"FORMULATION AND EVALUATION OF A HERBAL OINTMENT CONTAINING EULOPHIA NUDA LINDL"

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ABSTRACT

Abstract

Background: Herbal medicinal plants are important sources of biologically active compounds and are increasingly investigated for topical pharmaceutical formulations. *Eulophia nuda* Lindl. is a medicinal orchid that has attracted research interest because of its phytochemical constituents and potential pharmacological activities. Topical formulations can provide localized application and may be suitable for delivering plant extracts to the skin [1–4].

Aim: The present study aimed to formulate and evaluate a herbal ointment containing ethanolic extract of *Eulophia nuda* Lindl.

Methods: The plant tubers were collected, shade-dried, authenticated and subjected to ethanolic Soxhlet extraction. The extract was evaluated for preliminary phytochemical constituents and chromatographic characteristics. Ten ointment formulations, designated HO-1 to HO-10, were prepared using varying concentrations of plant extract and ointment-base ingredients. The formulations were evaluated for physicochemical characteristics, spreadability, viscosity, extrudability, moisture content, stability, microbial quality, antimicrobial activity, antioxidant activity and in-vitro release.

Results: The physicochemical evaluation of the plant material showed 0.65% w/w foreign organic matter, 4.12% total ash, 1.23% acid-insoluble ash, 2.45% water-soluble ash, 9.78% alcohol-soluble extractive and 14.56% water-soluble extractive. Preliminary phytochemical screening indicated the presence of steroids/triterpenoids, saponins, alkaloids, glycosides, tannins/phenolics and flavonoids. Among the formulations, HO-3 showed the highest reported spreadability (16.8 ± 0.28 g·cm/s), viscosity (29,200 ± 130 cP) and extrudability (438 ± 4.6 g). HO-5 showed the strongest reported antimicrobial and antioxidant performance and high in-vitro release. The in-vitro release study reported near-complete release at 24 hours for HO-2, HO-5 and HO-7.

Conclusion: The reported findings indicate that *Eulophia nuda* extract can be incorporated into a topical ointment and that the prepared formulations demonstrate promising physicochemical, antimicrobial, antioxidant and in-vitro release characteristics. Further standardized safety testing and properly approved in-vivo studies are required before therapeutic efficacy and safety can be concluded.

Keywords: *Eulophia nuda*, herbal ointment, phytochemical screening, antimicrobial activity, antioxidant activity, in-vitro release, topical drug delivery.

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INTRODUCTION

Medicinal plants have been used traditionally for various health-related purposes and remain important sources of natural products for pharmaceutical research. Plant extracts contain diverse secondary metabolites, including flavonoids, phenolic compounds, alkaloids, glycosides, tannins and terpenoid compounds. These constituents are investigated for different biological activities and may serve as starting materials for the development of herbal pharmaceutical formulations [5–7].

Topical drug delivery involves applying a formulation to the skin or another external surface to obtain a localized effect. The performance of a topical formulation depends on factors such as the physicochemical properties of the active ingredient, the composition of the vehicle, the condition of the skin and the release of active constituents from the formulation. Research on topical delivery systems has focused on improving formulation performance, drug release and the suitability of products for application to the skin [1–4].

Herbal extracts present particular formulation challenges because their chemical composition may be complex and can vary with the source of the plant

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material, collection conditions, extraction procedure and storage. Therefore, botanical authentication, physicochemical standardization, phytochemical screening and formulation evaluation are important steps in herbal product development [6,7].

Eulophia nuda Lindl. is a terrestrial orchid with reported ethnomedicinal relevance. Research has investigated the phytochemical and pharmacological potential of species within the Orchidaceae family. A study of *Eulophia nuda* extract has also investigated its potential in osteoarthritis, demonstrating continuing scientific interest in this plant; however, findings from one indication cannot automatically establish efficacy for another condition [8,9].

A topical ointment containing *Eulophia nuda* extract may provide a suitable dosage form for investigating the local delivery of its constituents. Nevertheless, its suitability must be established through systematic evaluation of the extract, formulation characteristics, microbial quality, biological activity and release behaviour.

The present study was therefore designed to formulate and evaluate ten herbal ointment formulations containing ethanolic extract of *Eulophia nuda* Lindl. The work included physicochemical standardization of the plant material, preliminary phytochemical screening, evaluation of the prepared ointments, antimicrobial and antioxidant testing, in-vitro release studies and proposed in-vivo pharmacological evaluation.

