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Quantification of Bioactive Compounds from Plant Extracts Using HPLC and Ultrasonication Methods for Preparing Sugar Free Tonsilitis Syrup

R. Caroline Jeba*, R. Sundeep, Mohamed Fardeen, M. Barath, M. Deva, B. Diwakar

Department of Biotechnology, Dr. M.G.R Educational and Research Institute, Maduravoyal, Chennai-600 095, India

E: mail: janeshjeba@gmail.com

ABSTRACT

This study focuses on neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), black pepper (*Piper nigrum*), curry leaves (*Murraya koenigii*), and pineapple (*Ananas comosus*) were selected for preparing sugar free tonsilitis syrup. The first step is the extraction of bioactive compounds was achieved using Soxhlet and ultrasonication methods, followed by High-Performance Liquid Chromatography (HPLC) for compound identification and quantification.

KEYWORDS

Ocimum sanctum, curcuma longa, Zingiber officinale,piper nigru,. Murraya koenigii, Ananas comosus, HPLC

Introduction

Herbal medicine has been famous for ages for its healing powers, particularly in traditional healthcare practices globally. In recent years, scientific research has examined the healing powers of various plants more intensely, particularly in blends that employ multiple ingredients to enhance health benefits. Curry leaves are a widely used culinary and medicinal plant in South Asian countries, particularly India. These leaves are rich in bioactive compounds such as carbazole alkaloids, flavonoids, and essential oils, which contribute to their wide range of pharmacological properties (Khanum et al., 2000). Herbs like **Turmeric** and **Ginger** are known for their anti-inflammatory and pain-relief properties. Turmeric's active compound, curcumin, helps reduce swelling and inflammation (Chainani-Wu, 2003), while ginger is a natural analgesic that eases pain and discomfort (Mishra et al., 2001). With the growing threat of antibiotic resistance, developing a herbal syrup provides a much-needed alternative to antibiotics, particularly for viral tonsillitis where antibiotics are ineffective (Ventola, 2015). Tulsi, also known as holy basil, is revered in Ayurvedic medicine for its spiritual and medicinal value. Tulsi inhibits COX-2 and lipoxygenase pathways and modulates pro-inflammatory cytokines. It also enhances both humoral and cell-mediated immunity (Singh et al., 2007). Black pepper is not only a common spice but also a well-researched medicinal plant. Piperine increases the bioavailability of drugs and other herbal constituents like curcumin by inhibiting enzymes involved in drug metabolism (Shoba et al., 1998). Pineapple is not only valued for its nutritional benefits but also for its medicinal properties, mainly due to the presence of the proteolytic enzyme bromelain.

ISSN 1019-6609
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Materials and Methods

The selected herbal plant materials—turmeric (Curcuma longa), ginger (Zingiber officinale), tulsi (Ocimum sanctum), neem (Azadirachta indica), pepper (Piper nigrum), . (Piper longum), curry leaves (Murraya koenigii), and pineapple crown (Ananas comosus)—were sourced from certified providers. Each part of the plants was selected based on its literature reported phytoconstituents and traditional medicinal value (Mukherjee, 2002; Kamboj, 2000).

Collection And Authentication Of Plant Materials

The selected medicinal plants used for the formulation of the polyherbal syrup were collected based on their traditional relevance and established therapeutic potential in the management of respiratory infections and microbial diseases. All the collected plant materials Murraya *koenigii*, *Curcuma longa*, *Piper nigrum*, *Ocimum sanctum, Azadirachta indica*, *Zingiber officinale*, *Ananas comosus* were subjected to botanical authentication by Prof. P. Jayaraman**,** Director of the Institute of Herbal Botany**,** Plant Anatomy Research Centre (PARC), West Tambaram, Chennai – 600045. The authentication was carried out based on morphological and taxonomic characteristics.

Drying And Cleaning

Green curry leaves, neem leaves, tulsi, pepper, ginger, and turmeric were first picked and washed thoroughly free of dirt, dust, and other impurities. Caution in washing was observed to preserve delicate leaves and phytochemical integrity (Jones & Kinghorn, 2005). Pineapple crown, ginger rhizomes, and turmeric rhizomes were sliced into uniform portions using sterile blades for even drying and preservation of integrity against microbial attack.

