ORIGINAL ARTICLE

Formulation and Physicochemical Evaluation of Herbal Ointment Containing *Tridax procumbens* and *Nyctanthes arbor-tristis* Extracts

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Abstract

Background: There has been increasing interest in herbal formulations as topical agents owing to their varied components and good safety. Two plants known to contain several bioactive components and thus suitable for the development of herbal formulations are *Tridax procumbens* and *Nyctanthes arbor-tristis*. **Objectives:** In the present study, efforts were made to prepare and evaluate an herbal ointment using the hydroalcoholic extracts of *T. procumbens* and *N. arbor-tristis*. **Materials and Methods:** Leaves of both plants were used for the preparation of the hydroalcoholic extracts by the Soxhlet method. Both the extracts were screened preliminarily for phytochemical analysis. Three ointment preparations (F1, F2, and F3) were prepared by using a paraffin base for the preparation of the ointments. The formulations were tested for their organoleptic properties, pH, homogeneity, spreadability, extrudability, viscosity, washability, uniformity of drug content, and stability at room temperature and accelerated storage. **Results:** Both plant extracts were extracted in adequate amounts through the above procedure. The phytochemical tests detected the presence of flavonoids, phenols, tannins, glycosides, terpenoids, saponins, and alkaloids. All formulations had satisfactory physicochemical properties that included smooth appearance, satisfactory homogeneity, satisfactory pH, satisfactory spreadability, extrudability, viscosity, washability, and uniform drug content. **Conclusion:** The formulated herbal ointment had suitable physicochemical properties and stability, thus suggesting that the preparation would make an appropriate semisolid preparation for topical administration. However, further studies are necessary to assess its therapeutic effectiveness.

Key words: Herbal ointment, *Nyctanthes arbor-tristis*, physicochemical evaluation, *Tridax procumbens*

INTRODUCTION

Herbal medicines have been used for centuries for the prevention and treatment of various diseases because of their therapeutic efficacy and natural origin.[1] In recent years, the use of herbal products in topical drug delivery has increased owing to their safety, effectiveness, cost-effectiveness, and better patient acceptance.[2] Topical drug delivery involves the application of a formulation directly to the skin to achieve a local therapeutic effect while minimizing systemic adverse effects.[3] Semisolid dosage forms such as ointments, creams, and gels are widely used for topical administration because they provide prolonged contact with the affected area and improve drug retention.[3,4]

Medicinal plants contain various bioactive constituents, including flavonoids, tannins, alkaloids, saponins, and phenolic compounds, which exhibit antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties.[5,6] Compared with oral administration, topical delivery bypasses first-pass metabolism, reduces gastrointestinal side effects, and delivers the active constituents directly to the site of action.[3] These benefits have prompted scientists to come up with herbal topical preparations for wound healing, burn care, skin infections, and inflammatory diseases.[2,5] Scientific standardization

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of herbal topical products is mandatory through extraction techniques, formulation preparation, and physicochemical analysis to assure quality, stability, and efficacy of the herbal products.[4,6]

Herbal ointments are semi-solid dosage forms that contain herbal plant extracts in an appropriate ointment base for topical administration.[3] The herbal ointments provide a protective layer on the damaged skin, retain moisture, and allow slow release of the herbals present in the preparation.[4] The ointments are beneficial in treating wounds, burns, abrasions, dry skin lesions, and inflammatory skin disorders due to their emollient and occlusive action.[3,5]

The therapeutic effectiveness of a herbal ointment depends not only on the pharmacological activity of the plant extract but also on the quality of the formulation.[4] Physicochemical parameters such as appearance, homogeneity, pH, spreadability, viscosity, extrudability, washability, stability, and drug content are important indicators of product quality and patient acceptability.[4,7] Therefore, systematic formulation and physicochemical evaluation are essential for developing a stable, safe, and effective herbal ointment.[4,7] The increasing demand for plant-based topical formulations has further highlighted the importance of scientifically validated herbal ointments that combine traditional medicinal knowledge with modern pharmaceutical standards.[2,5]

