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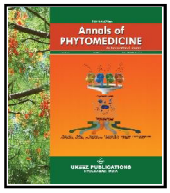

Siddha Formulation : Open Access

Assessment of Thaalaga parpam: A herbomineral formulation through systematic spectroscopic analysis, acute toxicity testing and *in vivo* anti-inflammatory activity

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Abstract

Siddha medicine as traditional medicine has gained much focus from the scientific community for its potential medicinal value and its standardization is necessary to use it as safety drug throughout the world. Thaalaga parapam (TP), a herbomineral drug known to use for the treatment of respiratory diseases is subjected to characterization through fourier transform infra red spectroscopy (FTIR). The characterization of the drug done by FTIR is used to find its functional groups. The acute oral toxicity test for the formulation was performed according to OECD guidelines 423. Further, the animal study was carried out for assessment of the acute anti-inflammatory action of TP by using the carrageenan-induced paw edema model. The animals were divided into 4 groups, carrageenan treated, carrageenan with TP at doses of 200 mg/kg/PO, 400 mg/kg/PO, and carrageenan with standard drug indomethacin (10 mg/kg/PO). Acute inflammation was determined by paw edema measurement. TP-treated groups showed a significant reduction in paw edema compared to carrageenan-treated rats. The current study concluded that the Siddha medicine, herbomineral TP has a safety profile and high efficacy to treat acute inflammation.

1. Introduction

Siddha medicines are the treasure house of mysterious science incorporated with several medicines for the ailment of various diseases (Kabilan and Murugesan, 2016; Sethumathi, *et al.*, 2021). The Siddha medical system was founded by a group of spiritual people called SIDDHARS, who were spiritually enlightened persons (Radhakrishnan, 2018). The formulations in Siddha medicine are purely herbal or metal or minerals or a combination of herbominerals (Vetha Merlin *et al.*, 2015). The medicines obtained from minerals have long shelf-life than herbal drugs and so, it is used for the treatment of chronic diseases. There are several methods employed for the purification of minerals and metals used in drugs for the welfare of human beings. Parpams are herbominerals and are powder in nature which is acquired by calcification of purified minerals, metals and animal products by precise technique (Suoboda and Prakriti, 1998). This method of preparation of Siddha medicine is used to convert metals or minerals into sulphide or oxide forms along with herbal treatment by a continuous high-temperature calcination process. The parpams thus obtained are very small particles and are given along with adjuvants such as ghee, milk, palm jaggery, butter, honey, *etc*., according to the type of disease. Adjuvants are used to enhance the biocompatibility of the drug. The method of preparation is very much important to make the drug exclusive (Shakya Akhilesh *et al.*, 2011). World Health Organisation (WHO) has recognized the traditional system of medicine for the healthcare of the community. So, now is the time to standardize the drugs of siddha for worldwide acceptance to ensure its safety. Thaalaga parpam (TP), a herbomineral Siddha formulation used for the treatment of various diseases such as fever, gonorrhea, respiratory diseases, bronchial asthma, sexually transmitted diseases, *etc*. So, the present study deals with the organoleptic characters, physicochemical properties, phytochemical analysis and FTIR analysis of TP to identify its functional group as a part of standardization as per the AYUSH guidelines. The fundamental aim of inflammatory response is to pinpoint and remove the harmful agents; secondarily, to eradicate damaged tissue components and to end in the healing of the affected tissues, organs, or system (Shaikh *et al.*, 2015).

Inflammation is the body's defense mechanism against pathogens, which is characterized by redness, swelling, itching, pain, and heat. The inflammation is responsible for the pathogenesis of many diseases including cancer, arthritis, cardiovascular dysfunction, and other life-threatening and debilitating disorders. The carrageenan-induced paw edema model is an acute model of inflammation that is widely used for screening novel anti-inflammatory compounds.

For centuries, people have been treated with TP for different disease conditions including asthma, epilepsy, and STD disorders. The current study evaluated the anti-inflammatory potentials of plant-derived Siddha-formulated drug, TP by using the carrageenan-induced paw edema model.

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2. Materials and Methods

2.1 Standard procedure for preparation of Thaalaga parpam

The constituents of this parpam are potassium nitrate and old tamarind (*Tamarindus indicus* L.), thaalagam (arsenic trisulphide) and sippichunnam (shell powder) (Thiagarajan, 2004 and Sarakku Suthisei Muraigal, 2008).

2.2 Collection, purification, and preparation of medicine

The old Tamarind (*T. indicus*) was collected from in and around Erode District, Tamil Nadu, India. The constituents were purified and the drug was prepared as per the Siddha literature in the Gunapadam Laboratory of Nandha Siddha Medical College and Hospital, Erode, Tamil Nadu, India.

2.3 Identification and authentication of the drug

The constituents were verified and accredited by the proficient authority of the Gunapadam Department, Nandha Siddha Medical College and Hospital, Erode, Tamil Nadu, India.

2.4 Drug profile

Dose: 65-130 mg twice a day for 10 to 12 days.

Adjuvant: Palm jaggery

Route: Enteral (Oral)

Indications: Tuberculosis, pneumonia, cough, pulmonary diseases, and kapha diseases.

2.5 Standardization of the drug

Standardization of Siddha drug is important to ensure its identity and to determine its quality and efficiency. This is determined by qualitative and quantitative analysis through its physicochemical investigations and instrumental analysis (Lohar, 2007)

2.6 Organoleptic character

Organoleptic characters like appearance, color, taste and odor of TP were noted using standard techniques (Harborne, 1973) (Table 1).

