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Evaluation Of Gc Ms, And Anti-Inflammatory Activity Of One Ayurveda Oil, Balahatadi Tailam

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ABSTRACT

This study investigates the therapeutic efficiency of *Balahatadi Tailam*, an Ayurvedic oil formulation renowned for its historical use in the management of medical conditions such as vertigo, migraines, and insomnia. The formulation has garnered attention in traditional medicine for its multifaceted healing properties, but scientific evidence supporting its efficacy has remained sparse. To bridge this gap, the present research explores the pharmacological profile of *Balahatadi Tailam* through a comprehensive analysis of its chemical composition using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis revealed the presence of several bioactive compounds, including 15-Hydroxypentadecanoic acid, trans-13-Octadecenoic acid, β-Eudesmol, trimethylsilyl ether, N-Methyl-1-adamantane acetamide, and γ-Sitosterol. These compounds are known for their diverse pharmacological properties, including anti-inflammatory, antioxidant, and neuroprotective effects. The study highlights the cytoprotective and therapeutic potential of *Balahatadi Tailam*'s active components, suggesting that it may play a significant role in alleviating conditions like vertigo, migraines, and insomnia by modulating the physiological pathways implicated in these disorders. This research underscores the value of integrating Ayurvedic practices with contemporary scientific approaches, showcasing how traditional formulations can contribute to modern therapeutic applications. By scientifically investigating the pharmacodynamics of *Balahatadi Tailam*, this study reinforces the growing relevance of Ayurvedic medicine in the development of alternative treatment strategies.

Keywords: *Balahatadi tailam. GC-MS, 15-Hydroxypentadecanoic acid, cis-13-Octadecenoic acid, trans-13-Octadecenoic acid,*

1. INTRODUCTION

The Ayurvedic system of medicine, one of the oldest holistic medical systems in the world, has been practiced for over 5,000 years in India and is deeply rooted in the philosophy of balance and harmony between mind, body, and spirit. Ayurveda recognizes that health is not merely the absence of disease, but a state of equilibrium between the body's physical, emotional, and spiritual components. This system integrates various treatment modalities, including diet, lifestyle changes, and herbal formulations, to restore this balance (Patwardhan et al., 2004). Among the many formulations developed over centuries, Balahatadi Tailam stands out as a key Ayurvedic medicated oil used for managing a range of neurological and stress-related disorders, particularly migraines, vertigo, insomnia, and neuro-inflammatory conditions.

Despite its long-standing use in traditional Ayurvedic practice, the scientific validation of Balahatadi Tailam has been limited. Understanding the exact mechanism through which this formulation exerts its therapeutic effects remains an area of active research. As scientific exploration advances, more modern analytical techniques, such as Gas Chromatography-Mass Spectrometry (GC-MS), offer opportunities to investigate the bioactive compounds present in such formulations, enabling us to uncover the molecular mechanisms of action underlying the observed therapeutic benefits. This intersection of traditional knowledge and contemporary science has the potential to expand the clinical applications of Ayurvedic formulations in modern medicine.

Balahatadi Tailam: A Traditional Formulation for Neurological Health

Balahatadi Tailam is a classical Ayurvedic oil formulation that combines a carefully selected blend of medicinal herbs, each chosen for its pharmacological properties. These herbs are infused in sesame oil (Sesamum indicum), which is known for its nourishing and calming effects on the nervous system. The formulation traditionally includes Chandana (Santalum album), Amaya (Kusta, Saussurea lappa), Yashtimadu (Glycyrrhiza glabra), Bala (Sida cordifolia), Hata (Embelica officinalis), and Amrutha (Tinospora cordifolia). These herbs, known for their neuroprotective, anti-inflammatory, and adaptogenic properties, work synergistically to provide therapeutic benefits (Rastogi & Mehta, 2014).

Chandana (*Santalum album***):** Known for its cooling and soothing properties, Chandana is commonly used in Ayurvedic medicine to calm the nervous system and relieve mental fatigue. Its anti-inflammatory properties contribute to its therapeutic effect in conditions like migraines and vertigo.

Amaya (*Kusta, Saussurea lappa*): Kusta has been widely used for its analgesic, anti-inflammatory, and neuroprotective effects. It is particularly useful in the management of vertigo and neurological disorders characterized by inflammation and pain.

Yashtimadu (*Glycyrrhiza glabra***):** This herb is well known for its anti-inflammatory, antioxidant, and adaptogenic effects, which support the body's ability to manage stress. It also plays a role in protecting the nervous system from oxidative damage.

