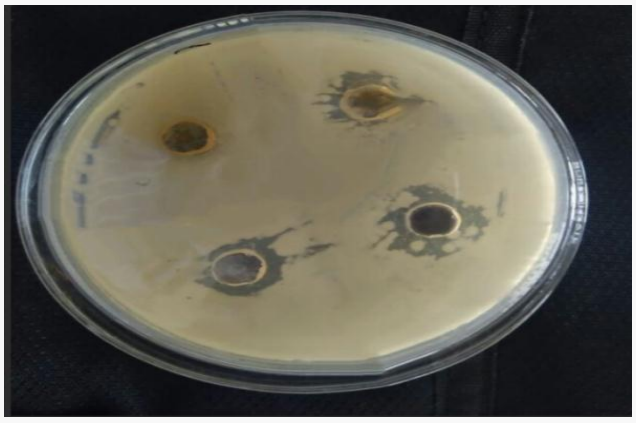

Fig. 9: Result of antibacterial activity of acacia *auriculiformis* gel containing bark and leaves extract.

Nurtrient agar plates using agar well diffusion method with ciprofloxacin solution (10µg) at the one well which served as a positive control. Zones of inhibition was measured for all the contents and activity was compared.

Zone of inhibition

Bacteria	**Diameter of zone of inhibition in mm**			
Escherichia-coli	**Bark extract**	**leaves extract**	**Standard (ciprofloxacine)**	**Gel (bark + leaves extract)**
	7mm	15mm	22mm	20mm

From the above observation is conclude that amongst the tested bacteria, the ethanolic extract of A*cacia auriculiformis* leaf and bark processes antibacterial activity against Escherichia. coil. while the ethanolic extract could be used to control infection caused by e.coil which supports the traditional use of the leaf of this plant.

In this antibacterial activity of A*cacia auriculiformis bark* and levaes extract was added in agar diffusion plate separately and incubate for 24 hr after they measured the zone of inhibition and the result was leaves activity are show more activity than bark extract.

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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