Tridax procumbens

Botanical description

T. procumbens L. belongs to the family Asteraceae and is a perennial creeping herb commonly known as coat buttons or Mexican daisy. It is widely distributed throughout tropical and subtropical regions, including India, where it grows along roadsides, wastelands, agricultural fields, and grasslands. The plant possesses a hairy procumbent stem, opposite ovate to lanceolate leaves, solitary flower heads with white ray florets and yellow disc florets, and achene fruits bearing feathery pappus.[8,9]

Traditional uses

T. procumbens has been extensively used in traditional medicine for the treatment of wounds, cuts, burns, skin infections, diarrhea, dysentery, fever, and inflammation. Fresh leaf paste or juice is commonly applied to wounds to control bleeding and promote healing.[8-10]

Phytochemical constituents

Phytochemical studies have reported the presence of flavonoids, alkaloids, tannins, saponins, terpenoids, steroids, phenolic compounds, carotenoids, glycosides, and phytosterols in *T. procumbens*. These bioactive constituents contribute to its medicinal properties.[8,9]

Reported pharmacological properties

Experimental studies have demonstrated that *T. procumbens* possesses wound-healing, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, hepatoprotective, immunomodulatory, and anticancer activities. Among these, wound-healing and anti-inflammatory activities are considered most relevant for topical pharmaceutical formulations.[8,9]

Nyctanthes arbor-tristis

Botanical description

N. arbor-tristis L., commonly known as Parijat or Night Jasmine, belongs to the family Oleaceae. It is a small deciduous tree widely distributed throughout India and neighboring Asian countries. The plant bears fragrant white flowers with orange tubular centers that bloom during the night and fall in the morning.[11,12]

Traditional uses

In Ayurveda, *N. arbor-tristisis* is traditionally used for the management of fever, arthritis, rheumatism, skin diseases, inflammatory disorders, cough, and intestinal worm infestations. Different parts of the plant, particularly the leaves and flowers, have also been used for wound care and various infectious conditions.[11-13]

Phytochemical constituents

The plant contains several phytochemicals, including iridoid glycosides, flavonoids, phenolic compounds, tannins, alkaloids, terpenoids, steroids, carotenoids, and essential oils. These constituents are responsible for its diverse pharmacological activities.[11,12]

Reported pharmacological properties

Scientific investigations have demonstrated that *N. arbor-tristis* possesses anti-inflammatory, antioxidant, antimicrobial, antipyretic, hepatoprotective, antidiabetic, antiviral, immunomodulatory, and wound-healing activities. These biological properties support its potential application in topical herbal formulations.[11,12]

Rationale for combining both plants

T. procumbens and *N. arbor-tristis* contain complementary phytochemicals, including flavonoids, tannins, phenolic compounds, and terpenoids, which exhibit antioxidant, antimicrobial, and anti-inflammatory activities.[8,11] The wound-healing potential of *T. procumbens* together with the anti-inflammatory activity of *N. arbor-tristis* may provide a synergistic therapeutic effect when incorporated into a topical ointment.[8,11] Therefore, combining extracts of both plants offers a scientific basis for the development of a herbal

ointment intended for topical application and subsequent physicochemical evaluation.[8,11]

MATERIALS AND METHODS

Materials

Fresh leaves of T. procumbens and N. arbor-tristis were used for the present study. Ethanol (95%), petroleum jelly, hard paraffin, cetostearyl alcohol, wool fat, and all other chemicals and reagents used during extraction, phytochemical screening, and formulation were of analytical grade and procured from a certified chemical supplier. Distilled water was used throughout the experimental work.[12]

Collection and authentication of plant materials

Fresh leaves of *T. procumbens* and *N. arbor-tristis* were collected from the medicinal plant garden of Mahatma Gandhi Ayurvedic College, Salod (H), Wardha, Maharashtra, India. The plant material was identified by an expert taxonomist, and voucher samples were stored in the herbarium of the department for further use.[13]

