

FORMULATION AND EVALUATION OF ANTIMICROBIAL HERBAL OINTMENT CONTAINING ETHANOLIC EXTRACT OF *JATROPHA* *GOSSYPIIFOLIA* (EUPHORBIACEAE)

Gowri A.*¹, Anitha P.², Abinaya B.², Esai Snekan M.², Harini M.², Nikson N.², Ponmari S.²,
Gnanaprakash R.²

¹M. Pharm., Associate Professor, Department of Pharmaceutical Chemistry, St. Mariam College of Pharmacy, Pudur,
Tirunelveli – 627851, Tamil Nadu, India.

²St. Mariam College of Pharmacy, Pudur, Tirunelveli – 627851, Tamil Nadu, India.

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***Corresponding Author: Gowri A.**

M. Pharm., Associate Professor, Department of Pharmaceutical Chemistry, St. Mariam College of Pharmacy, Pudur, Tirunelveli – 627851,
Tamil Nadu, India. DOI: <https://doi.org/10.5281/zenodo.23416647>

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ABSTRACT

Jatropha gossypifolia L. (Euphorbiaceae) is a medicinal plant reported to contain diverse secondary metabolites with potential antimicrobial activity. The present study aimed to prepare and evaluate an antimicrobial herbal ointment containing an ethanolic leaf extract of *J. gossypifolia*. Fresh leaves were authenticated, shade-dried, powdered and extracted with 90% ethanol using a Soxhlet apparatus. The ethanolic extract was subjected to qualitative phytochemical screening and incorporated into an ointment base containing white soft paraffin, hard paraffin, cetostearyl alcohol and anhydrous lanolin, with methyl paraben and propyl paraben as preservatives. The extract showed positive tests for alkaloids, flavonoids, coumarins/lignans and terpenoids. Antimicrobial activity against *Escherichia coli* was evaluated by an agar diffusion method and compared with Gentamicin. At 1.5 mg/mL, the reported zone of inhibition was 35 mm for the ethanolic extract compared with 20 mm for Gentamicin. The formulated ointment was green, smooth and homogeneous, with a pH of 5.95, a spreadability time of 3 seconds, good washability and no observed irritation during the reported 24-hour test. These findings indicate that the ethanolic leaf extract has promising in-vitro antimicrobial activity and can be incorporated into a topical herbal ointment. Further studies using additional microorganisms, standardized antimicrobial assays, stability testing and appropriate safety evaluation are required before pharmaceutical or clinical application.

KEYWORDS: *Jatropha gossypifolia*; herbal ointment; ethanolic extract; antimicrobial activity; phytochemical screening; *Escherichia coli*.

INTRODUCTION

Microbial infections are caused by pathogenic microorganisms, including bacteria, viruses, fungi and parasites, and remain an important cause of morbidity. The increasing emergence of antimicrobial resistance has created a need to investigate alternative and complementary antimicrobial sources. Natural products and medicinal plants contain diverse phytochemicals that may contribute to antimicrobial activity.

Jatropha gossypifolia L. (Euphorbiaceae), commonly known as bellyache bush or black physic nut, is a medicinal shrub traditionally used for various purposes. Different parts of the plant have been reported to contain alkaloids, flavonoids, lignans, phenolic compounds, saponins, tannins, terpenoids and other constituents. Previous literature summarized in the dissertation indicates antimicrobial, anti-inflammatory and wound-related pharmacological potential of the plant.

Topical semisolid preparations such as ointments provide a convenient means of delivering active substances to the skin. Ointments may provide protective and therapeutic effects and are evaluated using physicochemical and microbiological parameters. Based on the reported phytochemical profile and traditional use of *J. gossypifolia*, the present study was undertaken to formulate and evaluate an antimicrobial herbal ointment containing its ethanolic leaf extract.

MATERIALS AND METHODS

Plant material and authentication

Fresh leaves of *Jatropha gossypifolia* L. were collected and authenticated by the Department of Plant Science, Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627012, Tamil Nadu, India. The leaves were washed with distilled water, shade-dried at room temperature (20–30 °C) for 10–14 days, pulverized to a coarse powder and stored in an airtight container.

Preparation of ethanolic extract

Forty grams of dried leaf powder were extracted with 400 mL of 90% ethanol using a Soxhlet apparatus for approximately 6 hours or until the siphon tube became colourless. The extract was filtered through Whatman No. 1 filter paper and concentrated on a water bath maintained below 50 °C until a semisolid mass was obtained. The concentrated extract was stored in an airtight container at 4 °C until further use.

Phytochemical screening

The ethanolic extract was qualitatively screened for selected phytochemical groups. Alkaloids were tested using Dragendorff's reagent; flavonoids using the lead acetate test; terpenoids using the Salkowski test; and coumarins using ferric chloride. Positive reactions were recorded according to the observed colour or precipitate formation.

Preparation of herbal ointment

The herbal ointment was prepared by the incorporation (slab) method. White soft paraffin, hard paraffin, cetostearyl alcohol and anhydrous lanolin were melted in descending order of their melting points and mixed to obtain the ointment base. The required quantity of ethanolic leaf extract was triturated on a clean ointment slab to obtain a smooth paste.

Polyethylene glycol was used as a penetration/levigation aid when required. Methyl paraben and propyl paraben were incorporated after dissolution in a small quantity of warm ethanol. The extract was incorporated into the base by geometric dilution with continuous spatulation until a homogeneous, lump-free ointment was obtained.

