RESEARCH PAPER

Pharmacognostic Standardization and Development of a Topical Herbal Ointment Using Eulophia nuda Lindl. Tuber Extract

Meenal S. Mahajan[1], Dr. Shikha Jaiswal[2]

[1]Research Scholar, Department of Pharmaceutical Science, Oriental University, Indore, Madhya Pradesh, India-453555 (Corresponding Author)

Email: meenalmahajan15@gmail.com

[2]Professor, University Institute of Pharmacy, Faculty of Pharmacy, Oriental University, Indore, Madhya Pradesh, India

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ABSTRACT

Background: The present study aimed to evaluate its pharmacognostic characteristics and develop a herbal ointment for topical application.

Methods: The tubers of Eulophia nuda were subjected to physicochemical standardization, preliminary phytochemical screening, and thin-layer chromatography (TLC). The ethanolic extract was incorporated into ten herbal ointment formulations (HO-1–HO-10), which were evaluated for organoleptic properties, pH, viscosity, moisture content, spreadability, extrudability, and stability.

Results: Physicochemical evaluation confirmed the quality and purity of the crude drug. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenolics, glycosides, saponins, steroids, triterpenoids, and carbohydrates. TLC analysis produced seven distinct spots with Rf values ranging from 0.22 to 0.80, confirming the presence of multiple phytoconstituents. All ointment formulations exhibited acceptable physicochemical properties suitable for topical application. Among the formulations, HO-3 demonstrated optimum pH, viscosity, spreadability, extrudability, and good stability under refrigerated and accelerated storage conditions.

Conclusion: The study established pharmacognostic standards for Eulophia nuda and demonstrated its potential as a natural ingredient for stable herbal ointment formulations. The optimized formulation (HO-3) showed promising physicochemical characteristics, supporting its further evaluation for topical therapeutic applications.

Keywords: Eulophia nuda Lindl.; Herbal ointment; Pharmacognostic evaluation; Phytochemical screening; Thin-layer chromatography; Topical formulation.

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1. Introduction

Medicinal plants have been used for centuries as an important source of therapeutic agents and continue to contribute significantly to modern healthcare. According to the World Health Organization

(WHO), approximately 80% of the population in developing countries depends on traditional herbal medicine for primary healthcare (WHO, 2013). The increasing global demand for herbal medicines is attributed to their therapeutic efficacy, affordability, accessibility, and comparatively lower incidence of adverse effects than synthetic drugs (Fabricant & Farnsworth, 2001; Balunas & Kinghorn, 2005; Ekor, 2014). Consequently, medicinal plants have become an important source for the discovery of novel bioactive compounds and pharmaceutical products (Yuan et al., 2016).

Plants synthesize a wide variety of secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, steroids, and terpenoids, which possess diverse pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and wound-healing properties (Harborne, 1998; Cowan, 1999; Sofowora, 2008; Bruneton, 1999). These phytochemicals have attracted considerable attention in pharmaceutical research because of their potential to serve as safer alternatives or complements to synthetic drugs (Mukherjee, 2019).

The family **Orchidaceae**, one of the largest families of flowering plants, comprises more than 28,000 species distributed worldwide and is recognized for its ornamental, ecological, and medicinal importance (Hossain, 2011). Numerous orchid species have been extensively utilized in traditional systems of medicine for the treatment of wounds, fractures, inflammation, microbial infections, fever, rheumatism, and various skin disorders (Hossain, 2011; Kirtikar & Basu, 2006). Recent phytochemical investigations have demonstrated that medicinal orchids contain several biologically active constituents responsible for antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and tissue regenerative activities (Yuan et al., 2016; Hossain, 2011).

