FORMULATION AND EVALUATION OF A HERBAL ROLL-ON BASED ON COMPARATIVE EXTRACTION METHODS AND PHYTOCHEMICAL SCREENING OF MENTHA PIPERITA AND CURCUMA LONGA

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Abstract

The present investigation is aimed to formulation and evaluation of a herbal roll-on, to compare the percentage value of *Mentha Piperita* extracts by using different solvent and extraction methods, phytochemical screening of constituents presents in dry leaves. Mentha piperita, commonly known as peppermint or Pudina, is a widely cultivated aromatic perennial herb valued for its culinary, medicinal, and cosmetic applications due to its high menthol content and cooling properties. Menthol (also known as mint camphor) is a cyclic monoterpene alcohol which is found as a major constituent in the essential oils of *Mentha* species and responsible for the distinctive smell and flavour of the plant. A Roll-on is a type of liquid preparation packed in a container with an applicator consisting of a revolving ball at the top of the dispenser. Mentha piperita, commonly known as peppermint, is a sterile hybrid perennial herb valued worldwide for its aromatic leaves and essential oil rich in menthol and menthone, and is cultivated and utilized for culinary fl avoring, traditional and modern medicinal applications addressing digestive, respiratory, muscular, and der matological conditions, as well as in oral hygiene products, confectionery, beverages, aromatherapy, and per sonal care, while thriving in moist, welldrained soils and spreading vegetatively through rhizomes.

(Key words: extraction, roll-on, mentha piperita, phytochemical screening, comparison)

INTRODUCTION

Herbal formulations are increasingly valued for their natural origin, minimal side effects, and broad therapeutic potential. Topical preparations such as roll-ons are particularly popular for pain relief, stress reduction, and inflammatory conditions because they allow direct application to affected areas, ensuring rapid absorption and localized action [1]. The present project, "Formulation and Evaluation of a Herbal Roll-On Based on Comparative Extraction Methods and Phytochemical Screening of *Mentha piperita* and *Curcuma longa*", integrates traditional knowledge with modern extraction and analytical techniques to develop an effective natural remedy.

Essential oils are concentrated hydrophobic liquids containing volatile aromatic compounds derived from plants. They are widely used in pharmaceuticals, cosmetics, and food industries due to their antimicrobial,

antioxidant, analgesic, and anti-inflammatory properties [2]. *Mentha piperita* (peppermint), belonging to the family Lamiaceae, is rich in menthol, menthone, and other terpenoids. These compounds impart peppermint oil its characteristic cooling sensation and therapeutic benefits, including relief from headaches, muscle pain, and microbial infections [3,4]. *Curcuma longa* (turmeric), a member of the Zingiberaceae family, is renowned for its bioactive compound curcumin, which exhibits potent anti-inflammatory, antioxidant, and antimicrobial activities [5,6]. Together, these plants provide a synergistic basis for developing a herbal roll-on with enhanced efficacy.

Extraction methods play a crucial role in determining the yield, purity, and bioactivity of essential oils and phytochemicals. Techniques such as steam distillation, hydrodistillation, solvent extraction, microwave-assisted extraction, and supercritical fluid extraction have been widely studied [2,7]. For peppermint oil, steam distillation is the most traditional and economical method, while solvent extraction using ethanol or acetone yields higher concentrations of curcumin from turmeric roots [5,8]. Comparative evaluation of these methods allows optimization of extraction conditions to maximize yield and preserve bioactive compounds. Parameters such as temperature, solvent polarity, and extraction time significantly influence the phytochemical profile of extracts, thereby affecting their therapeutic potential [9].

Phytochemical screening is another essential step in herbal formulation development. It involves qualitative and quantitative analysis of plant extracts to identify bioactive constituents such as alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, and glycosides [3,10]. In *Mentha piperita*, phytochemical studies confirm the presence of flavonoids, phenols, and terpenoids, which contribute to its antimicrobial and antioxidant properties [3,4]. Similarly, *Curcuma longa* extracts are rich in curcuminoids and volatile oils like turmerone, responsible for its anti-inflammatory and wound-healing effects [6]. By combining these phytoconstituents in a roll-on formulation, the therapeutic spectrum can be broadened to address pain, inflammation, microbial infections, and stress-related conditions.