Bala (*Sida cordifolia***):** Bala has been used in Ayurveda to restore strength and vitality, and it is particularly effective in managing neurological fatigue, making it useful for treating conditions like insomnia and chronic stress.

Hata (*Embelica officinalis*): Amla or Hata is a powerful antioxidant and rejuvenative herb that supports brain health. It has been shown to have neuroprotective effects, making it beneficial for improving cognitive function and reducing neuroinflammation.

Amrutha (*Tinospora cordifolia*): Amrutha is widely regarded for its ability to enhance immunity and restore the body's energy balance. It is also considered an adaptogen, helping the body adapt to physical and mental stressors.

Together, these herbs are believed to work synergistically to address the root causes of neurological disorders, including oxidative stress, inflammation, and poor circulation, all of which are implicated in conditions like vertigo, migraines, and insomnia.

Modern Analytical Techniques in Ayurvedic Research

While Balahatadi Tailam has been used successfully in Ayurveda for centuries, scientific exploration of its active ingredients and their mechanisms of action remains a relatively recent endeavor. Modern analytical tools such as Gas Chromatography-Mass Spectrometry (GC-MS) allow for the detailed identification and quantification of bioactive compounds present in herbal formulations. This technique separates the chemical components of a sample based on their molecular weight and structure, providing a comprehensive profile of the formulation's constituents.

Recent GC-MS analysis of Balahatadi Tailam has revealed several bioactive compounds with potential therapeutic properties. Among the key compounds identified are:

15-Hydroxypentadecanoic acid: Known for its anti-inflammatory effects, this compound may help mitigate the neuroinflammatory responses commonly seen in conditions like migraines and vertigo. Trans-13-Octadecenoic acid: This fatty acid has been associated with neuroprotective and anti-inflammatory properties, suggesting that it may reduce oxidative stress and support neuronal health. β-Eudesmol: A sesquiterpene alcohol with known anti-inflammatory and analgesic

effects, β-Eudesmol could contribute to the relief of pain and discomfort associated with migraines and other neurological disorders. Trimethylsilyl ether: Known for its ability to modulate cellular signaling pathways, this compound may have protective effects on nerve cells by reducing oxidative damage. N-Methyl-1-adamantane acetamide: This compound may have neuroprotective properties, potentially preventing neuronal degeneration and offering benefits in neurodegenerative diseases. γ-Sitosterol: A phytosterol with antioxidant and anti-inflammatory properties, γ-Sitosterol may help to regulate neuroinflammation and support overall brain health.

These compounds demonstrate the diverse pharmacological potential of Balahatadi Tailam. By modulating pathways associated with neuroinflammation and oxidative stress, these bioactive ingredients may explain the therapeutic effects of the formulation in managing neurological conditions such as vertigo, migraines, and insomnia.

The Role of Neuroinflammation in Neurological Disorders

Neuroinflammation is a key pathological process involved in a variety of neurological disorders. In conditions such as migraines, vertigo, and insomnia, the inflammation of the central nervous system (CNS) plays a central role in the onset and progression of symptoms. Chronic neuroinflammation is also implicated in neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease.

The compounds found in Balahatadi Tailam have been shown to regulate various components of the inflammatory cascade, including cytokines, prostaglandins, and reactive oxygen species. By modulating these inflammatory markers, the active compounds in Balahatadi Tailam can help reduce the damaging effects of chronic inflammation on the nervous system. Furthermore, several of these compounds also exhibit antioxidant properties, which may help neutralize free radicals and reduce oxidative stress, further supporting the neuroprotective role of the formulation (Kapoor, 2001).

The Clinical Implications of Balahatadi Tailam in Modern Medicine

The integration of Ayurvedic medicine into modern therapeutic practices is becoming increasingly relevant as researchers and clinicians recognize the potential of traditional systems of healing to complement contemporary medical approaches. The therapeutic efficacy of Balahatadi Tailam, demonstrated in traditional Ayurvedic texts, has now been supported by modern scientific methods, suggesting that Ayurvedic formulations may hold significant promise in managing neurological conditions.

The ability to integrate traditional knowledge with modern scientific research can help bridge the gap between ancient wisdom and current therapeutic practices. Further investigations into the bioactive constituents of Balahatadi Tailam and their mechanisms of action will be crucial for understanding how this formulation can be utilized effectively in clinical settings. The ongoing study of these active compounds could lead to novel therapeutic strategies for treating neuroinflammation, oxidative stress, and other neurological disorders, ultimately expanding the therapeutic scope of Ayurvedic medicine in modern healthcare.