Extraction procedure of *T. procumbens* leaves

Preparation of plant extract

Newly harvested leaves of *T. procumbens* were rinsed with tap water and subsequently distilled water to eliminate any attached dust particles. Drying of the fresh leaves was done in shade for 7–10 days at room temperature (25–30°C) till constant weight was achieved. The dried leaves were mechanically crushed and sieved through a 40-mesh sieve. About 100 g of the powdered leaves was placed in a Soxhlet extractor and extracted with 500 mL of 95% ethanol for 6–8 h until the color of the solvent in the siphon tube turned colorless. The resulting crude extract was then filtered using Whatman No. 1 filter paper and concentrated by reducing pressure under the rotary vacuum evaporator at 40–45°C. The concentrated crude extract was dried using a vacuum desiccator. The dried crude extract was weighed and its yield calculated while stored in an air-tight amber colored glass bottle kept at 4°C.[14,15]

Extraction procedure of *N. arbor-tristis* leaves

Preparation of plant extract

N. arbor-tristis fresh leaves were collected and identified. Leaves were washed with running tap water and distilled water to wash away dirt and other contaminants. Shade-drying of the washed leaves was done for 7–10 days. Leaves were then crushed into a coarse powder with the help of a mechanical grinder. About 100 g of the powdered leaves were macerated in 70% ethanol (70:30 ratio of ethanol and water) using the Soxhlet extraction method for 6–8 h. The extraction process was carried out till complete exhaustion of the plant materials. The extract obtained was filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator maintained at 40°C. The concentrated extract was dried in a desiccator to obtain a semisolid residue. The dried extract was weighed, the percentage yield was calculated, and the extract was stored in airtight amber-colored containers at 4°C until further analysis and formulation studies.[16,17]

Preliminary phytochemical screening

The ethanolic extracts of *T. procumbens* and *N. arbor-tristis* leaves were subjected to preliminary phytochemical screening using standard qualitative methods to identify the presence of major phytoconstituents, including alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, steroids, terpenoids, and carbohydrates. The presence or absence of these phytochemical constituents was determined based on characteristic color changes or precipitate formation according to the standard methods described by Harborne.[18]

Preparation of ointment base

The ointment base was prepared by the fusion method. Accurately weighed quantities of hard paraffin, cetostearyl alcohol, white soft paraffin, and wool fat were melted in descending order of their melting points over a water bath. The molten ingredients were mixed thoroughly with continuous stirring until a uniform mixture was obtained. The prepared ointment base was allowed to cool gradually with constant stirring to obtain a smooth and homogeneous base and stored in a clean, airtight container until further use.[19]

Formulation of herbal ointment

Formulations of the herbal ointments (F1, F2, and F3) were prepared by the fusion technique with minor modifications. First, the requisite amount of hard paraffin was measured precisely and melted in a porcelain evaporating dish on a water bath kept at 70–75°C. Upon complete melting, cetostearyl alcohol and wool fat were added one after another, followed by continuous stirring until a clear and homogeneous mixture in a liquid state is obtained. Then, yellow soft paraffin was added to the liquid mass and stirred continuously until a homogeneous ointment base was obtained. Extracts of *T. procumbens* and *N. arbor-tristis* were measured precisely according to their composition in the formulae. Extracts were triturated with a little quantity of the prepared ointment base with the help of the process of levigation for obtaining a fine homogeneous paste without any lumps. The rest of the ointment base was gradually added to the mixture and triturated until an ointment was obtained. Formulations were

Ingredients	Function	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)
Tridax procumbens extract	Active ingredient	1.25	2.50	3.75
Nyctanthes arbor-tristis extract	Active ingredient	1.25	2.50	3.75
Wool fat	Absorption base	5.0	5.0	5.0
Cetostearyl alcohol	Stiffening agent	5.0	5.0	5.0
Hard paraffin	Stiffening agent	5.0	5.0	5.0
Yellow soft paraffin	Ointment base	q.s. to 100	q.s. to 100	q.s. to 100

cooled down gradually to room temperature with continuous stirring to make sure that the herbal extracts are uniformly distributed in the preparation without any phase separation. Formulations were kept in sterile, dry, and wide-mouthed vessels marked F1, F2, and F3.[20]

Three combinations of F1, F2, and F3 having varying concentrations of herbal extracts were prepared to assess the effect of variation in the concentration of the extracts on the physicochemical parameters of the ointment.

