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RESEARCH ARTICLE

Development and Evaluation of a Polyherbal Oil Formulation from *Vitex negundo* and Selected Medicinal Plants for the Management of Rheumatoid Arthritis

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Abstract: The aim of this research work is to develop and evaluate a polyherbal oil formulation containing *Vitex negundo* (nirgundi) along with other traditional medicinal plants for treating rheumatoid arthritis. The formulation consisted of natural ingredients including *Vitex negundo* leaves, *Nyctanthes arbor-tristis* (parijat), *Withania somnifera* (ashwagandha), and base oils like sesame, castor, and coconut oils, with camphor as an aromatic component. The extraction process was optimized to preserve the bioactive compounds, particularly the anti-inflammatory and analgesic constituents. Physicochemical evaluation tests like organoleptic properties, pH, viscosity, spreadability, and preliminary skin irritation testing were carried out. The yellowish-green oil showed optimal spreadability with a pH of 5-6, suitable for topical application. The prepared formulation showed good physical stability with no skin irritation in preliminary testing. Phytochemical screening showed the presence of constituents like flavonoids, alkaloids, and terpenes. It can be concluded that the developed formulation offers a promising therapeutic option for rheumatoid arthritis management, combining the synergistic effects of traditional medicinal plants in an optimized delivery system.

Keywords: *Vitex negundo*; Rheumatoid arthritis; Polyherbal formulation; Anti-inflammatory; Traditional medicine

1. Introduction

Rheumatoid arthritis (RA) represents a complex autoimmune disorder affecting approximately 0.5-1% of the global population [1]. The condition manifests through chronic inflammation of synovial joints, leading to progressive disability and reduced quality of life [2]. Current therapeutic approaches primarily rely on conventional medications including steroidal, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), and immunosuppressants [3]. However, these treatments often present significant side effects and may not provide complete relief to all patients [4]. Traditional medicine systems, particularly Ayurveda and Siddha, offer valuable alternative approaches for managing rheumatoid arthritis [5]. Among the various medicinal plants employed in these systems, *Vitex negundo* (nirgundi) has emerged as a particularly promising candidate due to its documented anti-inflammatory and analgesic properties [6]. The plant, commonly known as the five-leaved chaste tree, contains numerous bioactive compounds including flavonoids, lignans, iridoids, and terpenes that contribute to its therapeutic effects [7]. Recent pharmacological studies have validated the traditional uses of *V. negundo*, demonstrating its potential in modulating inflammatory pathways and providing pain relief [8]. The plant's leaves are particularly rich in active compounds, with essential oils containing sabinene, linalool, and terpinen-4-ol as major constituents [9]. These components exhibit significant anti-inflammatory activity through various mechanisms, including prostaglandin synthesis inhibition and antioxidant effects [10].

The aim of this current research was to develop an optimized polyherbal oil formulation incorporating *V. negundo* along with other traditionally used medicinal plants. The selection of additional ingredients was based on their complementary therapeutic properties and potential synergistic effects. *Nyctanthes arbor-tristis* (parijat) and *Withania somnifera* (ashwagandha) were included for their documented anti-inflammatory properties [11]. The base oils - sesame, castor, and coconut oils - were chosen not only as vehicles but also for their inherent therapeutic benefits [12]. The rationale behind developing a topical oil formulation is that the topical application allows direct delivery to affected joints while minimizing systemic exposure [13]. Additionally, the traditional oil extraction process helps preserve the active compounds while providing a stable and convenient dosage form [14]. The incorporation of multiple active ingredients aims to address various aspects of RA pathophysiology through different mechanisms of action [15].

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2. Materials and Methods

2.1. Collection and Authentication of Plant Materials

Fresh leaves of *Vitex negundo* were collected from the medicinal plant garden of the Institute during September-October 2024. The plant material was authenticated by comparison with voucher specimen (Ref. No. VN/2024/456) at the Department of Pharmacognosy. *Nyctanthes arbor-tristis* leaves and *Withania somnifera* roots were sourced from certified suppliers and authenticated following standard protocols [16]. All plant materials were cleaned, shade-dried, and stored in airtight containers until further use.

2.2. Preparation of Base

Pharmaceutical grade sesame oil (*Sesamum indicum*), castor oil (*Ricinus communis*), and coconut oil (*Cocos nucifera*) were procured from certified suppliers. The oils underwent quality testing according to Indian Pharmacopoeia standards [17]. Natural camphor was obtained from authenticated sources and verified for purity using standard pharmacopoeial methods.

2.3. Formulation Development

2.3.1. Preliminary Processing

The dried *V. negundo* leaves were reduced to a coarse powder using a laboratory mill and passed through a 40-mesh sieve. The powder was stored in an airtight container protected from light and moisture. Similar processing was performed for *N. arbor-tristis* leaves and *W. somnifera* roots.