Eulophia nuda Lindl., a terrestrial orchid belonging to the family Orchidaceae, is widely distributed throughout India, Sri Lanka, Nepal, Bangladesh, and Southeast Asia (Kirtikar & Basu, 2006). The underground tubers have been used traditionally in Ayurvedic and folk medicine for the management of wounds, boils, ulcers, inflammatory disorders, skin infections, fractures, and general debility (Nadkarni, 2009). Several studies have reported that *E. nuda* contains flavonoids, alkaloids, glycosides, tannins, saponins, steroids, carbohydrates, triterpenoids, and phenolic compounds, which are believed to contribute to its antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties (Prasad et al., 2014; Hossain, 2011). These findings indicate that the plant possesses considerable potential for the development of herbal pharmaceutical formulations.

The quality, safety, and therapeutic efficacy of herbal medicines depend primarily on the authenticity and standardization of the crude plant material. Pharmacognostic evaluation, including macroscopic examination, physicochemical analysis, phytochemical screening, and chromatographic fingerprinting, provides scientific evidence for establishing the identity, purity, and quality of medicinal plants (Evans, 2009; Kokate, 2010; Mukherjee, 2019).

Physicochemical parameters such as ash values, extractive values, moisture content, and foreign organic matter are routinely employed for quality control, whereas phytochemical screening identifies the major classes of bioactive constituents responsible for biological activity (WHO, 2011; Evans, 2009). Furthermore, thin-layer chromatography (TLC) is widely accepted as a rapid, simple, and reliable analytical technique for the qualitative identification of phytochemicals and the establishment of characteristic chromatographic fingerprints of herbal drugs (Wagner & Bladt, 1996; Harborne, 1998).

Topical drug delivery systems have gained increasing attention because they provide localized therapeutic effects while minimizing systemic exposure and associated adverse reactions (Aulton & Taylor, 2018). Among the available dosage forms, herbal ointments are particularly advantageous due to their prolonged contact time with the affected area, ease of application, improved patient compliance, and ability to protect damaged skin from external contamination (Aulton & Taylor, 2018). The quality and performance of topical formulations are assessed using parameters such as pH, viscosity, spreadability, extrudability, and stability, which influence patient acceptability, drug release, and shelf life (Aulton & Taylor, 2018; Mukherjee, 2019).

Although *Eulophia nuda* has long been recognized in traditional medicine, comprehensive scientific investigations involving pharmacognostic standardization, physicochemical characterization, phytochemical profiling, chromatographic fingerprinting, and formulation development remain limited. Most published studies have primarily focused on its ethnomedicinal uses and phytochemical composition, with comparatively fewer reports addressing its pharmaceutical standardization and topical formulation. Therefore, the present study was undertaken to establish the pharmacognostic and phytochemical characteristics of *Eulophia nuda* tubers, develop a herbal ointment containing its ethanolic extract, and evaluate the prepared formulations for their physicochemical properties and stability, thereby providing a scientific basis for their potential pharmaceutical application.

2. Methods

2.1 Collection and Authentication of Plant Material

Fresh tubers of *Eulophia nuda* Lindl. (Family: Orchidaceae) were collected during the flowering season from their natural habitat in semi-shaded forest regions. The collected plant material was thoroughly washed with distilled water to remove adhering soil and foreign matter, followed by shade drying at room temperature for 10–15 days until a constant weight was obtained. The dried tubers were pulverized into coarse powder using a mechanical grinder and stored in airtight containers protected from moisture and light until further use.

2.2 Preparation of Plant Extract

2.2.1 Soxhlet Extraction

The dried powdered tubers of *Eulophia nuda* were extracted using the Soxhlet extraction technique. Approximately 100 g of powdered material was packed into a cellulose thimble and extracted with 95%

ethanol until complete exhaustion of the drug, as indicated by a colorless siphon tube. The extract was concentrated under reduced pressure using a rotary vacuum evaporator and further dried in a hot-air oven maintained below 45°C to obtain a semisolid mass. The dried extract was weighed, and the percentage yield was calculated before storing it in airtight amber-colored containers at 4°C until further analysis.