Antimicrobial activity

The dissertation reports evaluation of antimicrobial activity against *Escherichia coli* using an agar diffusion method. A standardized bacterial inoculum of approximately 0.5 McFarland turbidity was used with Mueller–Hinton agar. Sterile filter-paper discs of approximately 6 mm diameter were placed on the inoculated agar, and the test preparation was applied to the discs. Ointment base without extract served as the negative control and Gentamicin served as the positive control. Plates were incubated at 37 °C for 24 hours, after which the zone of inhibition was measured in millimetres.

Evaluation of the ointment

The formulated ointment was evaluated for colour and odour, consistency, pH, spreadability, washability, non-irritancy and solubility. The pH was determined using a digital pH meter after preparing the reported aqueous sample. Spreadability was assessed by the slide-separation method, and washability and non-irritancy were evaluated as described in the dissertation.

RESULTS AND DISCUSSION

Phytochemical screening

The ethanolic extract showed positive reactions for alkaloids, flavonoids, coumarins/lignans and terpenoids. The results are summarized in Table 1.

Table 1: Phytochemical Screening.

Phytochemical class	Test	Result
Alkaloids	Dragendorff's test	+
Flavonoids	Lead acetate test	+
Coumarins/lignans	Ferric chloride test	+
Terpenoids	Salkowski test	+

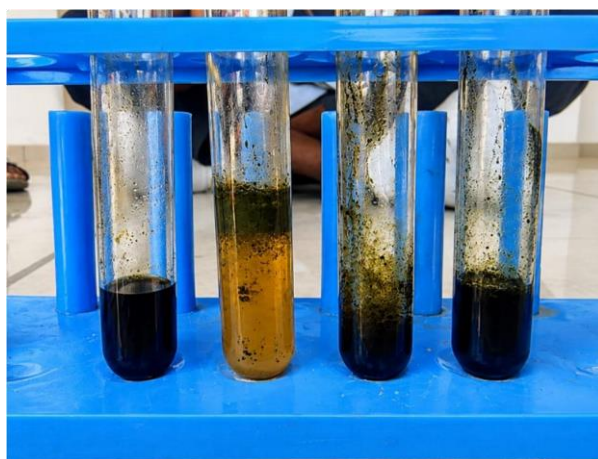


Figure 1: Chemical tests of the extract.

The observed phytochemical profile is consistent with the literature summarized in the dissertation, which reports several secondary-metabolite classes in *J. gossypifolia*. These constituents may contribute to the biological activity of the plant extract.

Antimicrobial activity

The reported antimicrobial evaluation against *Escherichia coli* showed inhibition by both the standard and test preparations. The ethanolic extract produced a zone of inhibition of 24 mm at 1 mg/mL and 35 mm at 1.5 mg/mL, whereas Gentamicin produced zones of 20 mm and 15 mm at the corresponding reported concentrations. The results are presented in Table 2.

Table 2: Report of the Anti-microbial evaluation.

Preparation	1 mg/mL (mm)	1.5 mg/mL (mm)	Test organism
Gentamicin	20	15	<i>Escherichia coli</i>
Ethanolic extract of <i>J. gossypifolia</i>	24	35	<i>Escherichia coli</i>

The reported increase in zone diameter for the extract from 24 mm to 35 mm with increasing concentration indicates greater inhibition at the higher tested concentration under the study conditions. However, because the study used a single reported organism and limited concentrations, the result should be interpreted as preliminary in-vitro evidence rather than evidence of clinical superiority over Gentamicin.

Physicochemical evaluation

The formulated ointment was reported to be green in colour with a characteristic odour and smooth consistency without grittiness. The measured pH was 5.95 and the spreadability test required 3 seconds for slide separation. The formulation was reported to be washable and non-irritant during the stated 24-hour observation. The reported solubility profile was soluble in alcohol, ether and chloroform.

Table 3: Physicochemical Evaluation of the antimicrobial herbal ointment.

Parameter	Observation
Colour	Green
Odour	Characteristic
Consistency	Smooth
pH	5.95
Spreadability	3 seconds
Washability	Good
Non-irritancy	Non-irritant
Solubility	Soluble in alcohol, ether and chloroform

Overall, the formulation exhibited the reported acceptable physical characteristics and preliminary antimicrobial activity. The dissertation itself identifies limitations including the use of a single test organism and a limited concentration range, and recommends broader microbiological validation, minimum inhibitory concentration/minimum bactericidal concentration studies and stability testing.

CONCLUSION

The ethanolic leaf extract of *Jatropha gossypifolia* demonstrated promising in-vitro antibacterial activity against *Escherichia coli* in the reported agar diffusion study and showed positive qualitative tests for alkaloids, flavonoids, coumarins/lignans and terpenoids. The extract was successfully incorporated into a herbal ointment that exhibited a green colour, smooth consistency, pH 5.95, good spreadability and washability, and no observed irritation in the reported preliminary test. The findings support further investigation of *J. gossypifolia* as a source for topical herbal formulations. Additional studies using multiple microorganisms, validated antimicrobial susceptibility methods,

quantitative MIC/MBC testing, stability studies and appropriate toxicological evaluation are required before conclusions regarding clinical usefulness can be made.

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