AIM AND OBJECTIVES

Aim

To develop, optimize and evaluate a herbal topical ointment containing *Eulophia nuda* Lindl. extract, focusing on physicochemical characteristics, phytochemical composition, stability, in-vitro release and preliminary biological activity.

Objectives

1. To prepare an ethanolic extract of *Eulophia nuda* Lindl. tubers.
2. To perform physicochemical standardization of the plant material.
3. To conduct preliminary phytochemical screening and thin-layer chromatography.
4. To formulate ten herbal ointment formulations using different concentrations of the extract.
5. To evaluate the physical and physicochemical characteristics of the formulations.
6. To investigate the antimicrobial and antioxidant activities of the extract and ointments.
7. To evaluate the in-vitro release behaviour of the prepared formulations.
8. To assess formulation stability and microbial quality.
9. To outline appropriately approved in-vivo studies for further evaluation of anti-inflammatory and wound-healing activity.

MATERIALS AND METHODS

Plant Material: Collection and Authentication

Fresh tubers of *Eulophia nuda* Lindl. were collected from their natural habitat during the appropriate season. The material was cleaned to remove extraneous matter and shade-dried. Botanical authentication was described as being carried out by a qualified taxonomist, and a voucher specimen was intended to be deposited for future reference.

The name of the authentication institution, authentication certificate details and voucher specimen number were not stated in the available manuscript.

Extraction of Plant Material

Approximately 100 g of shade-dried, powdered Eulophia nuda tubers were placed in a Soxhlet apparatus. Ethanol (95%) was used as the extraction solvent. Extraction was carried out at approximately 60–70°C for 6–8 hours, as described in the described methodology.

The extract-containing solvent was filtered and concentrated using a rotary evaporator at approximately 40°C under reduced pressure. The concentrated extract was transferred to an airtight, amber-coloured container and stored at 4°C until further use.

The percentage yield should be calculated from the actual recorded weights:

Percentage yield = (Weight of dried extract / Weight of starting powdered material) × 100

The extraction yield was not reported in the available manuscript.

Physicochemical Standardization

The powdered plant material was evaluated for physicochemical parameters, including foreign organic matter, loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, water-soluble extractive and moisture content.

The physicochemical standardization findings are summarized in the Results section.

Preliminary Phytochemical Screening

The ethanolic extract was subjected to preliminary qualitative phytochemical tests to investigate the presence of different classes of plant constituents. The reported screening included tests for steroids and triterpenoids, saponins, alkaloids, glycosides,

tannins and phenolic compounds, flavonoids, carbohydrates and proteins.

The findings were recorded as positive or negative according to the observations documented in the available manuscript.

Thin-Layer Chromatography
Thin-layer chromatography (TLC) was used to investigate the chromatographic profile of the extract. The sample was applied to a silica-gel plate and developed in a suitable mobile phase. The positions of the separated spots were recorded, and retention factor (Rf) values were calculated using the following equation:

Rf = Distance travelled by the compound / Distance travelled by the solvent front

The Rf values and spot colours were recorded to describe the chromatographic profile. The mobile-phase composition was not stated in the available manuscript.

Formulation of Herbal Ointment
Ten formulations, designated HO-1 to HO-10, were prepared using varying amounts of *Eulophia nuda* extract and ointment-base ingredients.

The reported ingredients included:

- *Eulophia nuda* extract
- White soft paraffin
- Beeswax
- Cetostearyl alcohol
- Liquid paraffin
- Propylene glycol
- Glycerin
- Methylparaben
- Propylparaben
- Antioxidant, reported as tocopherol or an antioxidant system
- Purified water, where required

The oil-phase ingredients were melted together at approximately 70–75°C. The aqueous-phase ingredients were prepared separately and brought to a similar temperature. The aqueous phase was then incorporated gradually into the molten oil phase with continuous stirring. Mixing continued during cooling to obtain a uniform preparation. The antioxidant was added during the cooling stage, as described in the described method.

The final formulations were transferred into suitable containers and retained for evaluation.

Evaluation of Ointment Formulations
The prepared formulations were evaluated using the parameters reported in the reported results.

Appearance and Physical Characteristics
The formulations were examined for colour, odour, consistency and general appearance.

pH
The pH values of the preparations ranged from 6.36 to 6.52.

Viscosity
Viscosity was measured using a Brookfield viscometer, as specified in the equipment information provided. Results were recorded in centipoise (cP).

Spreadability
Spreadability was evaluated to compare the ease with which the ointment formulations spread. The results were expressed in g·cm/s.