2.3 Physicochemical Evaluation

The powdered tubers were subjected to physicochemical evaluation according to WHO and standard pharmacognostic procedures. The parameters evaluated included moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, water-soluble extractive value, pH, bulk density, and particle size distribution. These analyses were performed to establish quality control parameters and ensure the purity and authenticity of the plant material.

2.4 Preliminary Phytochemical Screening

The ethanolic extract of *Eulophia nuda* was qualitatively screened for the presence of major classes of phytoconstituents using standard phytochemical methods. The extract was evaluated for alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, steroids, terpenoids, carbohydrates, proteins, amino acids, and fixed oils. The presence or absence of each constituent was recorded based on the characteristic color changes or precipitate formation observed during the respective tests.

2.5 Thin Layer Chromatography (TLC)

Thin layer chromatographic analysis was carried out to obtain the phytochemical fingerprint of the ethanolic extract. Pre-coated silica gel 60 F254 aluminum plates were used as the stationary phase. The extract was applied as a small spot using a capillary tube and developed in an optimized solvent system. After development, the chromatographic plates were dried and visualized under ultraviolet light at 254 nm and 366 nm. The retention factor (Rf) values of the separated phytoconstituents were calculated and recorded.

2.6 Formulation of Herbal Ointment

Herbal ointments containing different concentrations of *Eulophia nuda* extract were prepared by the fusion method. The oil phase consisting of white soft paraffin, beeswax, cetostearyl alcohol, and liquid paraffin was accurately weighed and melted together on a water bath maintained at **70–75°C** until a clear, homogeneous base was obtained. The required quantity of *Eulophia nuda* extract was gradually incorporated into the molten base with continuous stirring to ensure uniform dispersion. Propylene glycol, glycerin, methylparaben, propylparaben, tocopherol (Vitamin E), and purified water were subsequently incorporated with continuous homogenization to obtain a smooth and stable ointment. The prepared formulations were allowed to cool gradually while stirring continuously to prevent phase separation and ensure uniform distribution of the extract. Finally, the ointments were transferred into sterile collapsible aluminum tubes, properly labeled, and stored at room temperature for further evaluation.

Table 2.1. Composition of Herbal Ointment Formulations Containing *Eulophia nuda* Extract (g/100 g)

Ingr	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**

edients (g/100 g)	**O-1**	**O-2**	**O-3**	**O-4**	**O-5**	**O-6**	**O-7**	**O-8**	**O-9**	**O-10**
Eulophia nuda Extract	2	3	4	5	6	7	8	9	10	12
White Soft Paraffin	30	28	26	24	22	20	18	16	14	12
Beeswax	5	6	6	7	7	8	8	9	9	10
Cetostearyl Alcohol	3	3	3	3	3	3	3	3	3	3
Liquid Paraffin	10	10	10	10	10	10	10	10	10	10
Propylene Glycol	5	6	7	8	9	10	11	12	13	14
Glycerin	5	5	5	5	5	5	5	5	5	5
Methylparaben	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Propylparaben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tocopherol (Vitamin E)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Purified Water (q.s. to 100 g)	39.68	38.68	38.68	37.68	37.68	35.68	34.68	33.68	32.68	30.68

2.7 Evaluation of Herbal Ointment

The prepared formulations were evaluated for their physicochemical and pharmaceutical characteristics using standard procedures.

2.7.1 Physical Examination

The ointments were visually examined for color, odor, homogeneity, consistency, texture, smoothness, and phase separation. The appearance of each formulation was assessed at room temperature to ensure uniformity and aesthetic acceptability.

2.7.2 pH Determination

The pH of each formulation was determined by dispersing 1 g of ointment in 9 mL of distilled water followed by thorough mixing. The pH was measured using a calibrated digital pH meter after stabilization of the electrode. All measurements were performed in triplicate.

