RESEARCH ARTICLE

Formulation and Evaluation of Antimicrobial Ointment from *Acacia auriculiformis* Leaves Extract

Umesh B. Telrandhe[1], Laxman G. Galat[2], Shailesha D. Chavan[2], Manish A. Kamble[3], Mahendra C. Gunde[3], Rajeshwar V. Khirsagar[2*]

[1]*Department of Pharmacognosy, Datta Meghe College of Pharmacy, DMIHER, Wardha, Maharashtra, India*
[2]*Department of Pharmaceutics, School of Pharmacy S.R.T.M.University, Nanded Maharashtra, India*
[3]*Department of Pharmacognosy, Kamla Nehru College of Pharmacy, Nagpur Maharashtra, India*

Received: 29th April, 2023; Revised: 10th June, 2023; Accepted: 21st June, 2023; Available Online: 25th June, 2023

ABSTRACT

The current experiment aims to create and test an ointment with an ethanolic extract from *Acacia auriculiformis* for antimicrobial action. The soxhalation procedure was used to create the ethanolic extract. After producing the ointment base, five batches of ointments were created by incorporating the extract into the base. Physical and chemical properties of each formulation, including color, flavor, pH, spreadability, consistency, solubility, and washability, were assessed. The formulation's stability at different temperature conditions was also assessed, and the results show that neither its irritancy nor its spreadability has changed. F5 was discovered to be the ideal formulation among the ointment batches. It has a larger zone of inhibition over both gram-positive and gram-negative bacteria.

Keywords: Ointment, *Acacia auriculiformis,* Parrafin wax, Antimicrobial.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.2.30

How to cite this article: Telrandhe UB, Galat LG, Chavan SD, Kamble MA, Gunde MC, Khirsagar RV. Formulation and Evaluation of Antimicrobial Ointment from *Acacia auriculiformis* Leaves Extract. International Journal of Drug Delivery Technology. 2023;13(2):665-669.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The development of human history demonstrates that traditional medicine is used for therapeutic purposes. According to recent statistics from the World Health Organization, 70 to 80% of the population relies mostly on animal and plant-based remedies because of inadequate or no access to medical care.[1] In addition to being utilized as traditional medicines, substances derived from wild plants and animals are also used as initial materials in the creation of contemporary allopathic and herbal therapies.[2] A straight, medium-sized, deciduous, or evergreen tree that can reach a height of 30 meters, *Acacia auriculiformis* is frequently seen in parks and by the sides of Indian roads. The word "*Acacia*" in its general form comes from the Greek word "akis," which signifies a point or a spike. In contrast, the Latin word "auricula" denotes a creature's external ear, and the word "forma" denotes a frame, figure, or shape. The Australian-born tree was initially planted to India in West Bengal in 1946. The tree contains large amounts of arabinose, rhamnose, galactose, glucuronic acid, and methyl glucuronic acid.[3] The plant has reportedly demonstrated a range of pharmacological properties, including antioxidant & anti-inflammatory[4], antibacterial & antifungal,[5] antimalarial,[6] antifilarial,[7] cestocidal,[8] antimutagenic & chemopreventive, [9] spermicidal,[10] hepatoprotective,[11] wound-healing,[12] and antidiabetic action.[13] Auriculoside, a flavan glycoside isolated from *A. auriculiformis*, was found to have central nervous system depressive action.[14] A useful tool for establishing plant quality control parameters is pharmacognostic standardization. The global adoption of herbal products in the contemporary medical system depends on standards and quality monitoring of plants.[15] As a result, every country has established a set of guidelines for maintaining the quality of herbal medicine. The current investigation's goal was to establish quality control standards for *A. auriculiformis* standardization.[16]

Topical Drug Delivery

Table 1 shows Conventional topical dosage forms

Table 1: Conventional topical dosage forms[17]

Solid	Dusting powders, Poultices, Plasters etc.
Liquid	Lotion, Solution, Suspension, Colloidions etc.
Semisolid	Ointments, Creams, Pastes, Gels etc.
Miscellaneous	NDDS, Transdermal delivery systems, Rubbing alcohol, Gauzes etc.