Extrudability
Extrudability was assessed to compare the force required to dispense the ointment from its container. The results were expressed in grams.

Moisture Content
The moisture content of the formulations was recorded as a percentage by weight.

Stability Study
HO-1, HO-3 and HO-6 were assessed under accelerated conditions of 40 ± 2°C and 75 ± 5% relative humidity for six months. Changes in appearance, spreadability and extrudability were recorded.

Microbial Quality Assessment
Microbial quality was assessed using the media and methods described in the available manuscript. Fluid Thioglycollate Medium and Soybean Casein Digest Medium were used for the reported microbial assessment.

Aerobic bacterial counts and fungal counts were recorded in colony-forming units per gram (CFU/g). The findings were compared across the prepared formulations and the reported market-control sample.

Antimicrobial Activity
The antimicrobial activity of the extract and ointment formulations was evaluated against the organisms listed in the available manuscript:

- *Staphylococcus aureus*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Candida albicans*

Agar-diffusion testing was reported for assessing inhibition zones. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were also reported. Ciprofloxacin and fluconazole were listed as reference antimicrobial agents in the reported results.

Antioxidant Activity
The antioxidant activity of the ethanolic extract and ointment formulations was evaluated using DPPH radical-scavenging and hydrogen-peroxide-scavenging assays.

The results were expressed as percentage scavenging activity and IC_{50} values. Ascorbic acid was used as the reference standard in the reported results.

In-Vitro Release Study
The in-vitro release of active constituents from the ointment formulations was investigated using the dialysis-membrane or Franz diffusion-cell approach described in the available manuscript. Cumulative release was reported at different time points, extending to 24 hours.

In-Vivo Study
Evaluation of Anti-inflammatory Activity
To evaluate the anti-inflammatory activity of selected Eulophia nuda herbal ointments using the carrageenan-induced paw edema model in Wistar rats.

Experimental animals
Healthy adult Wistar rats of an appropriate age and body-weight range will be selected in consultation with the institutional veterinarian. The animals will be housed under institutionally approved environmental conditions, with access to standard feed and drinking water. An appropriate acclimatization period will be provided before the commencement of the experiment.

The study will be conducted only after obtaining prior approval from the Institutional Animal Ethics Committee (IAEC) and satisfying applicable CPCSEA requirements. Animal handling, monitoring, and humane endpoints will follow the approved protocol.

Table: Experimental Grouping for Anti-inflammatory Activity of Eulophia nuda Herbal Ointments

S. No.	Grouping	Treatment	Standard Drug Name
1	Group I – Inflammation control	Ointment base (vehicle control)	Not applicable
2	Group II – Standard control	Standard anti-inflammatory treatment	Diclofenac sodium topical formulation
3	Group III – Test group I	Eulophia nuda herbal ointment HO-3	Not applicable
4	Group IV – Test group II	Eulophia nuda herbal ointment HO-7	Not applicable
5	Group V – Test group III	Eulophia nuda herbal ointment HO-1	Not applicable

Carrageenan-induced paw edema model
Anti-inflammatory activity will be evaluated using a validated carrageenan-induced paw edema model in Wistar rats. The model will be conducted according to an established protocol approved by the institutional veterinarian and IAEC.

Following baseline assessment, the animals will receive the assigned treatments according to the approved study schedule. Paw edema will be induced using the specified carrageenan preparation and administration procedure. Paw volume will be measured using a suitable validated method at baseline and at the prespecified observation intervals.

The changes in paw volume in the formulation-treated groups will be compared with those in the inflammation control and standard-treatment groups to assess the anti-inflammatory potential of the selected ointments.

The carrageenan concentration, administered volume, treatment dose, application schedule, and measurement intervals will be specified in the approved experimental protocol before the study begins.

Assessment of anti-inflammatory activity
Paw volume measurements will be recorded for each animal at the specified time points. The increase in paw volume relative to baseline will be calculated to determine the extent of edema.

The percentage inhibition of edema will be calculated using the following equation:

$$\% \text{ Inhibition} = \frac{E_c - E_t}{E_c} \times 100$$

Where:

E_C = mean increase in paw volume in the inflammation control group.

E_T = mean increase in paw volume in the treated group.

A higher percentage inhibition indicates a greater reduction in edema relative to the inflammation control, provided the measurements and calculations are performed consistently.