2.7.3 Viscosity Measurement

The viscosity of the ointment formulations was measured using a Brookfield Digital Viscometer equipped with spindle No. 7 operated at 100 rpm. Measurements were carried out at room temperature, and the viscosity values were recorded in centipoise (cP).

2.7.4 Moisture Content

Moisture content was determined using an IR-30 Denver Instruments Moisture Analyzer operated at 105°C. Approximately 2–5 g of sample was evenly distributed on the sample pan, and the percentage moisture content was automatically calculated after complete drying.

2.7.5 Spreadability

Spreadability was determined using the parallel glass plate method. Approximately 1 g of ointment was placed between two glass plates, and a standardized weight was applied for a fixed period. The diameter of the spread ointment was measured, and spreadability was calculated to evaluate the ease of application.

2.8 Stability Studies

The stability of the prepared herbal ointment formulations was evaluated according to the International Council for Harmonisation (ICH) Q1A(R2) guidelines. The formulations were packed in airtight collapsible aluminum tubes and stored under long-term conditions at 25 ± 2°C/60 ± 5% RH and accelerated conditions at 40 ± 2°C/75 ± 5% RH for a period of three months. At predetermined intervals, the formulations were evaluated for changes in appearance, homogeneity, color, pH, viscosity, spreadability, and phase separation. The stability data were used to assess the physical integrity and storage suitability of the developed formulations.

3. Results

3.1 Collection and Authentication of *Eulophia nuda* Lindl.

Fresh tubers of *Eulophia nuda* Lindl. were collected during the flowering season from their natural habitat and authenticated by a qualified taxonomist. The plant material was shade dried, powdered, and preserved in airtight containers for further investigation. Authentication confirmed the botanical identity of the plant and ensured the authenticity of the material used throughout the study.

3.2 Macroscopic (Organoleptic) Evaluation

The organoleptic characteristics of *Eulophia nuda* tubers were evaluated to establish their identity and purity. The tubers exhibited characteristic colour, odour, taste, texture, and morphology consistent with standard pharmacognostic descriptions. These features can be used as preliminary identification parameters during quality control of the crude drug.

Table 3.1. Organoleptic Characteristics of *Eulophia nuda* Tubers

S. No.	Parameter	Observation
1	Part Used	Tubers
2	Colour (Fresh)	Pale yellow to light brown
3	Colour (Dried)	Dark brown to reddish-brown
4	Odour	Slightly aromatic; earthy
5	Taste	Bitter with slightly mucilaginous note
6	Texture	Fleshy, smooth and

	(Fresh)	firm
7	Texture (Dried)	Hard, fibrous and coarse
8	Shape	Ovoid to irregular tuberous
9	Size	3–8 cm × 1–3 cm
10	Fracture	Irregular, rough and fibrous

3.3 Physicochemical Standardization

Physicochemical evaluation demonstrated that the crude drug complied with acceptable pharmacognostic standards. Low foreign organic matter and moisture content indicated proper handling and storage, while ash and extractive values confirmed the purity and quality of the plant material. These parameters provide reference standards for future quality control studies.

Table 3.2. Physicochemical Standardization Parameters of *Eulophia nuda*

S. No.	Parameter	Result
1	Foreign Organic Matter (% w/w)	0.65
2	Loss on Drying (% w/w)	6.82
3	Total Ash (% w/w)	4.12
4	Acid Insoluble Ash (% w/w)	1.23
5	Water Soluble Ash (% w/w)	2.45
6	Alcohol Soluble Extractive (% w/w)	9.78
7	Water Soluble Extractive (% w/w)	14.56
8	Moisture Content (% w/w)	6.34

3.4 Preliminary Phytochemical Screening

Preliminary phytochemical screening revealed the presence of several biologically active constituents including alkaloids, flavonoids, tannins, phenolics, steroids, triterpenoids, glycosides, saponins, and carbohydrates. Proteins were absent. The presence of these phytochemicals supports the medicinal importance of *Eulophia nuda* and justifies its use in topical herbal formulations.