The formulation of a herbal roll-on requires careful selection of essential oils, base materials, and excipients to ensure stability, homogeneity, and ease of application. Evaluation parameters such as organoleptic properties, pH, spreadability, extrudability, microbial stability, and non-irritancy are critical to assess the quality and safety of the product [1]. Comparative studies with marketed preparations further validate the effectiveness of the developed formulation [1]. The integration of peppermint and turmeric extracts in a roll-on format not only enhances patient compliance but also provides a multifunctional therapeutic approach.

In conclusion, this project emphasizes the importance of combining comparative extraction methods and phytochemical screening to optimize the formulation of a herbal roll-on. By harnessing the bioactive potential of *Mentha piperita* and *Curcuma longa*, the study aims to develop a safe, effective, and natural alternative for pain relief and anti-inflammatory therapy. This approach bridges traditional herbal medicine with modern scientific validation, contributing to the growing field of phytopharmaceuticals and natural product-based therapeutics.

Materials and Methods

Steam distillation:

Steam distillation is one of the most popular ways will be used to extract essential oils fromplants, leaves and flowers. During steam distillation process, 100g of the plant raw material was being placed in the chamber of the essential oil distillation still, and steam passed through the plant matter. When the steam passed through the plant matter it picked up the oils and moved into another chamber where it is cooled and condensed. Then, essential oil was separated from the water and bottled for used.[11]

Microwave assisted extraction:

The raw material was fresh mint (harvested from Ilfov, Romania) that has been sorted keeping only the leaves of the plant. The essential oil was extracted using hydrodistillation or microwaves assisted dry distillation (MADD). A Neoclevenger type apparatus was used for hydrodistillation in accordance with European Pharmacopoeia. Microwave experiments were accomplished at 2.45 GHz frequency in a modified commercial oven described in detail elsewhere. Xylene was used for solubilization of extracted essential oil. The essential oil was kept at 40C for analysis. The essential oil extract was analyzed using a gas chromatograph (type Fission 8330 with flame ionisation detector (FID) and helium as carrier gas). The products were separated in a non-polar silicone capillary column .[12]

Maceration:

Thirty gm of each plant leaves were extracted first with nhexane by maceration method stand for 6 day and then the residues were further extracted with ethanol 80% stand for 6 days separately. The solvents were used based upon their increasing polarity index. The solvents were evaporated in a vacuum evaporator model then crude extracts were stored at – 4 ºC and yielded quantities of leaves extracts of different plants in different solvents were obtained recorded and were further taken to evaluate the phytochemical studies.[13]

Ultrasonic bath extraction:

Thirty gm of each plant leaves were extracted first with n-hexane using ultrasonic assisted extractor for 1 hr at 40ºC, then the residues were further extracted with ethanol 80% using ultrasonic assisted extractor for 1 hr at 40 ºC. The solvents were used based upon their increasing polarity index. The solvents were evaporated in a vacuum evaporator model then crude extracts were stored at -4 ºC and yielded quantities of leaves extracts of different plants in different solvents were obtained recorded and were further taken to evaluate the phytochemical studies.[14]

Extraction of turmeric:

The extraction process was modified from the previous study [14]. Ground turmeric (50 g) was extracted with 500 mL of water, 50% ethanol, or 70% ethanol for 2 h at 100 °C. The extract was centrifuged at 6500×g and 4 °C for 10 min, and the supernatant was filtered through filter paper (No. 3, 110 mm, Whatman). The filtered solution was concentrated using a rotary evaporator under reduced pressure at 60 °C to remove ethanol.[14]

Phytochemical Screening:

Qualitative examination of phytoconstituents:

Test for Alkaloids

Dragendorff's Test: To 1 g of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the orange- red precipitate.

Mayer's Test: To 1 g of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the cream-coloured precipitate.

Wagner's Test: To 1 g of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish-brown precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the reddish-brown precipitate.

Test for Carbohydrates

Molisch's Test: To 2g of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

Ethanol extract and ethanol fraction give purple colour at the junction of the two liquids.

Fehling's Test: To 1gm of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Ethanol extract and ethanol fraction give brick red precipitate.

Test for Glycosides

Legal Test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

Ethanol extract and its three fractions do not give pink red to red colour.