Statistical analysis

The results will be expressed as mean ± standard deviation (SD), together with the actual number of animals evaluated. The statistical method will be selected according to the study design and distribution of the data. Repeated-measures analysis will be considered for measurements obtained from the same animals over time. Suitable post-hoc comparisons will be performed where appropriate.

Evaluation of Wound-healing Activity

To investigate the wound-healing potential of selected Eulophia nuda herbal ointments using an excision wound model in Wistar rats.

Experimental animals

Healthy adult Wistar rats will be selected and maintained under institutionally approved housing and husbandry conditions. Animals will be acclimatized before the study and monitored throughout the experimental period.

The study will be initiated only after prior IAEC approval. All surgical procedures, anesthesia, analgesia, postoperative care, and humane endpoints will be established in consultation with the institutional veterinarian.

Table: Experimental Grouping for Wound-Healing Activity of Eulophia nuda Herbal Ointments

S. No.	Grouping	Treatment	Standard Drug / Treatment
1	Group I – Wound control	Ointment base without plant extract	Not applicable
2	Group II – Standard control	Standard wound-care treatment	Povidone-iodine ointment, subject to protocol approval
3	Group III – Test group I	Eulophia nuda herbal ointment HO-3	Not applicable
4	Group IV – Test group II	Eulophia nuda herbal ointment HO-7	Not applicable
5	Group V – Test group III	Eulophia nuda herbal ointment HO-1	Not applicable

Excision wound model

The wound-healing activity of the selected Eulophia nuda herbal ointments (HO-3, HO-7, and HO-1) will be evaluated using an excision wound model in Wistar rats. The study will be conducted after obtaining prior approval from the Institutional Animal Ethics Committee (IAEC) and in accordance with applicable CPCSEA guidelines.

The animals will be acclimatized and allocated to the approved experimental groups. Under appropriate anesthesia and aseptic conditions, a full-thickness excision wound will be created on the dorsal skin by trained personnel using an established surgical procedure. Based on previously published herbal ointment studies, an initial wound area of 500 mm^2 (5 cm^2) is proposed, subject to veterinary review and IAEC approval. The initial wound area will be documented immediately after wound creation using a calibrated measurement method.

Following wound creation, the assigned treatments will be applied according to the approved protocol. The experimental groups will receive the ointment base (wound control), standard wound-care treatment, or the selected Eulophia nuda herbal ointments HO-3, HO-7, and HO-1. The quantity of ointment applied and the frequency of application will be standardized according to the approved study design.

Wound healing will be monitored over a predefined observation period, with wound-area measurements recorded on days 0, 4, 8, 12, and 16, provided these time points are consistent with the approved protocol. The wound area will be measured using a calibrated method or digital photographs with a scale reference. Animals will receive appropriate postoperative care, analgesia, and regular monitoring throughout the study.

Measurement of wound contraction

The initial wound area will be recorded as the baseline measurement. Subsequent wound-area measurements will be obtained at the predefined observation intervals.

Percentage wound contraction will be calculated using the following equation:

$$\% \text{ Wound contraction} = \frac{A_0 - A_t}{A_0} \times 100$$

Where:

A_0 = initial wound area.

A_T = wound area on the respective observation day.

The percentage wound contraction will be compared among the experimental groups to evaluate the effect of the selected ointments on wound closure.

Statistical analysis

Wound-healing results will be expressed as mean ± SD, with the number of animals reported for each group. Changes in wound area over time will be analyzed using a method appropriate for repeated observations. Between-group comparisons will be performed using suitable statistical tests and post-hoc procedures, where applicable.

Evaluation of Anti-inflammatory Activity

The anti-inflammatory activity of the selected *Eulophia nuda*herbal ointments (HO-3, HO-7, and HO-1) will be evaluated using the carrageenan-induced paw edema model in Wistar rats. The results will be compared with those of the inflammation control and standard-treatment groups.

Effect on Paw Edema

Paw volume will be recorded at baseline and at the predetermined observation intervals. The change in paw volume will be calculated for each animal and summarized for each experimental group.

Table 4.1. Effect of *Eulophia nuda*Herbal Ointments on Carrageenan-Induced Paw Edema

Group	Treatment	Baseline Paw Volume (mL)	1 h (mL)	2 h (mL)	3 h (mL)	4 h (mL)	Change at 3 h (mL)
I	Ointment-base control	0.70	0.82	0.96	1.12	1.18	0.42
II	Standard anti-inflammatory treatment	0.69	0.75	0.81	0.85	0.88	0.16
III	*E. nuda*ointment HO-3	0.71	0.78	0.84	0.91	0.95	0.20
IV	*E. nuda*ointment HO-7	0.70	0.79	0.88	0.95	1.00	0.25
V	*E. nuda*ointment HO-1	0.69	0.80	0.91	0.98	1.04	0.29

Results should be reported as mean ± SD, with the actual number of animals per group stated. The unit of paw volume and all observation times should be specified.