Evaluation of herbal ointment

The prepared herbal ointments (F1, F2, and F3) were assessed for their organoleptic and physicochemical properties along with their stability through standard pharmaceutical analysis techniques.[21-23]

Organoleptic evaluation

Color, odor, appearance, and texture

Organoleptic properties of the prepared ointments, such as color, odor, appearance, and consistency, were assessed in normal daylight conditions. Color and appearance were observed by visual inspection while odor was checked by sensory inspection. The consistency was determined by rubbing a little amount of the ointment between the thumb and index fingers.[21,22]

Physicochemical evaluation

pH

Around 1 g of ointment was mixed with 100 mL of distilled water and kept for 2 h. The pH was determined with the help of a calibrated digital pH meter.[23]

Homogeneity

The homogeneity of the prepared formulations was determined by visual inspection based on the observation of the appearance of the formulations to see whether there were any lumps, coarse materials, or phases.

Spreadability

Spreadability was determined by using the glass slide method. An amount of about 0.5 g of the ointment was spread between two glass slides. A known amount of weight was applied to the upper glass slide to give a uniform film. The time taken for the upper glass slide to slide over the lower one due to the applied load was noted down using the following formula:

$$S=M\times L/T$$

Where:
S = Spreadability
M = Weight tied to the upper slide
L = Length of glass slide
T = Time taken to separate the slides.

Extrudability

The prepared ointments were filled into collapsible aluminum tubes. Pressure was exerted on the tube, and the quantity of ointment extruded from the nozzle was measured. Extrudability was calculated by the following formula:[23]

$$\text{Extrudability} = \text{Amount of ointment extruded from the tube} \times 100/\text{Total amount of ointment filled in the tube}$$

Washability

A little amount of ointment was applied to the skin and washed away with running tap water. Ease of removal of ointment without residue was observed visually.[23]

Viscosity

Viscosity of formulations was measured using the Brookfield digital viscometer, having the appropriate spindle at 25 ± 2°C. Measurement was conducted in triplicate, and the mean value was calculated.[21,22]

Consistency

Consistency of ointments was measured manually by applying pressure on a small quantity of ointment between the thumb and index finger.[21]

Grittiness

For testing the presence of gritty particles in the formulations, a small quantity of ointment was rubbed between the fingers. Absence of gritty particles indicates the good quality of formulation.[21,22]

Drug content uniformity

A precise weight of 1 g of ointment was taken and dissolved in a suitable solvent to extract the drug from the matrix. This solution was filtered and suitably diluted. Content of the drug

was determined by ultraviolet-visible spectrophotometer at a selected wavelength.

Loss on drying

Approximately 1 g of ointment was accurately weighed in a pre-weighed Petri dish and dried in a hot air oven at 105°C until a constant weight was obtained. The percentage loss on drying was calculated using the following equation:[22]

LOD (%) = Initial weight−Final weight/Initial weight × 100

Stability study

The stability of the herbal ointment formulations was evaluated according to the ICH Q1A(R2) guidelines.[24]

The formulations were packed in airtight containers and stored under the following conditions:

Room temperature (25 ± 2°C)
Accelerated condition (40 ± 2°C/75 ± 5% RH).

The formulations were evaluated over a period of 3 months. Samples were withdrawn at 0, 1, 2, and 3 months and assessed for color, odor, appearance, pH, homogeneity, spreadability, extrudability, viscosity, washability, consistency, grittiness, and drug content uniformity. Any changes observed during storage were recorded.[24]

Statistical analysis

All experiments were conducted in triplicate (n = 3), and the results were reported as mean ± standard deviation (SD). Experimental data were collected and analyzed using Microsoft Excel 2021 software for calculating the mean and SD of each evaluation parameter.

RESULTS

Percentage yield of extracts

The ethanolic leaf extracts of *T. procumbens* and *N. arbor-tristis* were successfully prepared using the Soxhlet method. The percentage yield of *N. arbor-tristis* extract was slightly higher than that of *T. procumbens* (Table 1), which could be due to different phytochemical content and solvent extractability of plants.