These cleaned and washed herbal materials were then spread over at a single layer on stainless steel trays in such a manner that no overlapping is ensured, thereby allowing proper circulation of air in between. Easy even drying of all materials could be achieved with this arrangement. These trays were then placed in a hot air oven maintained at 60°C. These were dried continuously for 24 hours until material became crispy as well as gave complete surface loss of moisture.

Pineapple is not dried in a hot air oven since its content of bromelain is heat-sensitive and declines above 50°C.

Once dried, oven samples were withdrawn and allowed to cool at room temperature (25–30°C) in a sterile environment. Condensation is therefore prevented, and the dried sample is protected from humidity within the environment.

Grinding And Powdering

The dried herbals were additionally grinded mechanically with a sterile mixer grinder to produce coarse to fine powder. Powders were sifted using a 60-mesh sieve to ensure equal particle size (Harborne, 1998), which aids in successful extraction in the Soxhlet process. Powdered samples were weighed (10 g) and packed in labelled, air-tight containers for further use.

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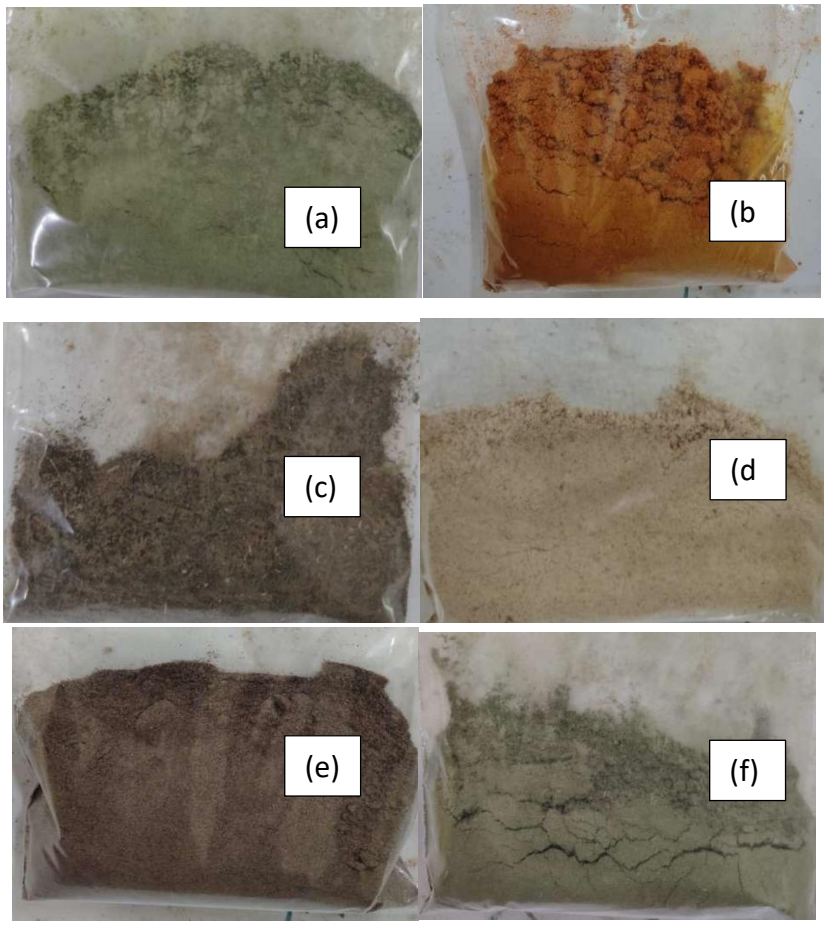

Fig 1. Powdered form of ingredients-(a)NEEM, (b)TURMERIC, (c)TULSI, (d)GINGER, (e)PEPPER, (f)CURRY LEAVES

Results And Discussion

Soxhlet Extraction Yield

Soxhlet extraction was employed for the efficient isolation of bioactive constituents from each herbal ingredient. This method ensured maximal recovery through continuous reflux and siphoning of solvents over extended periods. The herbs were first oven-dried at 60–70°C for 24 hours, ground into a fine powder, and packed in thimbles. Extraction was carried out using ethanol based on the solubility profiles of the phytoconstituents.