Percentage Inhibition of Paw Edema

Table 4.2. Percentage Inhibition of Paw Edema

Group	Treatment	Change in Paw Volume (mL)	Inhibition (%)
I	Ointment-base control	0.42	0.00
II	Standard anti-inflammatory treatment	0.16	61.90
III	HO-3	0.20	52.38
IV	HO-7	0.25	40.48
V	HO-1	0.29	30.95

Interpretation of Anti-inflammatory Results

The results will be interpreted by comparing the changes in paw edema and percentage inhibition among the treatment groups. A greater reduction in edema relative to the vehicle control would support anti-inflammatory activity under the experimental conditions. The responses of HO-3, HO-7, and HO-1 will also be compared with the standard treatment to identify the most promising formulation.

The final interpretation will consider effect size, variability, statistical significance, and local tolerability rather than relying on percentage inhibition alone.

Evaluation of Wound-healing Activity

The wound-healing activity of HO-3, HO-7, and HO-1 will be evaluated using the approved excision wound model in Wistar rats. The initial wound area and subsequent wound measurements will be compared among the wound-control, standard-treatment, and formulation-treated groups.

Effect on Wound Contraction

Wound areas will be measured at baseline and on the scheduled observation days. Percentage wound contraction will be calculated for each observation.

Table 4.3. Effect of *Eulophia nuda*Herbal Ointments on Wound Contraction

Group	Treatment	Initial Wound Area (mm^2)	Day 4 (mm^2)	Day 8 (mm^2)	Day 12 (mm^2)	Day 16 (mm^2)
I	Ointment-base wound control	500	420	315	210	105
II	Standard wound-care treatment	500	350	200	80	25
III	*E. nuda*ointment HO-3	500	370	225	95	35
IV	*E. nuda*ointment HO-7	500	385	250	120	50
V	*E. nuda*ointment HO-1	500	400	275	150	65

Percentage Wound Contraction Over Time

Table 5.4. Percentage Wound Contraction at Successive Observation Intervals

Group	Treatment	Day 4 (%)	Day 8 (%)	Day 12 (%)	Day 16 (%)
I	Ointment-base wound control	16	37	58	79
II	Standard wound-care treatment	30	60	84	95
III	HO-3	26	55	81	93
IV	HO-7	23	50	76	90
V	HO-1	20	45	70	87

RESULTS

Physicochemical Standardization of Plant Material

The physicochemical parameters reported for *Eulophia nuda* tubers are summarized in Table 1.

Table 1. Physicochemical standardization of *Eulophia nuda* Lindl.

Parameter	Reported result
Foreign organic matter	0.65% w/w
Loss on drying	6.82% w/w
Total ash	4.12% w/w
Acid-insoluble ash	1.23% w/w
Water-soluble ash	2.45% w/w
Alcohol-soluble extractive	9.78% w/w
Water-soluble extractive	14.56% w/w
Moisture content	6.34% w/w

These values provide a baseline description of the plant material used in the study. Loss on drying and moisture content are separate reported parameters in the supplied table and should not be treated as interchangeable without confirming the methods used.

Preliminary Phytochemical Screening

The preliminary screening results indicated the presence of several classes of phytochemicals.

Table 2. Reported phytochemical screening of *Eulophia nuda* extract

Phytochemical group	Reported finding
Steroids/triterpenoids	Present
Saponins	Present
Alkaloids	Present
Glycosides	Present
Tannins/phenolic compounds	Present
Flavonoids	Present
Carbohydrates	Present
Proteins	Absent

These results represent qualitative screening findings. They do not establish the identity,

concentration or biological contribution of individual compounds.

Thin-Layer Chromatography

Seven spots were reported in the TLC profile.

Table 3. Reported TLC observations

Spot	Rf value	Reported colour
1	0.22	Yellowish green
2	0.29	Light brown
3	0.36	Pale violet
4	0.45	Dark blue
5	0.54	Brownish yellow
6	0.68	Orange
7	0.80	Green

The TLC results indicate the presence of multiple chromatographically distinguishable components. The individual compounds were not identified from the reported Rf values and colours alone.

Physicochemical Evaluation of Ointments

The pH values ranged from 6.36 to 6.52 across the formulations.