**Author for Correspondence: rajksagar53@gmail.com*

Characteristics of an Ideal Ointment[18]

- It should be stable both chemically and physically.
- The ointment's base shouldn't have any medicinal properties.
- The active component in the ointment base should be evenly dispersed after being finely split.
- The cream should be smooth and without grain.

Plant Profile

Botanical Name: *Acacia auriculiformis* Benth.
Synonyms: *A. moniliformis* Griseb.
Common Name: Australian Baval, Australian Acacia, Ear leaf Acacia Plant.
Family: Fabaceae

Geographical Description

A. auriculiformis is a fast-growing, crooked, gnarly tree in the family Fabaceae. It is also known as auri, ear leaf acacia, Papuan wattle, tan wattle, and akashmoni in Bengali. It is indigenous to Papua New Guinea, Indonesia, and Australia. It can reach a height of 30 metres (98 feet).[19]

MATERIALS AND METHOD

All additional ingredients, including butylated hydroxyanisole, methylparaben, and citric acid, were obtained from Research Lab Fine Chemical Ind. Mumbai. White bees wax, hard paraffin, cetosteryl alcohol, and paraffin wax were purchased from SD Fine Chemcal Limited (Table 2).

Collection of Plant Material

A. auriculiformis plant specimens were harvested in sacks from a field near Butibori, Nagpur District, Maharashtra State, India. It was recognised and authenticated by R.T.M.N. University Nagpur's department of botany [M.S.].

Method extraction process

1. The filter paper bag with the finely curled leaves inside was put inside a soxhlet apparatus containing ethanol.
2. Heat was applied to the extraction solvent in the flask, causing the vapors to condense.
3. The filter paper bag containing the powdered leaves received drips of the condensed extract.
4. The liquid in the chamber syphon was collected into a flask when the level reached the top of the syphon tube.
5. The syphon tube was drained after doing this operation repeatedly. Crude extract was obtained in the form of a gummy or semisolid substance after the collected extract was entirely evaporated by rotary evaporator.[13]

Formulation of an ointment

Table 2: Preparation of an ointment base[18]

Sr. No.	*Ingredient's Name*	*Quantity (gm)*
1.	White bees wax	0.5
2.	Hard parrafin	0.5
3.	Cetostearyl alcohol	0.5
4.	Parrafin wax	8.5

Table 3: Herbal ointment formulation

Batch	*A. auriculiformis* extract (gm)	Ointment base (gm)
F1	0.3	10
F2	0.6	10
F3	0.9	10
F4	1.2	10
F5	1.5	10

Procedure for formulation of an ointment

- First, weigh the grated hard paraffin that will be placed in an evaporating dish on a water bath to prepare the ointment foundation. The remaining ingredients should be added and carefully stirred to assist the mixture melting and integrating uniformly after the hard paraffin has melted. The ointment base should now cool.
- A smooth paste that is two or three times the weight of the base is made by mixing an exact amount of *A. auriculiformis* extract with the ointment base. An additional base is then gradually added until the ointment is homogeneous and is then transferred to a suitable container (Table 3).[20]

RESULT AND DISCUSSION

Physicochemical Characteristic

Color - Pale Green
Odor - Characteristics
Consistency- Smooth and no signs of greed were seen.

pH

A Total of 25 mL of water was used to dissolve 2 g of ointment. A digital pH meter was used to calculate the solution's pH value. Before usage, the pH meter was calibrated. The pH was determined while the electrodes were submerged in the fluid. The formulation's pH should match the pH of the skin. The produced ointment's pH value was within the allowed range of 5–7.

Skin Irritation Test

One of the most crucial factors in the evaluation of a topical formulation is the skin irritancy test. On the skin of the hand, each composition was tested by self-application. A 2 cm area was designated, and ointment was administered to the skin every 6 hours for 24 hours while looking for any signs of allergy.

Viscosity

The viscosities of all formulations were measured, and it was found that they varied between 2314.61 and 2851.93 cps at 5 rpm. The pseudo-plastic flow was visible in every mixture. The average of the three data was used to estimate the standard deviation.