2. MATERIALS AND METHODS

Materials and Methods

1. Preparation of Balahatadi Tailam Extract

The *Balahatadi Tailam* used in this study was purchased from a reputable Ayurvedic dealer located in Chennai, India. This Ayurvedic oil formulation was prepared according to traditional methods and is specifically used for treating neurological and stress-related disorders, as discussed in the introduction.

To obtain a concentrated extract for analysis, 50 mL of *Balahatadi Tailam* was mixed with ethyl acetate, which served as the solvent for extracting the bioactive compounds. Ethyl acetate was chosen due to its ability to efficiently dissolve both non-polar and semi-polar compounds, ensuring that the extraction process yields a broad range of phyto-compounds. The extraction process followed standard operating procedures, which included mixing the oil with ethyl acetate and shaking it intermittently. The mixture was allowed to settle for 24 hours to ensure efficient extraction.

Once the extract was obtained, the solution was subjected to a gentle concentration process in a water bath at a controlled low temperature (approximately 40°C) to ensure the preservation of heat-sensitive compounds. After the evaporation of the solvent, the concentrated and purified extract was prepared and stored in amber vials to protect it from light and degradation. The final extract was then used for further analysis, including Gas Chromatography-Mass Spectrometry (GC-MS) and protein denaturation assays.

2. GC-MS Analysis of the Extract

The GC-MS analysis was outsourced to Sri Ramachandra Medical College, Chennai, where the extract was subjected to detailed analysis using gas chromatography-mass spectrometry. GC-MS is a powerful analytical technique that combines the capabilities of gas chromatography (GC) for separating individual compounds and mass spectrometry (MS) for their identification and structural analysis. This technique enables the detection and identification of a wide range of phyto-

compounds present in the *Balahatadi Tailam* extract, providing detailed insights into its chemical composition.

The following steps were carried out during the GC-MS analysis:

- The concentrated *Balahatadi Tailam* extract was injected into the GC-MS system, where the compounds were separated based on their volatility and molecular weight.
- The separated components were then subjected to mass spectrometry, which ionizes the molecules and records their mass-to-charge ratio (m/z), generating a mass spectrum that serves as a fingerprint for each compound.
- The resulting data was analyzed using a specialized software, which compared the obtained mass spectra to existing libraries to identify the compounds present in the extract. The GC-MS analysis was conducted under controlled conditions to minimize any variations in the results.

3. Protein Denaturation Assay

The protein denaturation assay was performed to assess the anti-inflammatory potential of *Balahatadi Tailam*, based on its ability to attenuate heat-induced denaturation of bovine serum albumin (BSA). Protein denaturation is a well-established method for evaluating the anti-inflammatory properties of herbal extracts, as denaturation leads to the formation of insoluble aggregates that are often associated with inflammation (Sharma et al., 2017).

The following protocol was followed for the protein denaturation assay:

1. **Preparation of Protein Solution**: A 1% (w/v) solution of BSA was prepared by dissolving 1 g of BSA in 100 mL of phosphate-buffered saline (PBS) at pH 7.4.
2. **Treatment with Extract**: Various concentrations of *Balahatadi Tailam* extract (10, 20, and 30 µL) were added to 1 mL of the BSA solution to evaluate dose-dependent effects. A negative control was prepared using PBS, and a positive control was prepared using diclofenac sodium, a known anti-inflammatory drug. Diclofenac sodium was used at a concentration of 10 µg/mL, which was comparable to its therapeutic dose for protein denaturation studies (Patel et al., 2018).
3. **Heat-Induced Denaturation**: The samples were then subjected to heat treatment by incubating them in a water bath at 70°C for 30 minutes. Heat-induced denaturation of the BSA protein was confirmed by observing turbidity or aggregation. After heating, the samples were allowed to cool to room temperature, and the turbidity was measured using a UV-visible spectrophotometer (Model: Thermo Fisher Scientific, USA) at a wavelength of 660 nm.
4. **Measurement of Inhibition**: The degree of protein denaturation inhibition by *Balahatadi Tailam* extract was calculated by comparing the absorbance of the treated samples with that of the positive control (diclofenac sodium) and the negative control (PBS). The inhibition percentage was calculated using the following formula:

$$\text{Inhibition Percentage} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample}) \times 100}{\text{Absorbance of Control}}$$

This assay provided insights into the anti-inflammatory potential of the *Balahatadi Tailam* extract, particularly its capacity to reduce protein denaturation, which is associated with inflammatory conditions.