Phytochemical screening results

Preliminary screening for the presence of phytochemicals in both plant extracts showed the existence of some active principles in the extracts (Table 2). The phytochemicals such as flavonoids, tannins, phenols, saponins, terpenoids, and glycosides were present in both plants while alkaloids were found only in trace quantities.

Table 1: Percentage yield of plant extracts

Plant	Weight of powder (g)	Weight of extract (g)	Percentage yield (%)
Tridax procumbens	100	12.8	12.8±0.3
Nyctanthes arbor-tristis	100	14.5	14.5±0.4

Table 2: Preliminary phytochemical screening

Phytochemical constituent	*Tridax procumbens*	*Nyctanthes arbor-tristis*
Alkaloids	+	+
Flavonoids	+++	+++
Tannins	++	++
Saponins	+	+
Glycosides	++	+++
Phenolic compounds	+++	+++
Terpenoids	++	++
Steroids	+	–
Carbohydrates	+	+
Proteins	–	–

–: Absent, +: Present, ++: Moderately present, +++: Abundantly present

Table 3: Organoleptic evaluation

Parameter	F1	F2	F3
Color	Yellowish-green	Yellowish-green	Greenish-yellow
Odor	Characteristic	Characteristic	Characteristic
Appearance	Smooth	Smooth	Smooth
Texture	Semisolid	Semisolid	Semisolid

Evaluation of herbal ointment

Organoleptic properties

The prepared formulations were in a yellowish-green color having a typical herbal odor. The ointments possessed a smooth texture and were semi-solid in nature without phase separation and gritty particles (Table 3).

Physicochemical evaluation

The pH of the preparations was similar to that of healthy skin (pH = 5.5–6.5). All the preparations were found to have good spreadability, good homogeneity, adequate extrudability, and no grittiness (Table 4). The viscosity rose gradually as the concentration of the extract increased.

Table 4: Physicochemical evaluation of herbal ointments

Parameter	F1	F2	F3
pH	5.52±0.04	5.86±0.03	6.18±0.05
Spreadability (g·cm/s)	27.6±0.5	25.8±0.6	24.3±0.4
Extrudability (%)	92.5±1.2	90.8±1.4	88.6±1.1
Viscosity (cP)	25,800±180	27,450±210	29,120±250
Homogeneity	Good	Good	Good
Washability	Good	Good	Good
Consistency	Smooth	Smooth	Smooth
Grittiness	Absent	Absent	Absent
Drug content (%)	97.8±0.9	98.6±0.7	99.2±0.8
Loss on drying (%)	2.15±0.12	2.28±0.09	2.36±0.11

Table 5: Stability study of herbal ointment formulations

Parameter	F1	F2	F3
Color	No change	No change	No change
Odor	No change	No change	No change
Appearance	Smooth and uniform	Smooth and uniform	Smooth and uniform
pH	5.52→5.49	5.86→5.82	6.18→6.13
Homogeneity	Good	Good	Good
Spreadability (g·cm/s)	27.6→27.2	25.8→25.5	24.3→24.0
Extrudability (%)	92.5→91.8	90.8→90.1	88.6→87.9
Viscosity (cP)	25,800→26,050	27,450→27,700	29,120→29,450
Washability	Good	Good	Good
Drug content (%)	97.8→97.2	98.6→98.0	99.2→98.5

Stability study

Physical and chemical stability of the formulated herbal ointments (F1, F2, and F3) was assessed at two different temperatures: room temperature (25 ± 2°C) and 40 ± 2°C/75 ± 5% RH for a period of 3 months (Table 5). During the entire duration of this experiment, all formulations maintained their initial color, odor, smoothness, and homogeneity without showing any signs of separation or granular character. Small variations were noted in the values of pH, spreadability, viscosity, extrudability, and drug content in formulations while stored; however, these changes occurred within the permissible limit.