Spreadability

After screening, it was discovered that the spreadability of an ointment was inversely proportional to the amount of hard paraffin, falling into three categories: low, moderate, and high. The ointment thickened and lost some of its spreadability as the

amount of hard paraffin rose. All formulations' spreadabilities were assessed, and it was found that formulation F5 spreads more easily than both other formulations and prototype formulations. The formula used to calculate spreadability was as follows.

$$\text{Spreadability (S)} = MxL/T$$

Where
S - Spreadability
M- Adding weight to the upper slide
L-Length of glass slide
T- Duration required for separating the slides

Solubility

Soluble in methanol, ethanol.

Washability

The ease of water washing was evaluated after applying the formulation to the skin.

Non-irritancy Test

Human skin was treated with a prepared herbal ointment, and the results were tracked.

Phytochemical Characterization of Leaves Extract

Chemical tests were carried out on the ethanolic extract to qualitatively determine phytochemical constituents (Table 4).

FTIR Study

IR study of pure extract was carried out in fourier transform infrared spectroscopy (Shimadzu). About 1-mg of extract was dispersed in KBr powder and kept in the sample holder and FTIR spectra were obtained by powder diffuse reflectance on FTIR spectrophotometer. It showed characteristic peaks at 3741.90, peak of O-H stretching (alkane) at 2918.30 and 2850.79, peak of CHO (aldehyde) stretching at 1375.25, peak of C-O stretching at 1165.00, peak of alkane at 723.31 (Figure 1 and Table 5).

FTIR studied the compatibility of extract with excipients (sucrose, HPMC, citric acid, calcium carbonate,). When compared to the characteristic peak values of pure extract, the FTIR spectra of all excipients with pure extract, displays the same characteristic peaks as pure and a small shift in peak values. The IR spectra of the entire sample showed the prominent characterizing peak of pure extract which confirmed that no chemical modification of the drug had been take place (Figure 2 and Table 6).

Table 4: Results of phytochemical characterization

Sr. No.	*Class of compound*	*Tests performed*	*Result*
1	Alkaloids	Meyer's Test	+ve
2	Flavonoids	Shinoda Test	+ve
3	Carbohydrates	Molish's Test	+ve
4	Tannins	Ferric Chloride Test	+ve
5	Saponins	Foam Test	-ve
6	Proteins and amino acids	Ninhydrin Test	-ve

Phytochemical Analysis: Present =(+Ve), Absent=(-Ve), results Of this study clearly indicate the presence of alkaloids, tannins, flavonoids, carbohydrates, in these extracts. But saponins, proteins and amino acids are absent.

