Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 15 (1), 2024; 172-179

The GCMS, antioxidant, and anti-inflammatory activity of the Ayurveda oil, *Balaguluchyadi Tailam*

Research Article

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Abstract

In Ayurvedic medicine, *tailas* are made by infusing the decoctions of holistic healing herbs in sesame or coconut oil. The traditional oil is known for its effectiveness in relieving various health issues. It is frequently used as massage oil for soothing muscular and joint pain, making it a valuable remedy for conditions like arthritis. The current study focuses on the GC MS analysis, antioxidant, and anti-inflammatory activities of one such ayurvedic oil *Balaguluchyadi tailam*. The oil is recommended for the treatment of neuropathy, gout, arthritis, cataracts, headaches, and neuritis that promote circulation and support the nervous system. Antioxidants, anti-inflammatory agents, and the amount of chemicals present in the sample were all examined in the oil using GC-MS analysis. The oil had strong reducing power activity, and the IC50 for the DPPH assay was 389.4 l/ml. The oil also had a significant anti-inflammatory effect. Numerous bioactive chemicals were present, according to the GC-MS study which has anti-inflammatory properties for which *Balaguluchyadi tailam* is recommended for providing instant relief from pain caused due to arthritis and rheumatoid arthritis. *Balaguluchyadi tailam* shortens the duration of the attack in the treatment method of abhyanga found to be extremely helpful for retaining *vata* and *rakta* balance.

Keywords: *Balaguluchyaditailam,* GC MS, Gout, Arthritis, Disulfide, di-tert-dodecyl, Dodecane, 1-iodo-beta-Sitosterol.

Introduction

Balaguluchyadi tailam is Ayurvedic herbal oil with a range of medicinal uses. This traditional oil, prepared with a base of sesame oil and a blend of Ayurvedic herbs, is known for its effectiveness in relieving various health issues. It is frequently used for soothing muscular and joint pain, making it a valuable remedy for conditions like arthritis. Additionally, it is applied topically to the skin to address skin disorders such as psoriasis and eczema. The oil is also employed for neurological disorders and may offer relief from headaches when applied to the forehead and temples.

The necessity of the hour is for effectiveness evaluation by contemporary standard methodologies of complementary and alternative medicine. This exercise will increase the credibility and acceptance of certain medication types. There has been some effort in this area, but much more needs to be done. The current work takes this ambition one step farther (1). The GC MS analysis of the Ayurvedic drug *Balaguluchyadi tailam* was carried out in the current investigation. *Balaguluchyadi tailam* is an oil-based Ayurvedic medication used to treat gout and arthritis. It treats neuropathy, neuritis, cataract, and headaches. The following lists the components of this drug and its preparation process.

Fine powders of 250 g each of Bala (*Sidacordifolia*; whole plant), *Guduchi* (*Tinospora cordifolia*; root, stem, leaf); *Surapadapa* (*Cedrus deodara*) heartwood are boiled with 12.288 litres of water and reduced to 3.072 litres of decoction. Fine powders of 16 g each of *Jata* (*Nardostachys jatamansi*: root/rhizome), *Amaya* (*Saussurea lappa* root), *Rakta Chandana* (*Pterocarpus santalinus*, heartwood, *Kunduru* (*Boswalia serrata)* exudate, *Nata* (*Valeriana walichii* root with rhizome and stolon), *Aswagandha* (*Withania somnifera*) root, *Sarala* (*Pinusrox burghii* heart wood), *Rasana* (*Pluchea lanceolata* rhizome) are mixed with water to make a paste. The decoction, the paste and 768 ml of sesame oil (*Sesamum indicum*) are mixed and heated till all the water content evaporates. The oil such obtained is filtered and packed to be used as medicine. *Sahasrayoga Tailayoga Prakarana -14.* Somarajan et al, 2019 have reviewed the various medicinal roles of *Balaguluchyadi tailam* and confirmed the same

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medicinal values (26). The following lists the components of this drug and its medical importance.

***Bala* (*Sida cordifolia*)**

S. cordifolia is used in the Ayurvedic system of medicine used in the treatment rheumatism, fever, asthma, nasal congestion, hypoglycemic conditions, liver ailments, and Parkinson's disease. It is also used as an analgesic drug, purgative, diuretic, aphrodisiac, and so on (27).