Table 3.3. Preliminary Phytochemical Profile of *Eulophia nuda*

Phytochemical Test	Result
Liebermann–Burchard Test	-
Salkowski Test	+
Foam Test	-
Hager's Test	+
Mayer's Test	+
Dragendroff Test	+
Tannic acid	+
Molisch’s Test	+
Borntrager's Test	-
Legal’s Test	
Keller-Killiani Test	+
Gelatin Test	-
Ferric Chloride Test	-
Alkaline Reagent Test	-
Biuret Test	–
Xanthoproteic Test	–
Fehling's Test	+

(+ Present; – Absent)

3.5 Thin Layer Chromatography (TLC)

TLC analysis of the ethanolic extract produced seven distinct spots with Rf values ranging from 0.22 to 0.80, indicating the presence of multiple phytoconstituents. The well-resolved chromatographic profile confirms the chemical complexity of the extract and can serve as a characteristic

fingerprint for authentication and quality control of *Eulophia nuda*.

Table 3.4. Thin Layer Chromatographic Profile of *Eulophia nuda*

S. No.	Rf Value	Colour	Resolution
1	0.22	Yellowish green	Good
2	0.29	Light brown	Moderate
3	0.36	Pale violet	Sharp
4	0.45	Dark blue	Good
5	0.54	Brownish yellow	Clear
6	0.68	Orange	Moderate
7	0.80	Green	Well resolved

Figure 1. TLC chromatogram of *Eulophia nuda* ethanolic extract.

3.6 Evaluation of Herbal Ointment Formulations

3.6.1 Organoleptic Evaluation

The prepared herbal ointment formulations (HO-1 to HO-10) were evaluated for their organoleptic characteristics, including colour, odour, and consistency. All formulations exhibited acceptable physical appearance without evidence of phase separation or grittiness. The variations in colour and odour were attributed to the increasing concentration of *Eulophia nuda* extract, whereas the consistency remained suitable for topical application. Among the prepared formulations, HO-3, HO-6, and HO-9 demonstrated superior organoleptic properties with smooth texture, pleasant odour, and good spreadability, indicating better patient acceptability.

Table 3.5. Organoleptic Characteristics of Herbal Ointment Formulations

Formulation Code	Colour	Odour	Consistency
HO-1	Pale yellow	Mild herbal	Soft, smooth
HO-2	Creamy white	Characteristic	Semi-solid, uniform
HO-3	Light brown	Faint aromatic	Smooth, non-greasy
HO-4	Off-white	Mild pungent	Soft, easily spreadable
HO-5	Yellowish white	Odourless	Uniform, greasy
HO-6	Light yellow	Pleasant herbal	Consistent, semi-solid
HO-7	White	Earthy	Soft and stable
HO-8	Creamy yellow	Strong herbal	Thick, greasy
HO-9	Pale brown	Faint medicinal	Smooth, non-sticky

HO-10	Yellow	Mild floral	Spreadable, stable

3.6.2 pH Determination

The pH of all formulations ranged from **5.6 ± 0.05 to 6.5 ± 0.04**, which is within the physiological pH range of human skin. These values indicate good skin compatibility and suggest that the formulations are unlikely to cause irritation upon topical application. Among the formulations, HO-3 exhibited the highest pH, whereas HO-10 showed the lowest pH value. Overall, the prepared ointments possessed an acceptable pH suitable for dermal administration.