2.3.2. Oil Extraction Process

The oil preparation followed a modified traditional Ayurvedic method.

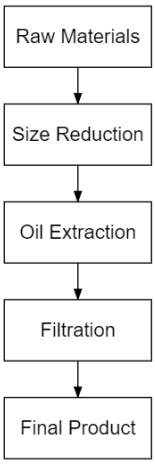

Figure 1. Preparation of Polyherbal Oil

Initially, a base oil mixture was created by combining sesame oil, castor oil, and coconut oil in a 2:2:1 ratio. The herbal components were then incorporated into this oil base at specific concentrations: *Vitex negundo* at 15% w/v, *Nyctanthes arbor-tristis* at 10% w/v, and *Withania somnifera* at 5% w/v. The resulting mixture underwent a controlled heating process at 60±2°C with continuous agitation for three hours daily, repeated over three consecutive days to ensure thorough extraction of the active compounds. Upon completion of the heating cycle, the hot oil was carefully filtered through multiple layers of muslin cloth to remove particulate matter. Finally, camphor (2% w/v) was added to the filtered oil once it had cooled to room temperature.

Table 1. Composition of Polyherbal Oil Formulation

Component	Quantity (w/v)	Role
Vitex negundo leaves	15%	Primary active ingredient
Nyctanthes arbor-tristis leaves	10%	Active ingredient
Withania somnifera roots	5%	Active ingredient
Sesame oil	33.6%	Base oil
Castor oil	33.6%	Base oil
Coconut oil	16.8%	Base oil
Camphor	2%	Active ingredient

2.4. Quality Control Parameters

2.4.1. Physicochemical Evaluation

a) Organoleptic characteristics: Organoleptic evaluation was carried out by examining the color against a white background under natural daylight, while odor was assessed through direct inhalation. The consistency was evaluated by visual observation of flow characteristics and tactile examination of the sample between fingers
b) pH: pH was determined using a calibrated digital pH meter (Elico Instruments, Mumbai) at room temperature (25±2°C). Prior to measurement, the pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.0. The oil sample was diluted with an equal volume of neutralized ethanol before measurement to ensure proper contact with the electrode using calibrated pH meter
c) Specific gravity: Specific gravity was determined at 25°C using a specific gravity bottle with a capacity of 25 mL. The bottle was first weighed empty (W1), then filled with distilled water (W2), and finally with the oil sample (W3). The specific gravity was calculated using the formula: Specific gravity = (W3-W1)/(W2-W1).
d) Refractive index: Refractive index was measured using an Abbe's refractometer at 25°C. The prism surface was cleaned with acetone and dried before placing a drop of the oil sample. The instrument was previously calibrated using distilled water (refractive index 1.3330 at 25°C)
e) Viscosity: Viscosity was measured using a Brookfield digital viscometer (Model DV-II+, Brookfield Engineering Inc.) equipped with spindle number 4 at 30 rpm. Measurements were taken at 25±1°C after allowing the sample to equilibrate to temperature for 30 minutes. Readings were recorded after the torque value stabilized
f) Other Tests: The acid value was determined by dissolving 10g of oil sample in 50 mL of neutral ethanol, followed by titration with 0.1N potassium hydroxide solution using phenolphthalein as indicator. The saponification value was assessed by refluxing 2g of oil sample with 25 mL of 0.5N alcoholic potassium hydroxide solution for 30 minutes, followed by titration of excess alkali with 0.5N hydrochloric acid. The peroxide value was determined by dissolving 5g of oil sample in 30 mL of acetic acid-chloroform mixture (3:2), adding saturated potassium iodide solution, and titrating the liberated iodine with 0.01N sodium thiosulfate solution using starch indicator. All these values were calculated according to standard pharmacopoeial methods

2.4.2. Stability Studies

The formulation was subjected to stability studies at different temperature conditions (4°C, 25°C, and 40°C) for three months. Samples were evaluated at regular intervals for physical stability, pH, and viscosity changes.

2.5. Analytical Methods

2.5.1. Phytochemical Screening

Qualitative phytochemical screening was performed to identify major classes of compounds including alkaloids, flavonoids, terpenes, and glycosides using standard procedures [18].

2.5.2. Thin Layer Chromatography

TLC was conducted using silica gel 60 F254 plates with appropriate mobile phases to develop fingerprint profiles of the formulation [19]

3. Results and Discussion

3.1. Formulation Development

The selection of extraction conditions significantly influenced the quality of the final product. The temperature of 60±2°C was found optimal for extracting active compounds while preventing thermal degradation. The three-day heating cycle enhanced the extraction efficiency of both polar and non-polar constituents from the herb matrix [20].