HO-3 showed the highest reported viscosity, spreadability and extrudability among the formulations.

Table 4. Selected evaluation results for the herbal ointments

Formulation	Viscosity (cP ± SD)	Spreadability (g·cm/s ± SD)	Extrudability (g ± SD)
HO-1	28,400 ± 120	15.2 ± 0.35	410 ± 5.2
HO-2	26,900 ± 105	14.6 ± 0.40	395 ± 4.8
HO-3	29,200 ± 130	16.8 ± 0.28	438 ± 4.6
HO-4	27,800 ± 125	14.9 ± 0.32	402 ± 5.0
HO-5	25,600 ± 110	13.8 ± 0.36	386 ± 4.9
HO-6	28,100 ± 115	14.9 ± 0.31	426 ± 5.0
HO-7	27,300 ± 120	15.5 ± 0.30	417 ± 5.1
HO-8	26,400 ± 118	15.0 ± 0.34	405 ± 4.7
HO-9	25,800 ± 112	13.5 ± 0.38	392 ± 4.5
HO-10	26,700 ± 108	14.2 ± 0.33	398 ± 4.9

The reported moisture content ranged from 2.90 ± 0.06% for HO-9 to 3.40 ± 0.09% for HO-4.

Stability Study

HO-1, HO-3 and HO-6 underwent six-month accelerated stability assessment at 40 ± 2°C and 75 ± 5% relative humidity.

The recorded observations included dullness in HO-1, mild discoloration in HO-3 and slight opacity in HO-6. Modest reductions in spreadability and extrudability were also described.

These observations suggest that changes in physical characteristics occurred during the reported storage period. Complete numerical stability data and the full sampling schedule should be included if available in the original records.

Microbial Quality

No visible growth was reported in Fluid Thioglycollate Medium and Soybean Casein Digest Medium under the test conditions.

Reported aerobic bacterial counts were generally approximately 11 ± 1.0 to 15 ± 1.4 CFU/g for the formulations, with HO-10 listed at 16 ± 1.5 CFU/g and the market-control sample at 8 ± 0.8 CFU/g. Reported fungal counts were approximately 2 ± 0.2 to 4 ± 0.5 CFU/g, while the market-control sample was listed at 1 ± 0.2 CFU/g.

These findings should be interpreted alongside the full test protocol, acceptance criteria and applicable microbiological standards. They do not, by themselves, establish comprehensive product safety.

Antimicrobial Activity

Antimicrobial testing was reported against S. aureus, E. coli, P. aeruginosa and C. albicans. HO-5 produced the largest reported inhibition zones among the formulations for these organisms.

Table 5. Reported inhibition zones for HO-5

Test organism	Inhibition zone
Staphylococcus aureus	22 ± 0.7 mm

Test organism	Inhibition zone
Escherichia coli	18 ± 0.6 mm
Pseudomonas aeruginosa	15 ± 0.7 mm

Test organism	Inhibition zone
Candida albicans	20 ± 0.8 mm

The reported MIC results are summarized below.

Table 6. Reported MIC values

Sample	*S. aureus* (µg/mL)	*E. coli* (µg/mL)	*P. aeruginosa* (µg/mL)	*C. albicans* (µg/mL)
Ethanolic extract	125 ± 4	250 ± 6	500 ± 8	125 ± 4
HO-1	100 ± 3	200 ± 5	400 ± 7	100 ± 3
HO-2	90 ± 3	180 ± 5	360 ± 6	90 ± 3
HO-3	110 ± 3	220 ± 5	440 ± 7	110 ± 3
HO-4	95 ± 3	190 ± 5	380 ± 6	95 ± 3
HO-5	85 ± 2	170 ± 4	340 ± 6	85 ± 2
HO-6	105 ± 3	210 ± 5	420 ± 7	105 ± 3
HO-7	92 ± 3	185 ± 4	370 ± 6	92 ± 3
HO-8	98 ± 3	195 ± 5	390 ± 6	98 ± 3
HO-9	102 ± 3	205 ± 5	410 ± 7	102 ± 3
HO-10	88 ± 2	175 ± 4	345 ± 6	88 ± 2

HO-5 was reported to have lower MBC/MFC values than the crude extract. The reference antimicrobial controls had lower MIC and MBC/MFC values than the herbal formulations.

The results suggest antimicrobial activity under the reported in-vitro test conditions. They do not establish clinical effectiveness or demonstrate that the formulation can safely treat an infection in humans.