ISSN 1019-6609
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Fig. 2 Ginger extraction

Fig. 3 Tulsi extraction

Fig. 4Pepper extraction

Fig. 5 Curry leaves extraction

Fig. 6 Turmeric extraction

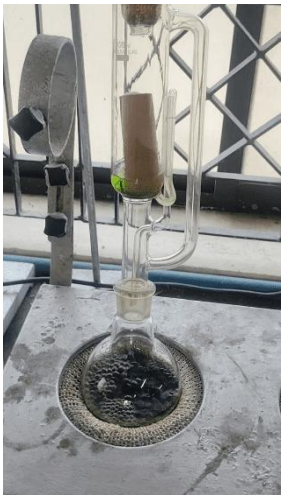

Fig. 7 Neem extraction

ISSN 1019-6609
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Table .1 Soxhlet Extraction of Ingredients

S. No	Ingredient Name	Botanical Name	Solvent Used	Extraction Time (hrs)	Physical Appearance of Extract
	Ginger	*Zingiber officinale*	Ethanol	6	Yellowish-orange
	Tulsi	*Ocimum sanctum*	Ethanol	6	Olive green
	Pepper	*Piper nigrum*	Ethanol	4	Brown
	Curry Leaves	*Murraya koenigii*	Ethanol	6	Dark green
	Turmeric	*Curcuma longa*	Ethanol	6	Dark orange
	Neem	*Azadirachta indica*	Ethanol	6	Green

Interpretation

The choice of solvent played a crucial role in the efficiency of extraction. Ethanol was particularly effective for ginger, neem, tulsi, curry leaves, and pepper, likely due to its higher polarity which favours the extraction of phenolic compounds and alkaloids such as gingerol, nimbin, piperine, flavonoids, terpenoids, and curcuminoids.

The yield was visually assessed based on extract appearance and will be further quantified in subsequent sections using HPLC. Each extract demonstrated a distinct coloration, indicative of the phytoconstituents present — for instance, the bright orange colour of turmeric extract corresponded with the presence of curcumin, a potent antioxidant and antimicrobial agent.

These extracts were then subjected to HPLC analysis to determine the presence and concentration of major bioactive markers.

Ultrasonication Of Pineapple Extract (Without Soxhlet)

Pineapple (Ananas comosus) was washed, dried, and ground to coarse powder. In contrast to the other herbs, bromelain is not resistant to heat. Therefore, ultrasonication was selected as a cold extraction method.