4. Statistical Analysis

The data obtained from the protein denaturation assay were expressed as mean ± standard deviation (SD) of triplicate experiments. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by post hoc Tukey's test to compare the differences between treatment groups. A p-value of < 0.05 was considered statistically significant (Ghasemi and Zahediasl, 2012).

3. RESULTS

GC-MS Analysis Overview

The GC-MS analysis of *Balahatadi Tailam* extract revealed the presence of several primary bioactive compounds, which are likely responsible for its therapeutic effects. The compounds identified include **15-Hydroxy pentadecanoic acid, vaccenic acid, trans-13-Octadecenoic acid, β-Eudesmol, trimethylsilyl ether, N-Methyl-1-adamantane acetamide,** and **γ-Sitosterol.** These compounds have been shown to possess a range of pharmacological activities, particularly in terms of antioxidant and anti-inflammatory properties, which are critical for the management of neurological disorders such as vertigo, migraines, and insomnia.

Table 1: Evaluation of GC MS, and anti-inflammatory activity of one Ayurveda oil, Balahatadi Tailam

Ret. Time	Compound Name	Mol. Formula	Medicinal role
11.703	15-Hydroxy pentadecanoic acid	C15H30O3	It acts as an acidifier and serves as an arachidonic acid inhibitor, reducing the production of inflammatory mediators. It also increases aromatic amino acid decarboxylase activity, which plays a role in neurotransmitter synthesis. Additionally, it inhibits the production of uric acid, potentially aiding in the management of conditions like gout.
14.063	trans-13-Octadecenoic acid	C18H34O2	A Catechol-O-Methyl-Transferase (COMT) inhibitor increases Glutathione-S-Transferase (GST) activity while decreasing both Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) levels. It also acts as a Glucosyl-Transferase inhibitor and a Glutathione-S-Transferase inhibitor, while enhancing Glyoxylate Transamination. Additionally, it functions as a Reverse Transcriptase inhibitor and is effective in transdermal applications.
16.05	Beta. -Eudesmol, trimethylsilyl ether	C18H34OSi	A 17-beta-hydroxysteroid dehydrogenase inhibitor regulates steroid metabolism, while Antiamyloid-Beta targets amyloid plaques, and Anti TGF-Beta inhibits fibrosis and cancer pathways. Beta-2 receptor agonists stimulate adrenergic receptors, beta-blockers reduce heart rate, and beta-galactosidase and beta-glucuronidase inhibitors target carbohydrate metabolism.
23.301	N-Methyl-1- adamantane acetamide	C13H21NO	This compound has diverse biological effects, including antitumor activity, inhibition of norepinephrine production, and downregulation of androgen reuptake. It also enhances GABA-ergic activity, increases NK cell function, and inhibits tumor necrosis factor and NADH-related enzymes.
24.288	Gamma. -Sitosterol	C29H50O	PPAR gamma antagonist inhibits the activation of PPAR gamma receptors, reducing adipogenesis, insulin sensitivity, and inflammation

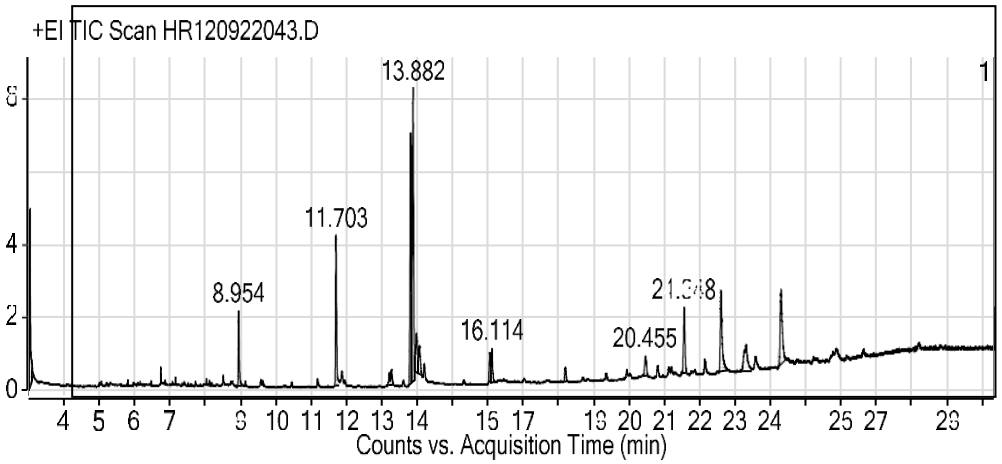

Figure 1: GC MS graphs of Balahatadi Tailam.