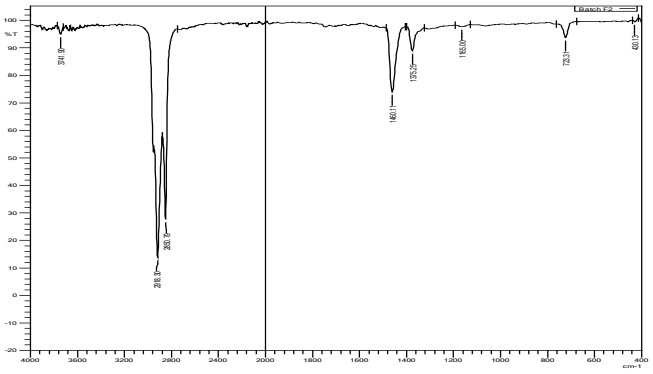

Figure 1: FTIR Spectra of ointment

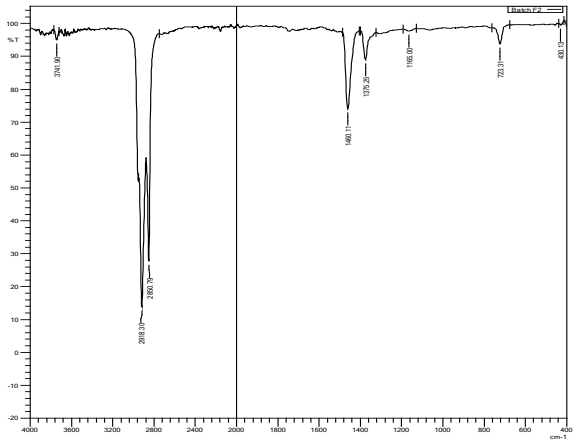

Figure 2: FTIR Spectrum of Extract

Table 5: FTIR analysis data of ointment

No.	*Peak*	*Intensity*	*Corr. Intensity*	*Base (H)*	*Base (L)*	*Area*	*Corr. area*
1	430.13	99.54	0.85	439.77	412.77	-0.171	13.494
2	723.31	93.73	5.39	761.88	677.01	193.099	122.136
3	1165	97.72	0.62	1192.01	1128.36	124.291	19.62
4	1375.25	89.01	8.58	1400.32	1325.1	397.649	211.01
5	1460.11	74.02	23.48	1485.19	1406.11	875.829	683.428
6	2850.79	27.77	38.3	2877.79	2748.56	2946.273	30.405
7	2918.3	13.95	41.15	2947.23	2877.79	4446.663	1356.93
8	3741.9	94.93	2.89	3770.84	3720.69	178.021	70.91

Table 6: FTIR analysis data of extract

No.	*Peak*	*Intensity*	*Corr. intensity*	*Base (H)*	*Base (L)*	*Area*	*Corr. area*
1	390.13	97.57	0.85	439.77	412.77	-0.171	13.494
2	700.31	90.70	5.39	761.88	677.01	193.099	122.136
3	1122	93.77	0.62	1192.01	1128.36	124.291	19.62
4	1298.25	87.01	8.58	1400.32	1325.1	397.649	211.01
5	2848.11	24.02	23.48	1485.19	1406.11	875.829	683.428
6	3701.79	92.77	38.3	2877.79	2748.56	2946.273	30.405

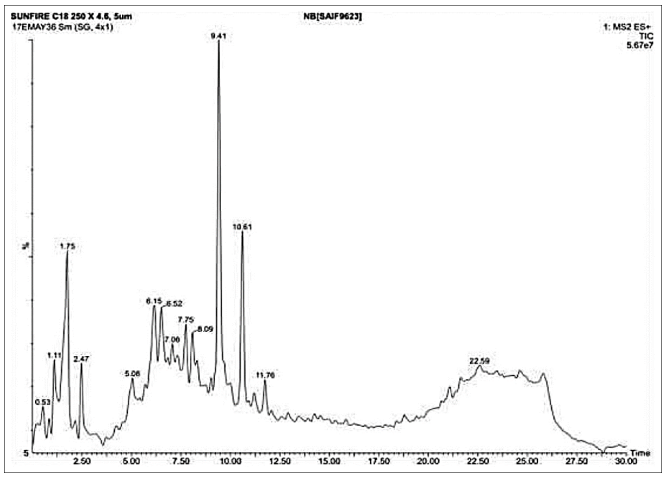

Figure 3: LC-MS/MS profile *A. auriculiformis* leaves extract

LC-MS/MS Analysis

The Waters ACQUITY FTN, Waters Alliance 2695 HPLC pump, with auto-sampler, quaternary pump system, and PDA detector type UPLC LG 500 nm, was used to carry out the LC-MS/MS analysis. For mass spectroscopy, the ESI detector's positive ionisation mode was utilised. With parameters of 250 mm x 4.6 mm x 5 pm, 3D channel range (200–450 nm), resolution (1.2 nm), and compensating reference 2D parameter range (310–410 nm), the reverse phase C18 column (SUNFIRE) was employed. The mobile phase used for the LC-MS/MS experiment was acetonitrile: formic acid in water (5:95), with the effective gradient running for 20 minutes at a flow rate of 1.5 mL/min (Figure 3).

The bioactive ethanol fraction was transferred to LC-MS/MS with an efficient gradient run to identify the bioactive chemicals present. Chemical (P-sitosterol) fragmentation pattern over retention duration of 9.414 minutes. B-sitosterol's previously reported molecular weight (413 amu) and the molecular ion peak 414 [M+H+ in the MS data] are similar. At 413 (m/z7, the base peak was discovered to be. The fragment peaks that are produced have the (m/z) 396 form.