Ultrasonication Parameters

Solvent Used: 50% ethanol in water

Sample quantity: 50 ml pineapple juice

Solvent Volume: 100 mL

Frequency: 20 kHz

Amplitude: 60%

Duration: 20 minutes

Temperature Control: Kept below 40°C in an ice-water bath

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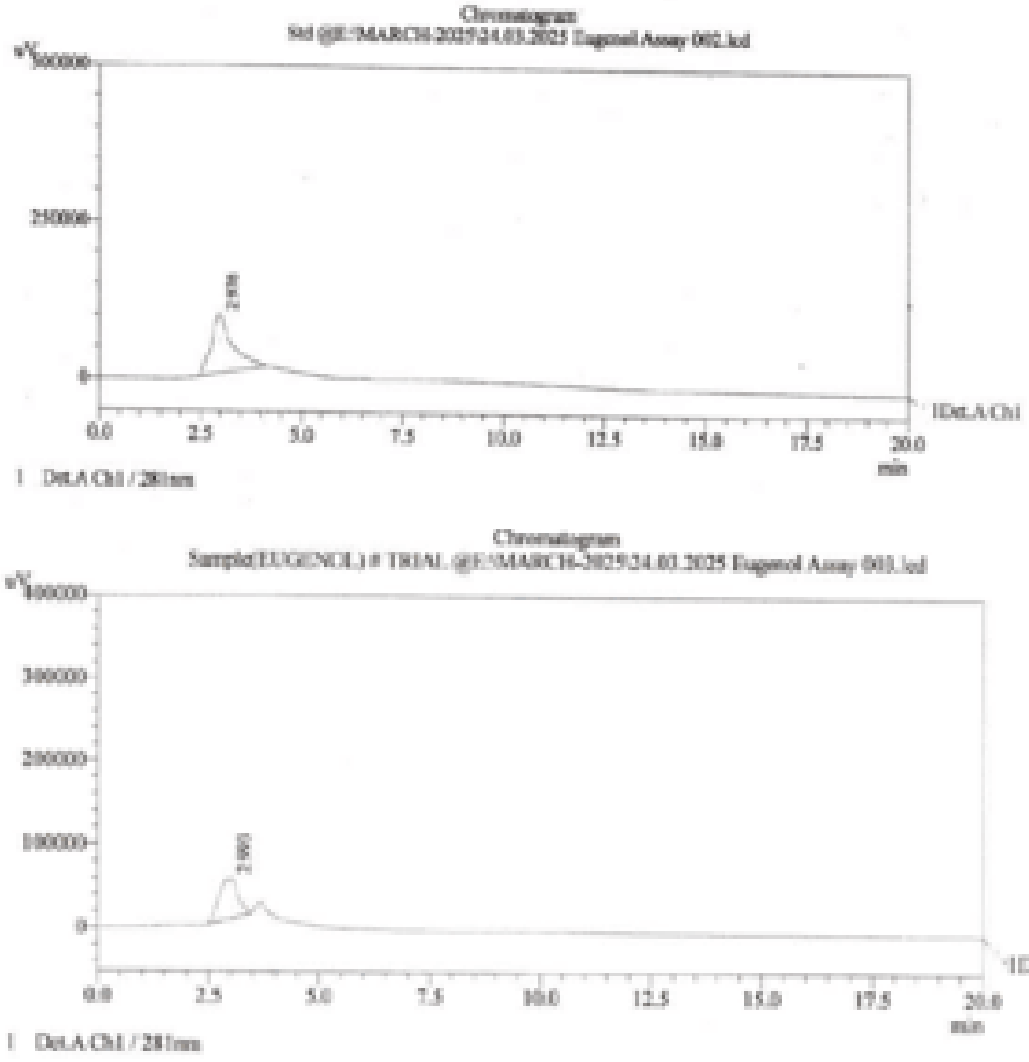
HPLC-Based Quantitative Analysis

Every extract was made either by Soxhlet extraction or by maceration employing suitable solvents. HPLC-grade solvents were employed to dissolve and inject reconstituted dry extracts in the HPLC system. The analytical-grade standard calibration reference compound was utilized and the weight of bioactive compound in each of the extracts determined from the retention time as well as area under the peaks.

Quantification of Tulsi



Quantification of Tulsi

Name	Retention time	Area	Height	Conc.
Std.	2.976	3557156	93960	0.3060
Sample	2.993	1431688	48557	1.0570