***Guduchi* (*Tinospora cordifolia*)**

This plant possesses enormous medicinal properties that help them to treat against diabetes, spasmodics, arthritis, inflammation, leprosy, stress, malaria, and so on. It also acts as a liver protectant, immunomodulation, and exhibits anti-neoplastic activities (28, 29).

***Surapadapa* (*Cedrus deodara*)**

The heart wood of this tree is used for treating illness related to skin, joint pains, bronchitic asthma, renal calculi, peptic ulcers, infections caused by microbes, neurological and immunological ailments (30).

***Jata* (*Nardostachys jatamansi*)**

The rhizome is used to treat CNS disorders, as a vasodilator, bronchodilator, spasmolytic etc. (31).

***Amaya* (*Saussurea lappa*)**

Amaya (Kusta) (*Saussurea lappa*) is studied quite extensively for its medicinal roles. There are reports of its use to treat diarrhea, tenesmus, dyspepsia, vomiting and inflammation (32). Nadda et al, 2020 have reported that Amaya has healing roles against liver ailments, diabetes, infections of fungi and helminthes, inflammations, and has immune-stimulant roles (33).

***Rakta Chandana* (*Pterocarpus santalinus* Linn)**

Soundararajan et al, 2016 have reported the heart wood has medicinal roles of *Pterocarpus santalinus*, such as anti-hyperglycemic, antipyretic, anti-inflammatory, anthelmintic, tonic, aphrodisiac, diaphoretic and as cooling agent (34).

***Kunduru* (*Boswellia serrata*)**

Boswellia serrata serves a variety of medical functions, including astringency, desiccation of ulcers, antiseptic, hemostasis, cicatrizant, memory enhancer, inhibition of phlegm secretion, tissue development, and ulcer healing (35).

***Nata* (*Valeriana walichii*)**

This plant is being used traditionally to cure diseases such as sleep disorder, hysteria, sedative, epilepsy. It is also used to cure rheumatism, low grade fever and as aphrodisiac. (36).

Sarala (*Pinus roxburghii*)

This plant is used to treat fevers, cough and cold, gynecological disorders, urinary problemsand disease related to skin, ear, and throat (Sinha and Tandon, 2018). (37)

***Rasana* (*Pluchea lanceolata*)**

The plant has been reported to have anti-inflammatory, analgesic, antipyretic, anti-arthritic and curative roles for dyspepsia, neurological disorders etc. (38)

***Til* (*Sesamum indicum*)**

Traditionally, Sesamum oil is the best edible oil in India. Being rich in polyunsaturated fatty acids, it has good antioxidant and anti-carcinogenic properties, thus provides protective effect on the liver. Miraj and Kiani, 2016 have reviewed the medicinal roles of this plant (39). Cheng et al, 2006 have shown the neuroprotective effects of Seasamin and sesamolin on cerebral ischemia (40).

Balaguluchyadi tailam is often used as a massage oil to relieve muscular and joint pain. It may help reduce inflammation and ease discomfort, this oil is sometimes recommended for individuals with arthritis to alleviate pain and improve joint mobility. The oil is believed to promote circulation and support the nervous system hence recommended for neurological disorders and conditions such as paralysis. It is also used in the case of for skin issues like psoriasis and eczema. It may help soothe and nourish the skin. When applied to the forehead and temples, the oil may provide relief from headaches and migraine and massaging it into the scalp can nourish the hair and promote overall scalp health. *Balaguluchyadi tailam* is rooted in *Ayurvedic* principles, where it is considered beneficial for conditions related to Vata and Kapha imbalances. This current study was carried out to study the various phytochemical compounds present in them, their uses and analyse the antioxidant and anti-inflammatory activities of the oil at various concentrations.

Materials and methods

The *Balaguluchyadi tailam* medication was bought from a typical Ayurvedic dealer in Chennai, India. In a separating funnel, 50 ml of *Balaguluchyadi tailam* were collected and extracted with ethyl acetate. The crude material after extraction was charged to study the Gas Chromatography- Mass Spectrophotometry (GC MS) patterns.