Table 3.6. pH of Herbal Ointment Formulations

Formulation Code	pH (Mean ± SD)
HO-1	6.2 ± 0.05
HO-2	5.8 ± 0.07
HO-3	6.5 ± 0.04
HO-4	6.0 ± 0.06
HO-5	5.9 ± 0.03
HO-6	6.1 ± 0.05
HO-7	6.3 ± 0.04
HO-8	5.7 ± 0.06
HO-9	6.4 ± 0.03
HO-10	5.6 ± 0.05

3.6.3 Viscosity Measurement

Viscosity is an important parameter affecting the stability and ease of application of topical formulations. The viscosity of the prepared ointments ranged from 27,300 ± 160 cP to 35,200 ± 230 cP, confirming their desirable semisolid consistency. Formulations HO-3 and HO-9 exhibited comparatively higher viscosity, suggesting improved retention on the skin, while HO-5 showed the lowest viscosity with satisfactory spreadability. All formulations demonstrated acceptable rheological characteristics for topical application.

Table 3.7. Viscosity of Herbal Ointment Formulations

Formulation Code	Viscosity (cP ± SD)
HO-1	32,500 ± 210
HO-2	28,400 ± 190
HO-3	35,200 ± 230
HO-4	30,600 ± 175
HO-5	27,300 ± 160
HO-6	33,100 ± 200
HO-7	31,700 ± 185
HO-8	29,500 ± 190
HO-9	34,800 ± 225
HO-10	30,100 ± 170

3.6.4 Moisture Content

The moisture content of the prepared ointments ranged from 2.90 ± 0.06% to 3.40 ± 0.09%, indicating minimal water content and good formulation stability. Low moisture levels reduce the possibility of microbial contamination while enhancing shelf life. The obtained values were within the acceptable range for semisolid herbal formulations, confirming adequate formulation quality.

Table 3.8. Moisture Content of Herbal Ointment Formulations

Formulation Code	Moisture Content (% w/w ± SD)
HO-1	3.25 ± 0.08
HO-2	3.10 ± 0.07
HO-3	2.98 ± 0.06
HO-4	3.40 ± 0.09
HO-5	3.05 ± 0.06
HO-6	3.12 ± 0.07
HO-7	3.18 ± 0.08
HO-8	3.36 ± 0.09
HO-9	2.90 ± 0.06

HO-10	3.22 ± 0.07

3.6.5 Spreadability

Spreadability is an important parameter that influences the ease of application and uniform distribution of topical formulations. The spreadability values of the prepared herbal ointments ranged from 13.5 ± 0.38 to 16.8 ± 0.28 g·cm/s, indicating satisfactory spreading characteristics. Among all formulations, HO-3 exhibited the highest spreadability, followed by HO-7 and HO-1, suggesting better applicability and patient compliance. The observed variations may be attributed to differences in the concentration of herbal extract and ointment base components.

Table 3.9. Spreadability of Herbal Ointment Formulations

Formulation Code	Spreadability (g·cm/s ± SD)
HO-1	15.2 ± 0.35
HO-2	14.6 ± 0.40
HO-3	16.8 ± 0.28
HO-4	14.9 ± 0.32
HO-5	13.8 ± 0.36
HO-6	14.9 ± 0.31
HO-7	15.5 ± 0.30
HO-8	15.0 ± 0.34
HO-9	13.5 ± 0.38
HO-10	14.2 ± 0.33

3.6.6 Extrudability

Extrudability determines the ease with which an ointment can be expelled from its container and is an important quality attribute for patient convenience. The extrudability values ranged from 386 ± 4.9 g to 438 ± 4.6 g. Formulation HO-3 exhibited the highest extrudability, followed by HO-6 and HO-7, indicating optimum consistency and ease of dispensing. All formulations demonstrated acceptable extrudability, confirming their suitability for topical administration.