ISSN 1019-6609
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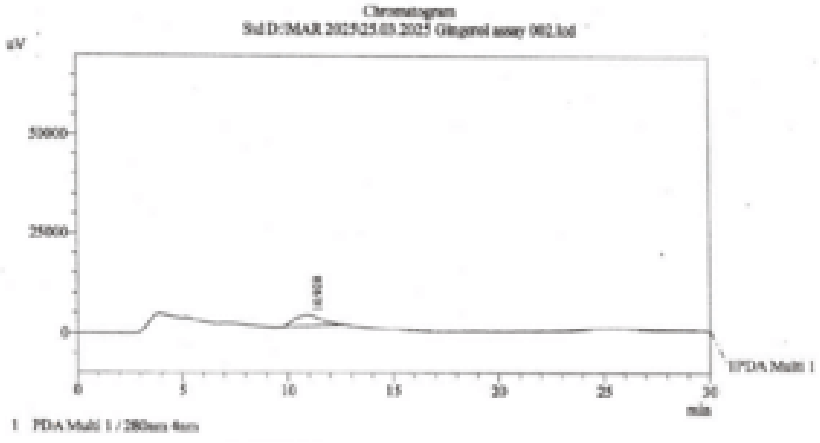
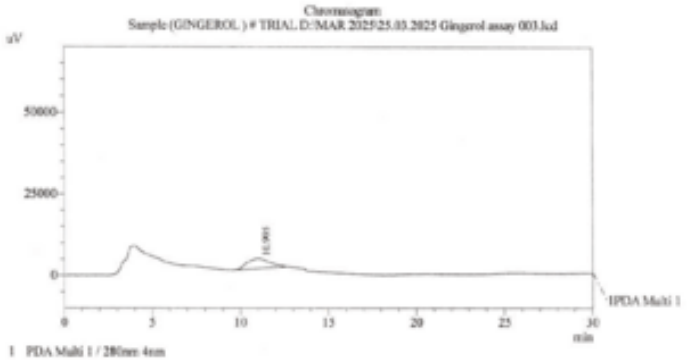
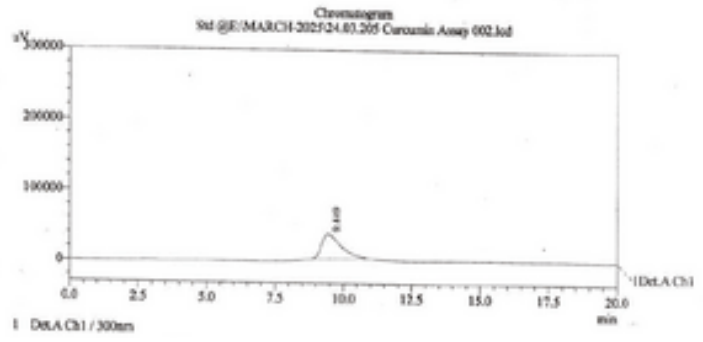
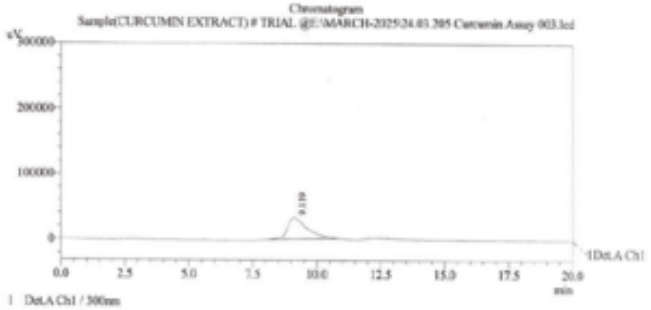
Quantification of Ginger