Instrument

Gas chromatography (Agilent: GC: (G3440A) 7890A. MS: 7000 Triple Quad GCMS,) was equipped with Mass spectrometry detector.

Sample Preparation

100 µl of the sample was dissolved in 1 ml of ethanol solvent. After agitation of the sample for 10 seconds the sample was taken for gas-chromatography for analysis.

GC-MS protocol

The column used for GC-MS was DB5 MS (30mm×0.25mm ID ×0.25 µm). The column consists of

5% phenyl, 95% methyl poly siloxane. 99.99% of helium was used as a carrier gas and the electron impact mode at 70 eV. The constant flow of the sample was 1ml/min. Temperature maintained in the injector region is 280 °C and in auxiliary region the temperature was maintained at 290°C. In the ion-source region the temperature was 280 °C. The temperature programmed in the oven region was 50 °C (isothermal for 1.0 min), with an increase of 40°C/min, to 170°C (isothermal for 4.0 min), then 10°C/min to 310°C (isothermal for 10min) fragments from 45 to 450 Da. The running time for GC is 32.02 min.

Anti-oxidant assay

DPPH is a stable free radical that can combine with an electron or a H^+ radical to generate a stable diamagnetic molecule. The reduction in the absorbance at 517 nm of DPPH radical caused by the interaction of antioxidant molecules with the radical, which causes the radical to be scavenged by hydrogen donation, was used to gauge the radical's ability to reduce. The reducing power test method was created because the FRAP assay is based on the notion that substances with reduction ability combine with potassium ferricyanide (Fe3+) to produce potassium ferrocyanide (Fe2+), which then interacts with ferric chloride to form ferric-ferrous complex, a compound with a maximum absorbance at 700 nm. The scavenging ability of the tailam was compared to the standard drug ascorbic acid. Both assays were conducted as per the protocol developed by Bhalodia et al., 2013 using ascorbic acid as standard antioxidant for both the experiments.

Anti-inflammatory activity

Following Gandhidasan et al (1991), a method for HRBC membrane stabilisation is used to investigate the in vitro anti-inflammatory effect of the extract. To get a fairly packed cell volume, the blood was centrifuged for 5 minutes at 2500 rpm. The supernatant was then collected, washed with sterile saline, and the process was repeated three times. 500 ml of sample at various concentrations and an equal volume of cell suspension were added, incubated for 30 minutes, and the absorbance was read in a spectrophotometer at 560 nm range to estimate the amount of haemoglobin in the suspension. The cells were reconstituted for 40% suspension with phosphate-buffered saline.

The protocol adopted for testing the ability of protein denaturation inhibition assay was followed with some minor modifications in the protocol suggested by Anyasor*et al.,* 2019. At different concentrations (200, 400, 600, 800, and 10,000 µl/ml), the effectiveness of the herbal formulation to stop heat-induced BSA denaturation was evaluated in contrast to the reference drug diclofenac sodium, distilled water as positive control and phosphate buffer sulphate (PH 7.4) as negative control. After being incubated at 37 °C for 20 minutes, the reaction mixtures were warmed to 70 °C for 5 minutes. After cooling, turbidity at 660 nm was measured using a UV-visible spectrophotometer. Using the O.D. values, the percentage inhibition of BSA denaturation and the IC50 value were calculated.

Results

The GCMS graphs of *Balaguluchyadi tailam* as in Figure 1 and illustrate the various peaks, its retention period, etc. The presence of many different types of chemicals in the GC MS profile of *Balaguluchyadi tailam* is shown in Table 1. By assessing the retention duration and fragmentation pattern with mass spectra in the NIST (National Institute of Standards and Technology) spectral library (version 1.10 beta, Shimadzu), the secondary metabolites in the profile were identified. As stated in Table 1, each active compound's potential medicinal applications were determined using Dr. Duke's phytochemical and ethnobotanical data base from the National Agriculture Library in the United States and other sources (41).