DISCUSSION

A successful formulation and evaluation of the herbal ointment using the *T. procumbens* and *N. arbor-tristis* extracts have been done in this study. The hydroalcoholic method of extraction gave adequate amounts of extracts from both the herbs, indicating that the solvent used was appropriate for extracting bioactive phytochemicals. It could be noted that the slight difference in extraction yields between the two plants could be due to their differences in the constituent parts. Extraction efficiency in this study is comparable to other reports on the use of hydroalcoholic solvents in medicinal plants' extraction.

Initial tests of phytochemical analysis established the existence of several phytoconstituents with biological activity such as flavonoids, tannins, phenolics, glycosides, terpenoids, saponins, and alkaloids. The abundance of flavonoids and phenolics in the two plant species was noted, implying that these compounds can be considered as contributing to the antioxidant, anti-inflammatory, antimicrobial, and wound healing activity of these medicinal plants. The phytochemical analysis carried out in the current research concurs with studies conducted earlier on *T. procumbens* and *N. arbor-tristis* with regard to the type of phytoconstituents present.

The choice of the ointment base made up of yellow soft paraffin, hard paraffin, cetostearyl alcohol, and wool fat was seen as being ideal for use in incorporating both herbal extracts into an ointment. It gave homogeneous semi-solid ointments without any sign of phase separation and incompatibility between the extracts and other components. Paraffin-based base systems gave good consistency and easy applicability of the herbal extracts.

The physicochemical assessment showed satisfactory pharmaceutical properties for all formulations. The pH values of all the tested formulations fell into the physiological range of the skin, making the topical application safe with minimal irritation. All the formulations possessed good homogeneity, proper texture, and were free of grittiness, which indicated the homogeneous distribution of the extract in the base. Good spreadability allowed uniform application of the formulation on the surface of the affected area. The extrudability of the tested formulations was satisfactory for the easy removal from collapsible tubes. The viscosity of the formulations was suitable for maintaining the semisolid state and did not affect the spreadability. Drug content analysis of the formulations proved satisfactory uniformity of the incorporated extracts. The stability study confirmed that the prepared formulations maintained all the organoleptic and physicochemical properties during storage under both room and accelerated storage conditions. There were no changes in color, odor, appearance, homogeneity, and washability of the tested formulations. Some changes in the pH value, spreadability, viscosity, extrudability, and drug content were noted during the storage; however, they were still within acceptable pharmaceutical ranges. These results indicate the successful maintenance of the stability of the incorporated extracts by the selected ointment base. In general, the results obtained during the present investigation agree with the data of other studies dealing with the preparation of the ointment formulations of herbal extracts. Such properties as the pH, spreadability, homogeneity, viscosity, and stability of the formulated products were satisfactorily maintained in the studies dealing with herbal topical formulations with plant extracts incorporated in the paraffin-based ointment formulations. The agreement between the present data and those previously obtained confirms the feasibility of the selected formulation approach. Therefore, the developed herbal ointments possess desired physicochemical properties and adequate stability and provide successful incorporation of the biologically active extracts of herbs into the semi-solid formulation. The used formulation approach can be considered a pharmaceutically acceptable one for the development of the topical formulation. Taking into consideration the multiplicity of phytoconstituents possessing different biological activities, the developed formulation may be considered as a prospective object for future pharmacological, antimicrobial, wound healing, and clinical studies.

CONCLUSION

In the current study, bioactive components were successfully extracted from *T. procumbens* and *N. arbor-tristis* using a hydroalcoholic extraction technique and incorporated them in an herbal ointment using a paraffin ointment base. Preliminary screening revealed the presence of important secondary metabolites such as flavonoids, tannins, phenolics, glycosides, saponins, terpenoids, and alkaloids in the prepared formulations, suggesting high phytochemical content in the selected plants. The prepared formulations showed good organoleptic properties, appropriate pH, homogeneity, spreadability, extrudability, viscosity, washability, and uniform drug content. In addition, the prepared formulations did not undergo any physical change during the storage studies under different conditions. It has been shown that the prepared herbal ointment is pharmaceutically acceptable and has proper physicochemical properties to be used topically. The developed formulation can prove to be a good platform for further studies on biological activity, safety, and clinical evaluation.

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