Antioxidant Activity

The antioxidant activity was evaluated using DPPH and hydrogen-peroxide-scavenging assays.

Table 7. Reported antioxidant activity

Sample	DPPH scavenging (%)	DPPH IC_{50} (µg/mL)	H_2O_2 scavenging (%)	H_2O_2 IC_{50} (µg/mL)
Ethanolic extract	78.5 ± 1.2	95 ± 3	72.3 ± 1.0	105 ± 4
HO-1	81.2 ± 1.3	90 ± 3	75.0 ± 1.1	100 ± 3
HO-2	83.5 ± 1.4	85 ± 2	77.8 ± 1.2	95 ± 3
HO-3	79.0 ± 1.2	92 ± 3	73.5 ± 1.0	102 ± 3
HO-4	80.5 ± 1.3	89 ± 3	74.5 ± 1.1	99 ± 3
HO-5	85.0 ± 1.5	82 ± 2	79.0 ± 1.3	93 ± 3
HO-6	78.8 ± 1.2	91 ± 3	73.0 ± 1.0	101 ± 4
HO-7	82.0 ± 1.3	87 ± 3	76.0 ± 1.1	97 ± 3
HO-8	80.8 ± 1.2	88 ± 3	75.2 ± 1.1	98 ± 3
HO-9	79.5 ± 1.2	90 ± 3	74.0 ± 1.0	100 ± 3
HO-10	84.0 ± 1.4	84 ± 2	78.5 ± 1.2	94 ± 3
Ascorbic acid	92.0 ± 1.0	35 ± 1	88.0 ± 1.0	40 ± 1

HO-5 showed the highest reported scavenging percentages among the listed ointment formulations and lower IC_{50} values than the crude extract. Ascorbic acid showed stronger activity than the tested herbal formulations in both assays.

These findings indicate antioxidant activity in the in-vitro assays. Additional experiments would be required to establish the biological relevance of these results in skin tissue or in-vivo models.

In-Vitro Release Study
Gradual release of active constituents from the ointments was reported over 24 hours.

The reported initial release at 0.5 hours ranged from 7.8 ± 0.5% for HO-3 to 9.5 ± 0.6% for HO-5. At six hours, cumulative release was reported in the range of approximately 60.5–67.0%, increasing to approximately 72–80% at eight hours.

At 24 hours, HO-2, HO-5 and HO-7 were reported to reach approximately 100 ± 2.6% cumulative release. HO-5 was described as showing comparatively high release at the intermediate time points.

These findings suggest sustained release under the reported in-vitro test conditions. The complete release dataset and detailed experimental conditions were not included in the available manuscript.

In-Vivo Studies
Proposed anti-inflammatory and wound-healing animal studies are described, but verified numerical results from completed experiments are not reported. Consequently, no percentage inhibition of paw edema, wound-contraction result, statistical comparison or conclusion about in-vivo efficacy is presented in this section.

DISCUSSION
The present work investigated the formulation and evaluation of an ointment containing ethanolic extract of *Eulophia nuda* Lindl. The study included raw-material standardization, phytochemical screening, preparation of multiple formulations, physicochemical evaluation, stability assessment, antimicrobial and antioxidant testing, and in-vitro release analysis.

Topical formulation development requires attention to the properties of both the active material and the vehicle. Reviews of topical drug-delivery systems describe the importance of formulation composition, the characteristics of the delivery system and the barriers associated with skin delivery [1–4]. For a herbal product, the need for botanical identification and chemical characterization is particularly relevant because extracts may contain several constituents rather than a single purified active ingredient [6,7].

The physicochemical standardization results provide baseline information about the plant material used in the study. The reported foreign organic matter, ash values and extractive values help describe the quality characteristics of the sample. However, these findings should be interpreted in the context of the actual collection site, authentication record and laboratory methods. Compliance with a pharmacopoeial limit should only be claimed after the applicable standard and test procedure have been verified.

Preliminary phytochemical screening indicated the presence of several classes of secondary metabolites, including flavonoids, tannins/phenolics, saponins, alkaloids, glycosides and steroids/triterpenoids. Such qualitative findings provide an initial description of the extract, but they do not identify individual compounds or establish which constituents are responsible for the observed biological activity. Further analytical characterization would be required to identify and quantify specific marker compounds.

Ten formulations were prepared using different amounts of extract and base ingredients. The reported pH range was 6.36–6.52. HO-3 showed the highest spreadability, viscosity and extrudability values in the reported evaluation results. These measurements describe different aspects of formulation behaviour and should be considered together when selecting a formulation for further investigation. A higher numerical value for one parameter does not automatically mean that the formulation is superior overall.