Quantification of Turmeric



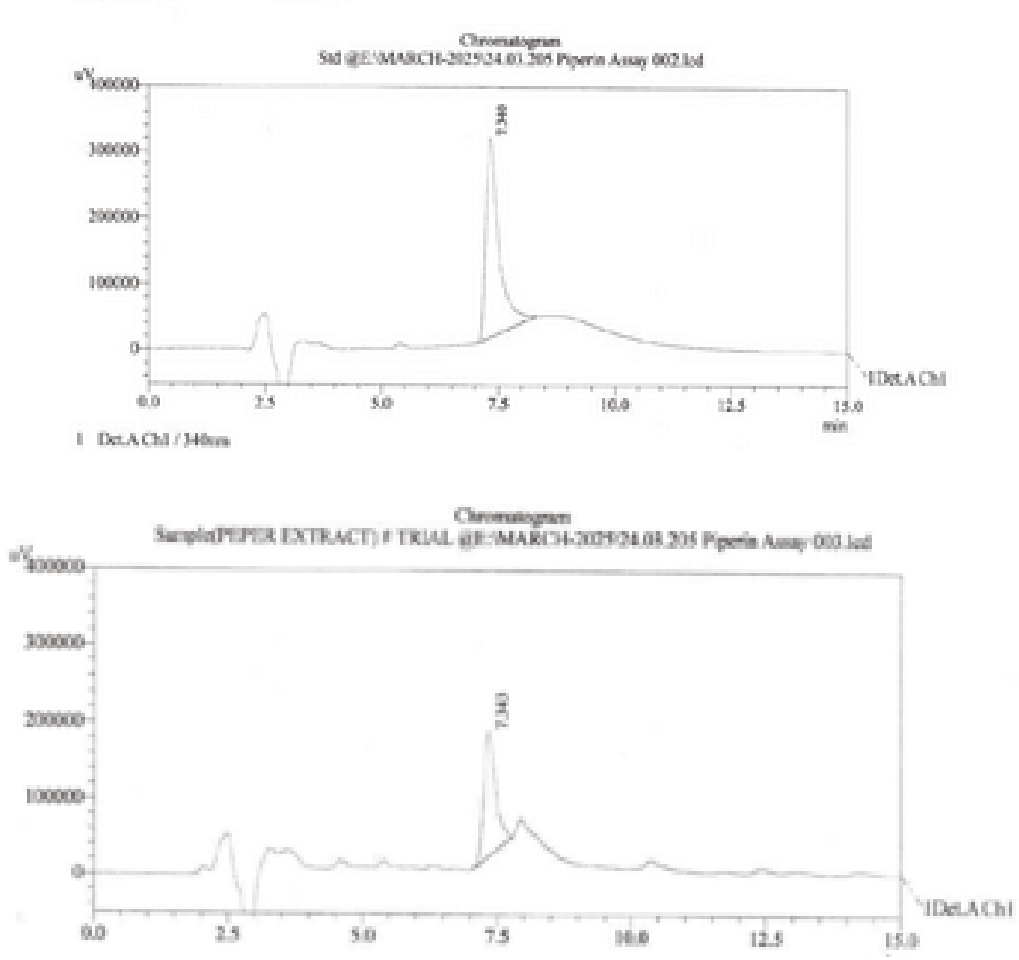


ISSN 1019-6609
eISSN 1853-4961

Quantification of Turmeric

Name	Retention time	Area	Height	Conc.
Std.	9.449	1058115	29295	0.0550
Sample	9.119	1720455	32289	12.0920

Quantification of Pepper



Quantification of Pepper

Name	Retention time	Area	Height	Conc.
Std.	7.340	5926506	301839	0.2200
Sample	7.343	2562970	163480	12.3410

ISSN 1019-6609
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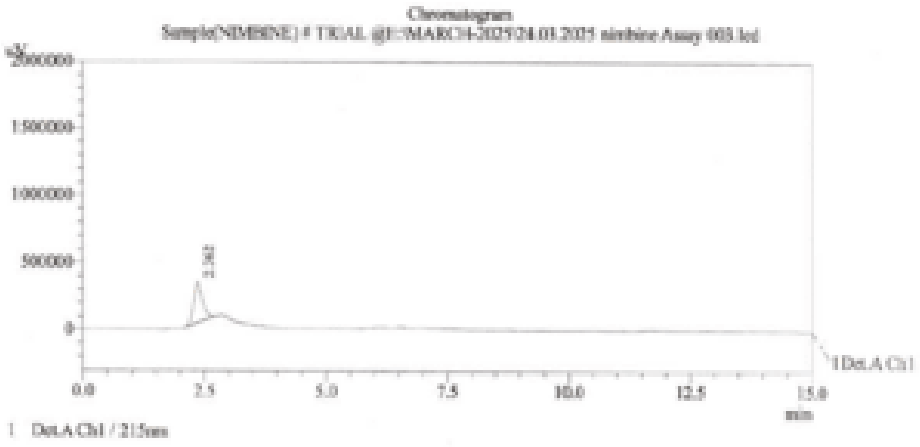
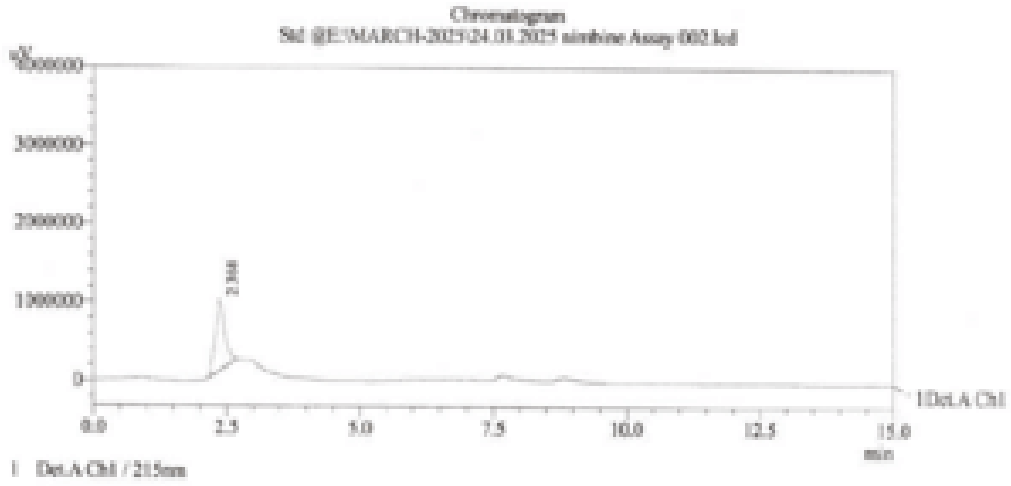
Quantification of Pineapple