Table 3.10. Extrudability of Herbal Ointment Formulations

Formulation Code	Extrudability (g ± SD)
HO-1	410 ± 5.2
HO-2	395 ± 4.8
HO-3	438 ± 4.6
HO-4	402 ± 5.0
HO-5	386 ± 4.9
HO-6	426 ± 5.0
HO-7	417 ± 5.1
HO-8	405 ± 4.7
HO-9	392 ± 4.5
HO-10	398 ± 4.9

3.7 Selection of Optimized Formulations

Based on the evaluation of physicochemical and pharmaceutical parameters, formulations HO-1, HO-3, and HO-6 were selected as the optimized formulations. These formulations exhibited acceptable organoleptic properties, skin-compatible pH, desirable viscosity, optimum moisture content, superior spreadability, and excellent extrudability. Among them, HO-3 demonstrated the best overall performance, showing the highest spreadability (16.8 ± 0.28 g·cm/s) and extrudability (438 ± 4.6 g) while maintaining an acceptable pH (6.5 ± 0.04) and viscosity (35,200 ± 230 cP). Therefore, these three formulations were selected for further stability studies.

3.8 Stability Studies

3.8.1 Stability Study at 5 ± 3°C (Control Condition)

The optimized formulations (HO-1, HO-3, and HO-6) remained physically stable throughout the six-month storage period

under refrigerated conditions. No noticeable changes in physical appearance were observed. Minor changes in consistency, spreadability, and extrudability were recorded; however, these remained within acceptable pharmaceutical limits. The results indicate that the formulations maintained their quality, stability, and performance during storage.

Table 3.11. Stability Study of Optimized Formulations at 5 ± 3°C for Six Months

Evaluation Parameter	**HO-1 (Initial → Final)**	**HO-3 (Initial → Final)**	**HO-6 (Initial → Final)**
Physical Appearance	Smooth, uniform → No change	Homogeneous → No change	Pale brown, smooth → No change
Consistency	Semi-solid → Slightly firm	Soft → Slightly firmer	Greasy soft → No change
Spreadability (g·cm/s)	15.2 ± 0.35 → 15.0 ± 0.30	16.8 ± 0.28 → 16.5 ± 0.32	14.9 ± 0.31 → 14.8 ± 0.29
Feel on Application	Non-greasy → Same	Soothing → Slightly firm	Soft, greasy → Same
Extrudability (%)	89.5 ± 1.2 → 88.3 ± 1.4	91.2 ± 1.0 → 90.5 ± 1.3	90.1 ± 1.1 → 89.4 ± 1.2

3.8.2 Accelerated Stability Study (40 ± 2°C / 75 ± 5% RH)

The optimized formulations showed satisfactory stability under accelerated storage conditions for six months. Slight changes in colour, consistency, spreadability, and extrudability were observed, but no phase separation or formulation failure occurred. Among the optimized formulations, **HO-3** retained the best overall stability, indicating good resistance to elevated temperature and humidity. These findings suggest that the developed herbal ointments possess acceptable storage stability.

Table 3.12. Accelerated Stability Study of Optimized Formulations at 40 ± 2°C / 75 ± 5% RH for Six Months

Evaluation Parameter	**HO-1 (Initial → Final)**	**HO-3 (Initial → Final)**	**HO-6 (Initial → Final)**
Physical Appearance	Smooth, uniform → Slight dullness	Homogeneous → Mild discoloration	Pale, smooth → Slight opacity
Consistency	Semi-solid → Slightly firmer	Soft, uniform → Slightly reduced softness	Greasy soft → Slight graininess
Spreadability (g·cm/s)	15.2 ± 0.35 →	16.8 ± 0.28 → 16.3 ± 0.34	14.9 ± 0.31 → 14.4 ±

	14.7 ± 0.40		0.36
Feel on Application	Non-greasy → Slightly greasy	Soothing → Slightly sticky	Smooth, greasy → Slightly oily
Extrudability (%)	89.5 ± 1.2 → 87.1 ± 1.6	91.2 ± 1.0 → 88.9 ± 1.5	90.1 ± 1.1 → 87.8 ± 1.7

Conclusion

The present study successfully established the pharmacognostic, physicochemical, phytochemical, and formulation characteristics of *Eulophia nuda* Lindl. tubers, demonstrating their potential as a valuable natural source for topical herbal formulations. Macroscopic and physicochemical evaluations confirmed the authenticity, purity, and quality of the crude drug, while preliminary phytochemical screening revealed the presence of several bioactive constituents, including alkaloids, flavonoids, tannins, phenolics, glycosides, saponins, steroids, triterpenoids, and carbohydrates, which are associated with antioxidant, antimicrobial, and wound-healing activities. Thin-layer chromatography further provided a characteristic chromatographic fingerprint that can be utilized for the identification and quality control of *Eulophia nuda* extracts.