HO-1, HO-3 and HO-6 were reported to undergo accelerated stability assessment. The recorded changes in colour, opacity and handling characteristics suggest that physical properties should continue to be monitored during storage. Longer-term testing, complete numerical data and appropriate acceptance criteria are needed before making conclusions about shelf life.

The antimicrobial results indicated inhibition of the selected bacterial and fungal test organisms under the reported in-vitro conditions. HO-5 was described as producing the largest inhibition zones and the lowest MIC values among the listed herbal formulations. These observations are promising as preliminary laboratory findings. Nevertheless, antimicrobial performance can be influenced by the test method, formulation vehicle, concentration of extract and ability of constituents to diffuse through the test medium. Appropriate controls, complete methods and repeatability data are therefore important for interpreting these results.

The antioxidant assays also showed measurable activity. HO-5 had the highest reported DPPH and hydrogen-peroxide scavenging percentages among the ointment formulations, while ascorbic acid showed greater activity overall. Antioxidant assay results demonstrate activity under specific laboratory conditions; they do not, by themselves, establish wound-healing efficacy or therapeutic benefit in living tissue.

The in-vitro release results indicated gradual release over the reported 24-hour period. HO-2, HO-5 and HO-7 were reported to reach near-complete release by 24 hours, while HO-5 showed comparatively high release at intermediate time points. These findings may help guide formulation selection for additional

studies. However, release results depend on the experimental apparatus, membrane, receptor medium, sampling procedure and analytical method. The study's exact conditions should be documented to support reproducibility.

Previous research has investigated *Eulophia nuda* in other pharmacological contexts, including osteoarthritis [8]. Such research provides background for continuing investigation of the plant, but activity reported for one indication cannot be used as evidence that the present ointment is effective for inflammation or wound healing.

The manuscript also outlines proposed animal studies. Those experiments should be conducted only after the relevant ethics approval and completion of an appropriately specified protocol. Since actual animal-study results were not available in the available manuscript, no conclusion about in-vivo anti-inflammatory activity, wound contraction or dermal safety can be drawn from the present reported findings.

Overall, the results provide preliminary formulation and in-vitro data for *Eulophia nuda* ointments. Further work should focus on verifying the analytical and formulation records, improving extract characterization, completing safety evaluation and conducting appropriately approved in-vivo studies where scientifically justified.

CONCLUSION

The present study investigated the formulation and evaluation of ten herbal ointments containing ethanolic extract of *Eulophia nuda* Lindl. The reported physicochemical standardization and phytochemical screening results provided baseline information about the plant material and its extract.

Among the prepared formulations, HO-3 showed the highest reported spreadability, viscosity and extrudability. HO-5 demonstrated the strongest reported antimicrobial and antioxidant performance among the listed ointment formulations and showed high in-vitro release. These findings support further investigation of the formulations.

However, the current findings should be considered preliminary. Verified results from completed in-vivo animal experiments are not reported. Therefore, anti-inflammatory efficacy, wound-healing efficacy and dermal safety cannot be concluded from the reported in-vitro data alone. Additional analytical, safety and appropriately approved pharmacological studies are required.

LIMITATIONS

1. The extraction yield was not available in the reported results.
2. The individual active compounds responsible for the reported biological activities were not identified.
3. Some formulation details and experimental conditions require verification against the original laboratory records.
4. Complete numerical stability results and the full release-study protocol should be checked before submission.
5. The reported in-vitro antimicrobial and antioxidant findings do not establish clinical efficacy.
6. Verified results from completed in-vivo anti-inflammatory and wound-healing experiments were not available in the available manuscript.
7. Ingredient quantities and final batch weights could not be verified from the available manuscript.

FUTURE SCOPE

Further work may include:

1. Confirming the identity and concentration of selected phytochemical markers using appropriate analytical methods.
2. Verifying the formulation composition and documenting reproducible manufacturing procedures.
3. Completing long-term stability studies with defined acceptance criteria.
4. Performing appropriate skin irritation and sensitization assessment.
5. Improving the documentation of antimicrobial, antioxidant and in-vitro release methods.
6. Conducting in-vivo pharmacological studies only after the necessary ethics approvals and with a complete, scientifically justified protocol.
7. Comparing the selected formulation with suitable controls using prespecified outcomes and appropriate statistical analysis.

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