Figure 1.Depicts the GC-MS profile of *Balaguluchyadi tailam*

DataFile	HR120922046.D	**SampleName**	*Balguluchyadi tailam*
SampleType		**Position**	15
InstrumentN	GCMSMSOnl	**Username**	
AcqMethod	ScreeningMethodNew.M	**AcquiredTime**	28-09-2022PM4:30:02
IRMCalibrationStatus	NotApplicable	**DAMethod**	Screening.m
FragmentorVoltageCollisio	0	**IonizationMode**	EI

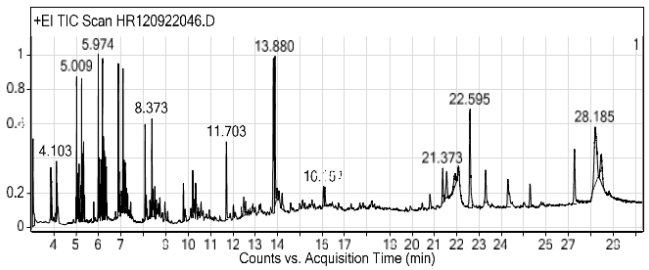


Table 1: Depicts the details of GC MS profile of *Balaguluchyadi tailam*

Ret. Time	Name of Compound	Mol. Formula	Mol. Wt.	% Peak Area	Possible Medicinal Role
3.85	Nonane,4,5-dimethyl-	C11H24	156.31	2.29	Not known
5.009	Nonane,2,2,4,4,6,8,8-heptamethyl-	C16H34	226.44	7.73	Not known
5.23	Hexadecane	C16H34	226.44	3.49	Not known

6.098	Disulfide,di-tert-dodecyl	C24H50S2	402.80	1.54	Antitoxin, dilates coronary blood vessels, promotes diuresis, digestion enhancer, increases SOD activity
6.206	Sulfurousacid,2-ethylhexylpentadecyl ester	C23H48O3S	404.70	1.95	Not known
8.068	Dodecane,1-iodo-	C12H25I	296.23	3.13	Antitoxin, Coronary dilator, promotes diuresis, digestion enhancer, increases SOD activity
8.432	Heneicosane	C21H44	296.6	1.70	Not known
10.197	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	296.6	2.58	Not known
10.33	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	C15H26O2	238.36	1.83	Oligosaccharide provider
11.703	15-Hydroxypentadecanoicacid	C15H30O3	258.39	4.29	Acidifier, Arachidonic acid supressor, promotes Aromatic Amino acid decarboxylase activity, suppresses the production of uric acid
13.815	9,12-Octadecadienoicacid(Z,Z)-	C18H32O2	280.44	9.65	Not known
13.88	Trans-13-Octadecenoicacid	C18H34O2	282.46	10.3	Catechol-O-Methyl-Transferase-Inhibitor, promotes GST Activity, suppresses GOT activity, Decreases GPT, Glucosyl-Transferase-Inhibitor, GST-Inhibitor, Promotes GXT, Reverse-Transcriptase-Inhibitor, acts as Transdermal agent
14.203	5-Methyl-Z-5-docosene	C23H46	322.6	1.36	Catechol-o- methyl Transferase inhibitor, methyl donor, methyl guanidine inhibitor, Increases Zinc bioavailability
16.054	7-Hexadecenal,(Z)-	C16H30O	238.41	1.28	Not known
16.112	5-Methyl-Z-5-docosene	C23H46	322.6	1.36	Catechol-o-methyl Transferase inhibitor, methyl donor, methyl guanidine inhibitor, promotes Zinc bioavailability
21.373	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	C15H22O	218.33	2.63	Not known
21.548	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	C33H54O3	498.8	2.48	Cholesteorlytic,17 β-hydroxysteroid dehydrogenase inhibitor, alcohol dehydrogenase supressor, succinate dehydrogenase inhibitor, oligosaccharide provider
21.941	Lauric anhydride	C24H46O3	382.6	1.74	Not known
22.595	1,3-Benzodioxole, 5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-	C20H18O6	354.35	9.31	Not known
23.288	Carbamic acid, N-[1,1-bis(trifluoromethyl)ethyl]-, 4-(1,1,3,3-tetramethylbutyl)phenyl ester	C19H25F6NO2	413.4	2.49	Not known
24.291	Beta.-Sitosterol	C29H50O	414.7	3.49	17 β-hydroxysteroid dehydrogenase inhibitor, Antiamyloid beta, Anti TGF beta, β-receptor agonist, Beta-adrenergic receptor blocker, beta blocker, β-galactosidase suppressor, β-glucuronidase inhibitor, ER beta binding agent