Name	Retention time	Area	Height	Conc.
Std.	4.085	22352	1434	0.5500
Sample	4.167	612	278	213.4500

Quantification of Neem





Quantification of Neem

Name	Retention time	Area	Height	Conc.
Std.	2.368	11335884	3902182	0.1300
Sample	2.162	3865022	310503	109.4300

ISSN 1019-6609
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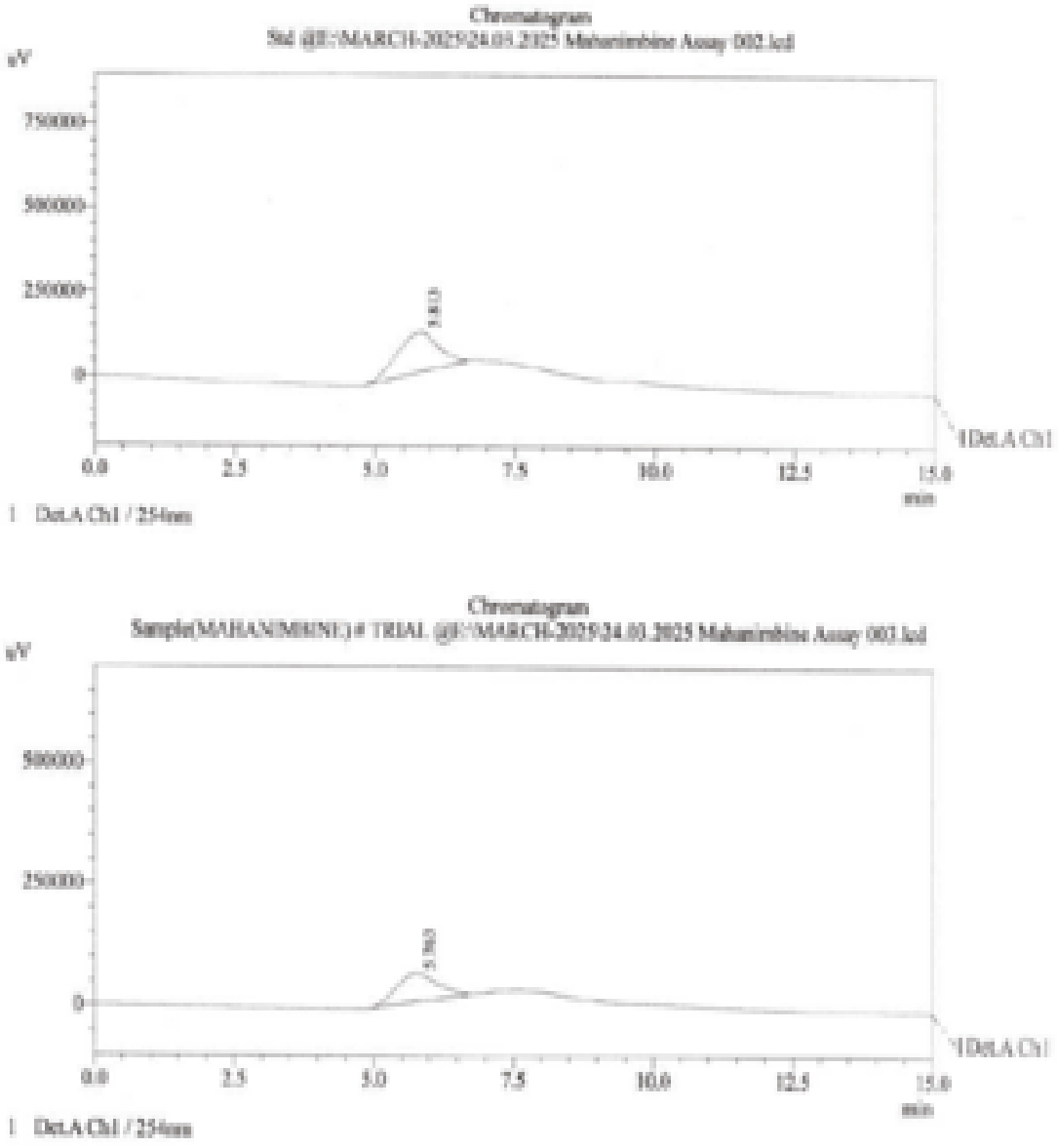
Quantification of Curry leaves