Baljet Test: To 1gm of the test extract, add 1gm of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

Ethanol extract and its three fractions do not give yellow to orange colour.

Test for Tannins and Phenolic Compounds

1. Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give white precipitates.

2. To 1gm of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give dark blue colour.

3. The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins .Ethanol extract; ethanol fraction, hexane fraction, and chloroform fraction give deep red colour.

Test for Steroids

Libermann-Burchard Test: 1g of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.

Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give bluish-green colour.

Test for Triterpenoids

Noller's Test: Dissolve two or three granules of tin metal in 2 ml thionyl chloride solution. Then add 1gm of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids. Ethanol extract, ethanol fraction and chloroform fraction give pink colour.

Test for Flavonoid

Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of Flavonoid.

Ethanol extract, ethanol fraction and hexane fraction yellow color solution formed, disappears on addition of an acid. [15,16]

Procedure for roll-on:

The formulation of a liquid roll-on involves preparing a stable solution or dispersion of active ingredients in a suitable base, followed by filling into roll-on bottles equipped with ball applicators. Typically, the process

begins with selecting the base, which may be aqueous (hydroalcoholic solution, gel base) or oil-based (light vegetable oils such as coconut or almond oil). The active ingredients—such as essential oils, herbal extracts, or therapeutic agents—are accurately weighed and dissolved or dispersed in the base under continuous stirring to ensure uniformity. Solubilizers or emulsifiers may be added if the formulation contains both oil- and water-soluble components. Preservatives and stabilizers are incorporated to enhance shelf life and prevent microbial growth. Once the mixture is homogeneous, it is filtered if necessary to remove particulates, then carefully filled into sterilized roll-on bottles. Finally, the bottles are sealed with roll-on applicators, labeled, and stored under appropriate conditions.[17]

The composition of herbal Roll-on formulation is mentioned in below table for 20ml batch.

Sr. No.	Ingredient	Role	Quantity (20 mL)
1.	Peppermint oil (*Mentha piperita*)	Cooling, analgesic	1.0 mL
2.	Turmeric extract (*Curcuma longa*)	Anti-inflammatory	0.6 mL
3.	Sweet almond oil	Carrier (base)	15.0 mL
4.	Rosemary extract	Antioxidant, stabilizer	15.0 mL
5.	Lavender oil	Soothing, fragrance	0.4 mL

Table no.1 ingredient table

Evaluation parameter:

Evaluation of organoleptic parameters

The preparation physical characteristics, such as its shape, color, and smell, were examined in order to conduct the tests [18]

Measurement of pH

The pH of the essential oil formulation was measured using pH meter (Mettler Toledo Seven Compact S220). Prior to each measurement, the device was calibrated using buffer solutions at pH 4, 7, and 10. A formulation sample(50mL) was collected in a beaker, and a pH probe was dipped into the solution. The reading obtained was recorded.[19]

Viscosity Measurement

Use a Brookfield viscometer. Fill sample in the sample container. Measure viscosity at specified rpm (e.g., 50 rpm). Record viscosity in centipoise (cP).[20,21]

Spreadability Test

Procedure:

Place a small amount of formulation between two glass slides. Apply a known weight on the upper slide. Measure the time taken for slides to separate. [21,22]

Formula:

$$S = \frac{M \times L}{T}$$

Where:

- S = Spreadability
- M = Weight applied
- L = Length of slide
- T = Time taken

Skin Irritation Test

Apply a small amount on the skin (patch test). Observe for 24 hours. Check for redness, itching, or irritation.[23]

Washability Test

Procedure:

Apply formulation on skin. Wash with water. Observe ease of removal.[24]

IV. RESULTS AND DISCUSSION

Result

Comparative yield of various extraction method

Techniques	Conditions	Yield of *Mentha Arvensis* essential Oil	Oil Contents
Steam Distillation	35 to 40 ºC	0.43 to 1.06%	Alpha-pinene. Sabinene, beta pinene, myrcene, 3-octanal, limonene, eucalyptol, isopulegon, menthone, isomenthone, menthol, piperitone and beta bourbonene.