25.275	2,2-Dimethyl-6-methylene-1- [3,5-dihydroxy-1- pentenyl] cyclohexan-1- perhydrol	C14H24O4	256.33	1.86	Not Known
27.257	3-(2-Ethyl-piperidin-1-ylmethyl)-8a-methyl-5-methylene-decahydro-naphtho[2,3-b] furan-2-one	C22H35NO2	345.52	4.64	Not known
28.185	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	C27H52O5	456.7	12.77	Acidifier, Arachidonic acid suppressor, promotes Aromatic Amino acid decarboxylase activity, deters production of uric acid, Urine acidifier
28.459	Hexadecanoic acid, 2-(octadecyloxy)ethyl ester	C36H72O3	563.00	4.12	Acidifier, deters Arachidonic acid, promotes Aromatic Amino acid decarboxylase activity, deters production of uric acid, Urine acidifier

Antioxidant activity

Figure 1: Shows the results of DPPH antioxidant activity of the *tailam*

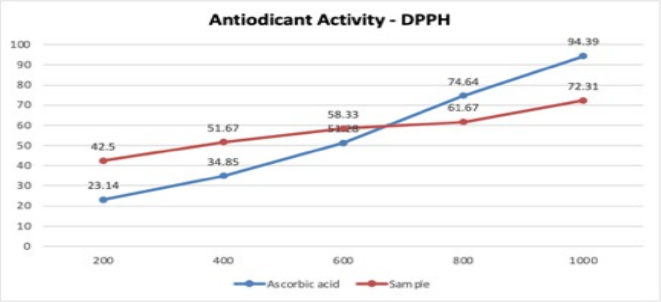
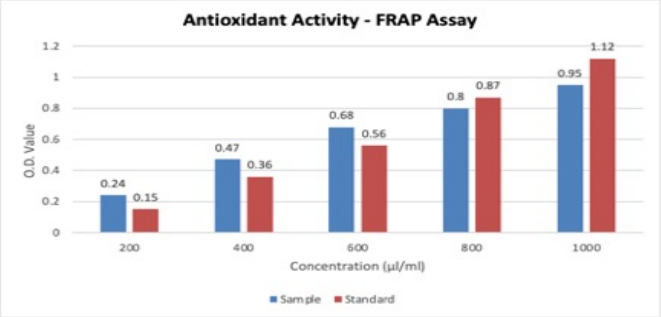


Figure 2: Shows the results of FRAP antioxidant activity of the tailam



To study the antioxidant activity, DPPH assay was performed and the IC50 for DPPH activity was determined to be 389.4081µl/ml. The oil sample showed the ability of reduce Fe3+ ions to Fe2+ ions thus showed to possess the free radical scavenging activity and reducing power activity.

Anti-inflammatory activity

Membrane stabilisation assay for HRBC:

Table 2 - showing the results of Membrane stabilisation assay for HRBC activity of the tailam

Concentration (µl/ml)	Negative Control (PBS)	Sample of RBC lysis (%)	Positive Control (Distilled Water)
200	0%	1.04	100%
400		3.02	
600		4.96	
800		7.2	
1000		8.64	

The experiment is carried out to determine whether the sample is compatible with HRBC. Distilled water is taken as positive control and is considered to be 100% haemolysis while phosphate buffer saline was taken as negative control and showed the highest compatibility to HRBC and showed no haemolysis.

Inhibition of BSA Denaturation Assay

Table 3: Showing the results of Inhibition of BSA Denaturation Assay of the *tailam*

Concentration (µl/ml)	% of Inhibition of Denaturation	
	Tailam sample	Diclofenac Sodium
200	12.86	11.24
400	32.54	23.46
600	55.02	36.48
800	64.68	45.6
1000	82.36	62.38
IC50 Value	**562.5 µl/ml**	**827.8778 µg/ml**

This test is done to see if the sample can stop standard BSA from becoming denatured. The anti-inflammatory medicine diclofenac sodium served as a positive control, and it was found that the sample efficiently inhibited protein denaturation. The sample and the reference drug's respective IC50 values were 562.5µl/ml and 827.88 µg/ml.