Antibacterial Activity Against Different Bacteria

The bacterial cultures were utilized to make the inoculum for the microorganisms. 15 ml of nutritious agar (Hi media) medium was applied to clean, sterile petri plates in order for them to freeze and solidify. 100 mL of the bacterial strain's broth was distributed uniformly on the medium using a spreading stick until it had completely dried. 6 mm diameter

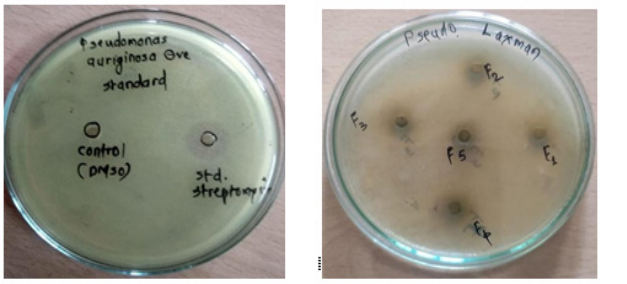

Figure 4: Zone of inhibition against *Pseudomonas aeuroginosa*

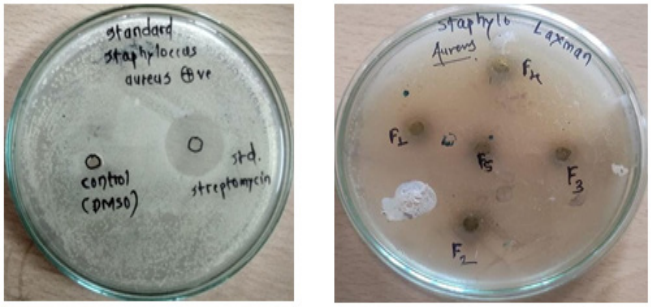

Figure 5: Zone of inhibition against *Staphylococcus aureus*

Table 7: Zones of Inhibition against *Pseudomonas aeuroginosa* & *Staphylococcus aureus*

Sr. No.	*Samples*	*Conc. (µL/mL)*	*Zone in diameter (mm) against Pseudomonas aeuroginosa*	*Zone in diameter (mm) against Staphylococcus aureus*
1	Control	50	00	00
2	Standard	50	18	20
3	F1	50	11	15
4	F2	50	11	16
5	F3	50	12	16
6	F4	50	14	17
7	F5	50	15	18

wells were drilled using a sterile cork borer. 50 mL was poured into each well. 24 hours at 37°C were spent incubating the petri plates. Streptomycin (1-mg/mL) was made as a positive control. The unfavorable control was DMSO. The widths of the zone of inhibition measured the antibacterial activity.

While all of the compounds examined for the semisolid ointments F1 to F5 have good antibacterial action against both gram + ve and gram -ve bacteria, Batch F5 exhibits more zone inhibition than the other batches. Zone of inhibition against

P. aeuroginosa is shows in Figure 4 and *S. aureus* in shows in Figure 5 and Table 7.

Stability Study

In accordance with ICH guidelines, a stability study was conducted on the batch F5 ointment formulation. For a period of one month, there were no noticeable variations in the optimized formulation's pH, spreadability, or viscosity.

Physicochemical Parameters

Table 8 shows Results of physicochemical characterization

Table 8: Results of physicochemical characterization

Physicochemical Parameter	Observation
Color	Pale green
Odor	Characteristics
Consistency	Smooth
pH	5–7
Spreadability (Seconds)	5 Seconds
Solubility	Soluble in alcohol
Washing ability	Good
Irritancy	Non irritant
Stability Study	Stable At 2, 25, 35°C

CONCLUSION

The formulation and evaluation of the herbal ointment were the aims of the current investigation. The soxhlet extraction method was used to create the herbal extracts to obtain a high yield without harming the chemical components or their activity. The levigation procedure was used to create the ointment, which made sure that the herbal extract and ointment base were blended evenly and stayed stable throughout storage. After looking into the physicochemical properties, it was determined that the spreadability, washability, solubility, and other characteristics are suitable. The formulation was also held for a four-week stability investigation at temperatures as low as 2°C and as high as 35°C. It shows a broader zone of inhibition against both gram-positive and gram negative bacteria.