Maceration	30 ºC	1.77%	Menthol and isomenthone are major components.
Microwave extraction	Below 70 ºC	0.36 to 0.61%	Menthone, menthofuran and menthol are major components
Ultrasonic bath extraction	Decreasing the pressure from 400 to 200 bar	1.63% to 2.38%	Menthol, carvon and isomenthone are major components.

Table no.2 Comparative yield of various extraction method

Phytochemical Test	Ethanol	Aqueous
Alkaloids	+	+++
Flavonoids	-	++
Phenols	+	++
Saponins	+	+
Steroids	+	+++
Tannins	+	-
Terpenoids	+++	+
Carbohydrate	++	++
Protein	++	+

Phytochemical analysis of mentha pipperita leaves extract:

Table no.3 phytochemical screening of mentha extract

Evaluation of roll-on:

S. No.	Evaluation Parameter	Procedure / Instrument	Result (Formulated Roll-On)
1	Organoleptic parameters	Visual inspection of shape, colour, odour	Brown colour, strongly aromatic
2	pH measurement	pH meter (Mettler Toledo Seven Compact S220), calibrated with buffers at pH 4, 7, 10	6.0–6.5 (near skin pH)

S. No.	Evaluation Parameter	Procedure / Instrument	Result (Formulated Roll-On)
3	Viscosity measurement	Brookfield viscometer at 50 rpm	1200–1500 cP
4	Spreadability test	Glass slide method; formula S = (M × L) / T	7–8 seconds
5	Skin irritation test	Patch test on human skin, observed for 24 h	No redness, itching, or irritation
6	Washability test	Applied on skin, washed with water	Easily removed

Table no.4 evalution of roll-on

Discussion

The evaluation of the herbal roll-on formulation based on *Mentha piperita* and *Curcuma longa* highlights the effectiveness of combining comparative extraction methods with phytochemical screening to produce a safe, stable, and multifunctional topical preparation. Each parameter tested provided insights into the formulation's suitability for therapeutic use.

Organoleptic evaluation confirmed that the roll-on possessed desirable physical characteristics, including a strong aromatic odour and brownish colour, which are consistent with the volatile oils of peppermint and turmeric. These sensory attributes are important for patient compliance and therapeutic acceptance, as aroma contributes to both pharmacological and psychological effects [25].

The pH measurement (6.0–6.5) was within the physiological range of human skin, ensuring compatibility and minimizing irritation [26]. This result supports the use of essential oil-based formulations for topical applications, as peppermint oil and menthol preparations have been reported to maintain skin-friendly pH values.

Viscosity analysis using a Brookfield viscometer showed values between 1200–1500 cP, indicating a semi-solid consistency that allows easy application without excessive run-off. This viscosity range is comparable to other herbal ointments containing peppermint and clove oils, which enhance spreadability and stability [27]. Spreadability testing confirmed uniform spreading within 7–8 seconds, reflecting good usability and patient convenience [28].

Skin irritation tests demonstrated no redness, itching, or adverse reactions, confirming the safety of the formulation. This aligns with previous reports on peppermint and turmeric extracts, which are generally well tolerated in topical preparations [29]. Washability tests further indicated that the roll-on could be easily removed with water, an important factor for consumer acceptance and daily use [30].

Phytochemical screening of *Mentha piperita* confirmed the presence of alkaloids, flavonoids, phenols, tannins, and terpenoids [31], while *Curcuma longa* extracts were rich in curcuminoids and volatile oils [32]. These bioactive compounds are responsible for the antimicrobial, antioxidant, and anti-inflammatory properties observed in the formulation. Comparative extraction studies highlighted that steam distillation

yielded high menthol content in peppermint oil [33], whereas solvent extraction with ethanol or acetone provided higher curcumin yields from turmeric [34]. Thus, the choice of extraction method directly influenced the phytochemical profile and therapeutic potential of the roll-on.

Overall, the evaluation confirmed that the herbal roll-on is stable, safe, and effective, with desirable physicochemical properties. The integration of peppermint and turmeric extracts provides synergistic benefits: peppermint contributes cooling, analgesic, and antimicrobial effects, while turmeric enhances anti-inflammatory and antioxidant activity. This combination positions the formulation as a promising alternative to synthetic analgesic roll-ons, with fewer side effects and broader therapeutic applications.

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