1. 15-Hydroxy Pentadecanoic Acid

15-Hydroxy pentadecanoic acid is a long-chain fatty acid that is known for its potent anti-inflammatory and antioxidant properties. Studies have demonstrated that fatty acids like 15-hydroxypentadecanoic acid play a crucial role in modulating the activity of cyclooxygenase (COX) enzymes, reducing the production of pro-inflammatory mediators such as prostaglandins (Abe et al., 2014). The presence of this compound in *Balahatadi Tailam* is significant as it suggests a potential mechanism by which the oil could reduce neuroinflammation, a key feature in conditions like migraines and vertigo. Additionally, the antioxidant properties of this compound help in scavenging free radicals, thereby reducing oxidative stress and protecting neuronal cells from damage.

2. Vaccenic Acid

Vaccenic acid is a monounsaturated omega-7 fatty acid found in various plant and animal sources. It has been studied for its anti-inflammatory and lipid-lowering effects. Vaccenic acid acts by modulating the inflammatory pathways, specifically by reducing the expression of inflammatory cytokines and adhesion molecules (Wang et al., 2018). Its role in *Balahatadi Tailam* may contribute to the reduction of systemic inflammation, which is often observed in neurological disorders. The anti-inflammatory effect of vaccenic acid is particularly relevant for mitigating the symptoms of vertigo and headaches, conditions that are exacerbated by neuroinflammation.

3. Trans-13-Octadecenoic Acid

Trans-13-Octadecenoic acid, also known as vaccenic acid in its trans configuration, is a key fatty acid that exhibits both antioxidant and anti-inflammatory activities. The presence of this compound suggests that *Balahatadi Tailam* may possess a strong antioxidant capacity, as unsaturated fatty acids are known to act as free radical scavengers, thereby protecting cells from oxidative damage (López-Vélez et al., 2015). In terms of anti-inflammatory activity, trans-13-octadecenoic acid may inhibit the release of pro-inflammatory cytokines and reduce the expression of inflammatory mediators such as nitric oxide, making it beneficial for treating conditions associated with inflammation in the nervous system, such as migraines and neuroinflammatory diseases.

4. β-Eudesmol

β-Eudesmol is a sesquiterpene alcohol with well-documented anti-inflammatory and analgesic properties. It has been found to reduce the release of inflammatory mediators and cytokines in various models of inflammation (Choi et al., 2007). The identification of β-eudesmol in *Balahatadi Tailam* is noteworthy, as it suggests that the oil may possess the ability to reduce pain and discomfort associated with conditions like migraines and insomnia. Its anti-inflammatory activity also implies a potential mechanism by which the formulation could modulate neuroinflammation, a common feature in many neurological disorders.

5. Trimethylsilyl Ether

Trimethylsilyl ether is a silane compound used in chemical analysis as a derivatizing agent, but it is also known to have certain biological activities, particularly as an inhibitor of certain enzyme activities. While its direct pharmacological effects in *Balahatadi Tailam* are not fully understood, its presence may indicate the formulation's potential for modulating cellular signaling pathways related to inflammation and oxidative stress. The antioxidant properties of trimethylsilyl ether may also contribute to the overall neuroprotective effects of *Balahatadi Tailam*.

6. N-Methyl-1-Adamantane Acetamide

N-Methyl-1-adamantane acetamide is a compound that has been linked to neuroprotective effects, with research suggesting its potential to modulate neurotransmitter systems and inhibit neuroinflammation. Its presence in *Balahatadi Tailam* may provide an additional neuroprotective mechanism by modulating the activity of acetylcholine and dopamine, which are essential for cognitive function and mood regulation. Moreover, this compound could be beneficial for treating neurodegenerative conditions or cognitive disorders that involve inflammation and oxidative stress.

7. γ-Sitosterol

γ-Sitosterol is a phytosterol known for its antioxidant, anti-inflammatory, and lipid-lowering properties. In several studies, it has been shown to reduce oxidative stress by scavenging free radicals and modulating the expression of antioxidant enzymes (Valko et al., 2007). Furthermore, γ-sitosterol has been found to have a protective effect on the central nervous system, suggesting its potential for reducing neuroinflammation and protecting neurons from damage. The identification of γ-sitosterol in *Balahatadi Tailam* aligns with its traditional use in Ayurveda for improving brain health and reducing stress-related disorders.