REFERENCES

1. Atanasov AG, Zotchev SB, Dirsch VM, et al. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov*. 2021;20(3):200-216. doi:10.1038/s41573-020-00114-z
2. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. *Molecules*. 2016;21(5). doi:10.3390/molecules21050559
3. Chester K, Zahiruddin S, Ahmad A, Khan W, Paliwal S, Ahmad S. Bioautography-based Identification of Antioxidant Metabolites of Solanum nigrum L. and Exploration Its Hepatoprotective Potential agChester, K. et al. (2017) 'Bioautography-based Identification of Antioxidant Metabolites of Solanum nigrum L. and Explorati. *Pharmacogn Mag*. 2017;13 (Suppl(62):179-188. doi:10.4103/pm.pm
4. Rangra NK, Samanta S, Pradhan KK. In vivo antiinflammatory potential of leaf extracts of *Acacia auriculiformis* benth. *Indian J Pharm Sci*. 2019;81(4):709-719. doi:10.36468/pharmaceutical-sciences.562
5. Rao AS, Shobha KL, Shetty MS, Pai K SR. In vitro antibacterial and antifungal activities of aqueous and ethanolic leaf extracts of *Acacia auriculiformis*. *Asian J Pharm Clin Res*. 2018;11(12):480-482. doi:10.22159/ajpcr.2018.v11i12.28853
6. Okokon JE. International Journal of Drug Development & Research Available online http://www.ijddr.in Covered in Official Product of Elsevier , The Netherlands. 2014;(August 2010).
7. Road RSCM. activity~ 1. Published online 1996:173-184.
8. Ghosh NK, Sinha Babu SP, Sukul NC, Ito A. Cestocidal activity of *Acacia auriculiformis*. *J Helminthol*. 1996;70(2):171-172. doi:10.1017/s0022149x00015340
9. Kaur K, Arora S, Hawthorne ME, Kaur S, Kumar S, Mehta RG. A correlative study on antimutagenic and chemopreventive activity of *Acacia auriculiformis* A. Cunn. and Acacia nilotica (L.) willd. ex del. *Drug Chem Toxicol*. 2002;25(1):39-64. doi:10.1081/DCT-100108471
10. Pal D, Chakraborty P, Ray HN, Pal BC, Mitra D, Kabir SN. Acaciaside-B-enriched fraction of *Acacia auriculiformis* is a prospective spermicide with no mutagenic property. *Reproduction*. 2009;138(3):453-462. doi:10.1530/REP-09-0034
11. Sharma N, Singh S, Singh SK. Review on Phytopharmacological Properties of *Acacia auriculiformis* A. Cunn. ex. Benth. *Inven Rapid Planta Act*. 2016;2016(1):1-6.
12. Singh S, Sharma N. Evaluation of wound healing activity of *Acacia auriculiformis* A. Cunn. stem bark. *Asian J Pharm Clin Res*. 2014;7(2):204-207.
13. Sharma D, Verma S, Kumar S, et al. Hydroethanolic leaf extract of *Acacia auriculiformis* exhibited antidiabetic and antioxidant activities. *Egypt J Basic Appl Sci*. 2022;9(1):372-382. doi:10.1080/2314808X.2022.2100674
14. Ahmadu AA, Lawal BA, Haruna A, Mustapha L. Tetrahydroxy Flavone from *Acacia auriculiformis* A. Cunn ex benth. (Fabaceae) with novel kinase activity. *Pharmacogn J*. 2019;11(3):559-563. doi:10.5530/pj.2019.11.89
15. Muyumba NW, Mutombo SC, Sheridan H, Nachtergael A, Duez P. Quality control of herbal drugs and preparations: The methods of analysis, their relevance and applications. *Talanta Open*. 2021;4:100070. doi:10.1016/j.talo.2021.100070
16. Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products: a review. *Futur J Pharm Sci*. 2020;6(1). doi:10.1186/s43094-020-00091-5
17. Rapalli VK, Singhvi G. Dermato-pharmacokinetic: assessment tools for topically applied dosage forms. *Expert Opin Drug Deliv*. 2021;18(4):423-426. doi:10.1080/17425247.2021.1856071
18. Maru AD, Lahoti SR. Formulation and Evaluation of Moisturizing Cream Containing Sunflower Wax. *Int J Pharm Pharm Sci*. 2018;10(11):54. doi:10.22159/ijpps.2018v10i11.28645
19. Names L, Description B. *Acacia auriculiformis* Fabaceae-Mimosoideae A. Cunn. ex Benth. Australian wattle. 2009;0:1-6.
20. Nalla A, Chinnala KM. Formulation and Evaluation of Herbal Ointment for. *Arav al World J Pharm Med Res*. 2017;3:113-117.