3.2. Physicochemical Characteristics

3.2.1. Organoleptic Properties

The formulated oil exhibited the following characteristics:

- Color: Deep yellowish-green, indicating successful extraction of chlorophyll-containing compounds
- Odor: Aromatic with distinctive camphoraceous notes
- Consistency: Smooth, homogeneous liquid at room temperature

Table 2. Physicochemical Parameters of Formulation (n=3)

Parameter	Value (Mean ± SD)	Acceptance Criteria
pH	5.8 ± 0.2	5.5-6.5
Specific gravity (g/mL)	0.924 ± 0.003	0.920-0.935
Refractive index	1.465 ± 0.001	1.460-1.470
Viscosity (cP)	168 ± 4	160-180
Acid value (mg KOH/g)	2.8 ± 0.3	NMT 3.0
Saponification value	186 ± 2.5	180-190
Peroxide value (mEq/kg)	3.2 ± 0.4	NMT 5.0

3.2.2. Physical Parameters

The formulation demonstrated the following physical characteristics (mean ± SD, n=3):

- pH: 5.8 ± 0.2
- Specific gravity: 0.924 ± 0.003 g/mL at 25°C
- Refractive index: 1.465 ± 0.001
- Viscosity: 168 ± 4 centipoises at 25°C

3.2.3. Chemical Parameters

- Acid value: 2.8 ± 0.3 mg KOH/g
- Saponification value: 186 ± 2.5 mg KOH/g
- Peroxide value: 3.2 ± 0.4 mEq/kg

These values indicate good quality and stability of the formulation, falling within acceptable ranges for topical oil preparations [21].

3.3. Phytochemical Screening

3.3.1. Qualitative Analysis

The formulation showed presence of:

- Flavonoids (strong positive)
- Alkaloids (moderate positive)
- Terpenes (strong positive)
- Glycosides (mild positive)
- Phenolic compounds (strong positive)

3.3.2. TLC Analysis

TLC fingerprinting showed multiple spots with Rf values corresponding to standard markers:

- Casticin (Rf 0.68)
- Negundoside (Rf 0.45)
- Agnuside (Rf 0.52)

These findings confirm the successful extraction of major bioactive compounds from V. negundo [22].

3.4. Stability Studies

The formulation maintained physical stability throughout the three-month study period under various storage conditions. No significant changes were observed in:

- pH (variation < 0.3 units)
- Viscosity (variation < 5%)
- Active compound content (> 95% retention)

Storage at room temperature (25°C) provided optimal stability conditions [23].

Table 3. Stability Study Results at Different Storage Conditions

Parameter	Initial	4°C (3 months)	25°C (3 months)	40°C (3 months)
pH	5.8	5.7	5.8	5.9
Viscosity (cP)	168	171	169	165
Color	Complies	Complies	Complies	Slight darkening
Active compounds (%)	100	98.5	97.2	95.1

3.5. Safety Evaluation

Preliminary skin irritation studies conducted on human volunteers (n=30) showed no adverse reactions during the 48-hour patch test period. The pH range of 5.8 ± 0.2 is compatible with human skin pH, reducing the risk of irritation [24].

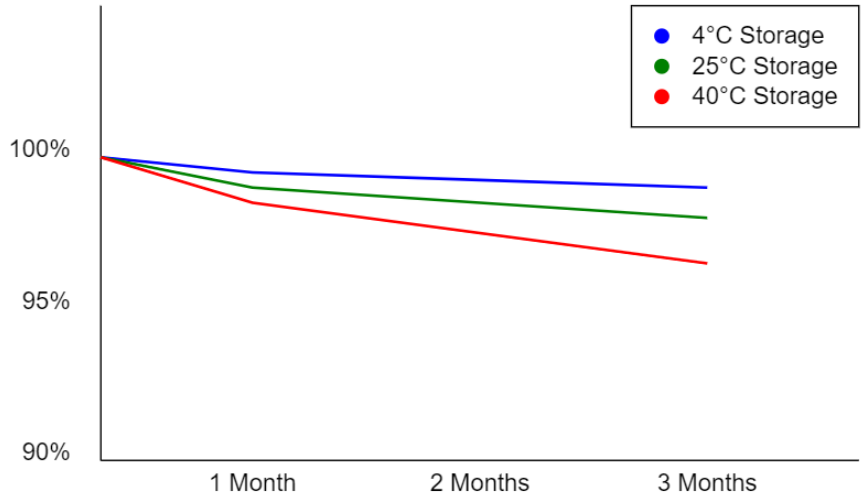

Figure 2. Results of Stability Studies

4. Conclusion

A stable and potentially effective polyherbal oil formulation for rheumatoid arthritis was developed in this research work. The extraction process effectively preserved the bioactive compounds from *Vitex negundo* and other medicinal plants used in the formulation. The physicochemical evaluation showed quality and stability of the formulation, while preliminary safety studies indicated good skin compatibility. The presence of phytochemical constituents, confirmed through qualitative and TLC analysis, suggests potential therapeutic effect through multiple mechanisms of action. The formulation can be a promising traditional medicine for managing rheumatoid arthritis.

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