Discussion

A good beginning point is to utilize two to four plants that best represent the desired activity, such as the renowned Trikatu formula of Ayurveda, which consists of three spicy herbs, a mixture that predominantly tastes bitter and can be used to treat a wide range of Pitta and Kapha problems. We add supplemental herbs to such a foundation recipe to alter or change its effects in different ways. Herbs can be added to intensify the effect, while balancing agents can be used to keep the effect from becoming too strong. In such as fashion *Balaguluchyadi tailam* is one such formulation with infusion of aqueous decoction of 11 herbal plant products in sesame oil. *Balaguluchyadi taila*m is used as an anti-inflammatory drug to help relieve pain and shorten the duration of the attack in the treatment method of abhyanga, an Ayurvedic therapy that entails massaging the entire body from head to toe with dosha-

specific warm herb-infused oil (50). Similarly, *balaguluchyadi kashyam* is one of the most important herbal formulations in treating arthritis (51).

From Figure 1 and Table 1, it is indicated that the presence of certain major biomolecules that have wide range of medicinal properties contributes to the medicinal role of *Balaguluchyadi tailam*. These molecules have antioxidant, anti-hypertensive, antibacterial roles, and support circulatory, neuromotor and respiratory functions.

Balaguluchyadi tailam is used as an anti-inflammatory drug to help relieve pain and shorten the duration of the attack in the treatment method of abhyanga, an Ayurvedic therapy that entails massaging the entire body from head to toe with dosha-specific warm herb-infused oil (43). Similarly, *Balaguluchyadi kashyam* is one of the most important herbal formulations in treating arthritis (45).

A case study by Sumayya and Sunilkumar(2023) reported that a 66-year-old patient at the Govt. Ayurveda College in Thiruvananthapuram with double vision, right eye discomfort, and unilateral full ptosis for the last two weeks. *Balaguluchyadi tailam* was applied to the head every morning for seven days as part of the therapy, and it was then massaged for 45 minutes. The condition was successfully treated with ayurvedic drugs and regular Jaloukaavacharana, which had a significant alleviation in treating neurological illness (42).

Tinospora cardifolia (Thunb.)Miers, *Sida cordifolia*, and *Cedrus deodara* are all found in *Balaguluchyadi kashyam*. Rats were used to study the anti-arthritic effects of *balaguluchyadi kashyam*. Results showed that collagen-induced arthritis rats had significantly higher levels of C-reactive protein, arthritis index, rheumatoid factor, and myeloperoxidase in serum and protein levels of TLR-4, myeloid differentiation factor 88, NF-kB, TNF-, IL-1, inducible nitric oxide synthase, cyclooxygenase-2, and prostaglandin E-2 in cartilage. These inflammatory mediators are down regulated after *balaguluchyadi kashyam* therapy. *Balaguluchyadi kashyam*, it is found, was successful in stopping the progression of rheumatoid arthritis (44).

Further, antioxidants that prevent oxidation, a chemical process that can result in the production of free radicals and anti-inflammatory agents which lessens bodily inflammation, including pain, swelling, and redness are found in this formulation. *Tinospora cordifolia, Pluchea lanceolata, Pterocarpus santalinus* which are a part of this composition have remarkable Anti-inflammatory and *Sesamum indicum, Saussurea lappa, Pluchea lanceolata, Pterocarpus santalinus* with significant antioxidant activity pays a vital contribution to the healing properties of this oil formulation. In this aspect *balaguluchyadi tailam*, it is advisable to consult authoritative Ayurvedic texts, practitioners, or scholarly articles on Ayurvedic medicine. Additionally, as with any herbal remedy, individual reactions may vary, and it is important to consult with an Ayurvedic practitioner or healthcare professional before using it for specific health concerns.

Conclusion

The molecules, as shown in Table 1 indicate several medicinal roles which could contribute to the effect of *Balaguluchyadi tailam* in curing diseases ranging from arthritis to neuropathy and various neurological illnesses. Thus, *Balaguluchyadi thailam* is used to treat neurological diseases, inflammatory pain, swelling, and stiffness linked to rheumatoid arthritis, gout, and other conditions, as well as muscular weakness, sluggish metabolism, overall body edema, and chronic weariness.

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