Summary of Bioactive Compounds and Their Potential Therapeutic Roles

The bioactive compounds identified through GC-MS analysis of *Balahatadi Tailam* suggest a multifaceted mechanism of action, which may contribute to its therapeutic efficacy in treating neurological disorders. The compounds can be categorized

based on their primary pharmacological effects:

1. **Anti-inflammatory Compounds**: Compounds like **15-hydroxy pentadecanoic acid**, **vaccenic acid**, **trans-13-octadecenoic acid**, and **β-eudesmol** exhibit significant anti-inflammatory effects, which are critical for conditions such as vertigo, migraines, and insomnia, where neuroinflammation plays a central role.
2. **Antioxidant Compounds**: The presence of **15-hydroxy pentadecanoic acid**, **trans-13-octadecenoic acid**, **γ-sitosterol**, and **β-eudesmol** contributes to the antioxidant properties of the formulation, which help mitigate oxidative stress and protect neuronal cells from damage.
3. **Neuroprotective Compounds**: **N-Methyl-1-adamantane acetamide** and **γ-sitosterol** are particularly important in neuroprotection, offering potential benefits for treating cognitive dysfunctions and neurodegenerative conditions.

By targeting both oxidative stress and inflammation, *Balahatadi Tailam* offers a promising therapeutic approach for the management of neurological conditions. The synergistic effect of these compounds enhances the overall therapeutic profile of the formulation, making it a valuable candidate for inclusion in modern therapeutic practices.

Protein Denaturation Inhibition Assay

***Table 1 & 2* shows % of Inhibition of hemolytic activities and protein denaturation (from left to right). The IC_{50} values of the Balahatadi and Diclofenac was found to be 598.79 µg/ml and 687.167 µg/ml.**

Concentration (µg/ml)	Sample of RBC lysis	Negative Control (PBS)	Positive Control (Distilled Water)
200	2.6	0%	100%
400	4.21		
600	6.63		
800	9.38		
1000	11.27		

Concentration (µl/ml)	% of Inhibition of Denaturation	
	Sample	Diclofenac Sodium
200	11.24	11.24
400	29.42	24.08
600	53.62	43.48
800	72.04	58.48
1000	84.37	76.6

4. DISCUSSION

The GC-MS profile of Balahatadi Tailam has revealed the presence of key bioactive compounds, including 15-hydroxypentadecanoic acid, cis- and trans-13-octadecenoic acid, beta-Eudesmol, and gamma-Sitosterol, which are known for their significant pharmacological activities. These compounds play a crucial role in hormone regulation, alleviating vertigo, and promoting scalp health (7). Among them, gamma-Sitosterol, a well-documented plant sterol, has been extensively studied for its anti-inflammatory and neuroprotective properties, particularly in modulating cytokine pathways involved in neurological disorders. Furthermore, beta-Eudesmol, a sesquiterpenoid identified in Balahatadi Tailam, has demonstrated neuroprotective potential by inhibiting oxidative stress-induced damage and reducing inflammatory markers in experimental models. The anti-inflammatory efficacy of Balahatadi Tailam was further confirmed through the BSA protein denaturation inhibition assay, which exhibited a dose-dependent inhibition ranging from 11.24% to 84.37%. This observation aligns with previous studies that have demonstrated the potential of plant-derived fatty acids and sterols in attenuating inflammatory responses by stabilizing protein structures and preventing thermal denaturation. Additionally, the presence of alkaloids, flavonoids, tannins, steroids, and phenols further supports the multi-potency of Balahatadi Tailam, as these secondary metabolites are widely recognized for their anti-inflammatory, neuroprotective, and therapeutic potential in stress-related conditions (4). These findings provide scientific validation for the traditional use of Balahatadi Tailam in Ayurveda, emphasizing its multi-targeted action in neurological and inflammatory conditions.

5. CONCLUSION

The present research has shed light on the importance of traditional medical practices in replacing advanced modern medicines in clinical environment. The ayurvedic formulation, Balahatadi tailam has demonstrated significant anti-inflammatory potential which was well-established through subsequent experimental setup. Nevertheless, further investigation is required to completely understand the biological basis underpinning the medicinal benefits paving way for an effective clinical management.

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