Quantification of Curry leaves

Name	Retention time	Area	Height	Conc.
Std.	5.813	6102440	118225	0.2008
Sample	5.763	2926076	56187	4.2256

ISSN 1019-6609
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Interpretation

1. Eugenol – 114.19 mg/g (Tulsi/Clove Extract)

Eugenol was the most prominent compound identified, with a relative concentration of 114.19 mg/g and an initial retention time (~2.99 min). Its powerful antimicrobial and antioxidant activities qualify it as the major active in the formulation (Marchese et al., 2017; Kamatou et al., 2012). Graphical inspections reveal its prevalence in all the graphics, supporting its pivotal therapeutic role. It is especially active against respiratory pathogens and oxidative stress. Its polarity is high, which accounts for its rapid elution in the chromatographic pattern.

2. Mahanimbine – 22.56 mg/g (Curry Leaf Extract)

Identified at 22.56 mg/g (RT ~5.76 min), Mahanimbine is a prominent contributor to antibacterial and anti-inflammatory activity (Bhandari, 2012; Khanum et al., 2000). Its high contribution to bar and pie charts positions it as an essential constituent in the control of broad-spectrum pathogens and immune enhancement. It adds to the formulation's healing and protective actions on respiratory tissue. Its peak area and shape confirm a solid, well-resolved presence in the extract.

3. Piperine – 17.65 mg/g (Black Pepper Extract)

Piperine at 17.65 mg/g (RT ~7.34 min) confirms mild antimicrobial activity and augmenting the intestinal permeability and bioavailability of other molecules such as curcumin (Shoba et al., 1998; Srinivasan, 2007). The same appears markedly in the charts, making a strong case for inclusion for the effects of synergy and bio-enhancement. It helps to optimize the bio efficacy of the whole system as a permeability enhancer. The prominent retention time with no tailing indicates high extractability and purity.

4. Curcumin – 7.32 mg/g (Turmeric Extract)

Found at 7.32 mg/g and eluting at the last time (~9.12 min), Curcumin is essential for its anti-inflammatory and antioxidant activities (Chainani-Wu, 2003; Aggarwal & Harikumar, 2009). Although in smaller amount, it is still pharmacologically important, with distinct chromatographic separation evident in the scatter plot. It helps in suppressing cytokine storms and inflammation in respiratory infections. Its slow elution time indicates its relatively low polarity.

5. Nimbine – 0.401 mg/g (Neem Extract)

Nimbine was detected at 0.401 mg/g (RT ~2.36 min). Although present in lower levels, it provides wide antimicrobial coverage, which increases the spectrum of the formulation (Biswas et al., 2002; Subapriya & Nagini, 2005). It acts against microbial adhesion and biofilm formation. The peak, though small, was sharp and well-resolved from other constituents.

6. Bromelain – 0.070 mg/g (Pineapple Extract)

At 0.070 mg/g (RT ~4.17 min), Bromelain is the least concentrated compound but provides significant mucolytic activity (Maurer, 2001; Pavan et al., 2012). It is present supporting respiratory relief, even at minimal concentrations, and is clearly the smallest in all graphical plots. It assists in thinning mucus and enhancing airflow in blocked lungs. Detection of the enzyme confirms the method sensitivity for bio actives with low concentrations.

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Conclusion

Individual quantitative HPLC analysis of extracts confirmed a diverse and effective phytochemical composition. These readings provide the platform for standardised formulation, and with it comes therapeutic effectiveness coupled with product quality consistency. Analysis of the antimicrobial activity of each of the ingredients is the subsequent step.

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