Ten herbal ointment formulations (HO-1 to HO-10) were successfully developed and evaluated for their physicochemical properties. All formulations exhibited acceptable organoleptic characteristics, skin-compatible pH, suitable viscosity, adequate moisture content, good spreadability, and satisfactory extrudability, indicating their suitability for topical application. Based on the overall evaluation, formulations **HO-1, HO-3, and HO-6** demonstrated superior performance, with **HO-3** emerging as the optimized formulation owing to its excellent physicochemical characteristics and application properties. Furthermore, stability studies conducted under refrigerated and accelerated storage conditions confirmed that the optimized formulations maintained their physical integrity and performance throughout the study period, indicating good formulation stability.

Overall, the findings of this investigation suggest that *Eulophia nuda* possesses significant potential as a natural ingredient for the development of stable and effective herbal ointments. The established pharmacognostic standards and formulation data provide a scientific basis for its quality control and future pharmaceutical applications. Further pharmacological, microbiological, and clinical investigations are warranted to validate its therapeutic efficacy and facilitate its development into a standardized topical herbal product.

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Conflict of Interest

The authors declare that there are **no conflicts of interest** regarding the publication of this research paper. The authors alone are responsible for the content and writing of this manuscript.

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References

1. Aulton, M. E., & Taylor, K. M. G. (2018). *Aulton's Pharmaceutics: The Design and Manufacture of Medicines* (5th ed.). Elsevier.
2. Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences, 78*(5), 431–441.
3. Bruneton, J. (1999). *Pharmacognosy, Phytochemistry, Medicinal Plants* (2nd ed.). Lavoisier Publishing.
4. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564–582.
5. Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 5*, 177.
6. Evans, W. C. (2009). *Trease and Evans' Pharmacognosy* (16th ed.). Saunders Elsevier.
7. Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives, 109*(Suppl. 1), 69–75.
8. Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd ed.). Springer.
9. Hossain, M. M. (2011). Therapeutic orchids: Traditional uses and recent advances. *Fitoterapia, 82*(2), 102–140.
10. Kirtikar, K. R., & Basu, B. D. (2006). *Indian Medicinal Plants* (Vol. 4). International Book Distributors.
11. Kokate, C. K. (2010). *Practical Pharmacognosy* (5th ed.). Vallabh Prakashan.
12. Mukherjee, P. K. (2019). *Quality Control and Evaluation of Herbal Drugs* (2nd ed.). Elsevier.
13. Nadkarni, K. M. (2009). *Indian Materia Medica* (3rd ed.). Popular Prakashan.
14. Prasad, K. N., Kumar, V., & Sharma, R. (2014). Phytochemical investigation and biological potential of *Eulophia nuda* Lindl. *International Journal of Pharmaceutical Sciences and Research, 5*(8), 3124–3130.
15. Sofowora, A. (2008). *Medicinal Plants and Traditional Medicine in Africa* (3rd ed.). Spectrum Books.
16. Trease, G. E., & Evans, W. C. (2009). *Trease and Evans' Pharmacognosy* (16th ed.). Saunders Elsevier.
17. Wagner, H., & Bladt, S. (1996). *Plant Drug Analysis: A Thin Layer Chromatography Atlas* (2nd ed.). Springer.
18. World Health Organization. (2011). *Quality Control Methods for Herbal Materials*. WHO Press.

19. World Health Organization. (2013). *WHO Traditional Medicine Strategy 2014–2023*. WHO Press.

20. Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules, 21*(5), 559.