2.7 Physicochemical analysis

Physicochemical characteristics of the herbomineral formulation were carried out as per the following standard procedure (Anonymous, 1966) (Table 2).

- **Loss on drying:** 5 g of the sample was weighed and heated in an oven at 105ºC for 5 h, then cooled in a desiccator and weighed. The percentage of loss in weight was then calculated.
- **Total ash content:** 2 g of TP was measured and placed in an ignited silica dish and kept in a muffle furnace at 450°C till it became free from carbon. The dish was cooled and weighed. The total ash percentage was calculated.
- **Water-soluble Ash:** To 25 ml of water, the ash was heated for 5 min, then insoluble ash was collected using filter paper, transferred to the silica crucible then heated for 15 min at 450°C and the residue was weighed until constant weight. The percentage of water-soluble ash was calculated.
- **Acid-insoluble ash:** The above-obtained ash was heated for 5 min with 25 ml of 1:1 dilute HCl, and the insoluble material was collected in a Gooch crucible on acidless filter paper and heated, cooled and weighed. The percentage of acid-insoluble ash is calculated.
- **Determination of pH:** 1 g of TP was weighed and taken in a 50 ml beaker then mixed with distilled water up to the mark and heated at 20-25°C to allow the suspension to settle down for an hour and the pH was calculated.

2.8 Phytochemical analysis

The preliminary screening of phytochemicals was done as per the standard procedure (Kokate *et al.*, 1995) (Table 3).

- **Detection of alkaloids:** A pinch of Thaalaga parpam, was dissolved in dilute hydrochloric acid and filtered. The filtrate was treated with Wagner's reagent. Brown or reddish precipitate shows the presence of alkaloids (Sri Bhuvaneswari *et al*., 2022).
- **Detection of phenols:** The parpam was treated with a few drops of ferric chloride (10%) solution. The appearance of bluish-black color shows the presence of phenols (Korach *et al*., 2020).
- **Detection of tannins:** The parpam was mixed with 1% gelatin solution along with sodium chloride. A white colour precipitate persists, the presence of tannins.
- **Detection of flavonoids:** To a few drops of 10% sodium hydroxide, TP is added, the intense yellow colour is formed with then with the addition of diluted hydrochloric acid it, becomes colorless, it indicates the presence of flavonoids (Lalitha *et al.*, 2022)

2.9 FTIR spectroscopic analysis

The TP was encapsulated in a potassium bromide pellet, to prepare translucent sample discs. The sample was loaded in an FTIR spectroscope (Shimadzu, IR affinity 1, Japan) with a scan range from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The FTIR spectral analysis was recorded at Nandha College of Pharmacy, Erode, Tamil Nadu, India (Ferguson, 1956).

2.10 Acute toxicity test

The oral acute toxicity study of TP was evaluated according to Organization for Economic Cooperation and Development (OECD) guideline 423. The rats were divided into two groups (n=6) group I was treated with 300 mg/kg of TP orally and group II was treated with a single oral dose of TP at a dose of 2000 mg/kg. We observed the mortality or adverse effects of TP on rats. Behavioural (grooming, immobility, anxiety, *etc.*), general examination (lacrimation, fur discoloration, *etc.*) respiratory, and neurologic changes, as well as changes in body weight, food and water consumption, were noted upto 24 h.

2.11 Anti-inflammatory activity

2.11.1. Experimental animals

For the experiment, around 150-200 g weighing wistar rats were used. They were kept at 24ºC for a 12:12 h light/dark cycle in polypropylene cages following the standard laboratory conditions. Proper commercial rat diet and water were provided to the animals. Before the study, the animals were quarantined and acclimatized to laboratory conditions for 7 days. The general health and suitability of the animals were observed during the study period. All the

investigational techniques and protocols used in this present study was reviewed by the Institutional Animal Ethics Committee (688/2/CPCSEA), following the norms of the IAEC. As per the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), animal care was given.

2.11.2 Carrageenan-induced rat paw edema

The anti-inflammatory activity of TP was evaluated in wistar albino rats (Winter *et al.*, 1963). Animals were fasted overnight and were divided into control, standard and different test groups. The wistar albino rats were divided into 4 groups, consisting of 6 animals in each group. Group II received standard indomethacin orally at the dose of 10 mg/kg. Before this, TP was given by oral route at the dose of 200 and 400 mg/kg to group III and IV, respectively. The required quantity of TP was weighed and made suspension with 0.5%w/v carboxy methyl cellulose. This suspension was administered orally. The rats in the control group (group I) received the vehicle solution without test compounds. After one hour of test drug administration, rats in all the groups were challenged with 0.1 ml of 1% carrageenan in the sub-plantar region of the right hind paw (Selvaraj *et al.*, 2014). Paw thickness was measured before and every 1 h up to 4 h using vernier caliper after the challenge of carrageenan. The percent inhibition of paw thickness for treated groups was calculated by comparing it with the mean paw thickness of the control group.

% Inhibition = 100 (1–Vt/Vc),

(Vc- Control mean paw thickness, Vt- Test mean paw thickness)

2.12 Statistical analysis

The data of each group are expressed as the mean ± standard mean error (SEM). Statistical analysis was carried out by a one-way analysis of variance (ANOVA) followed by Dunnett's test. The $p < 0.05$ was considered statistically significant.

3. Results

3.1 Preliminary phytochemical analysis

Organoleptic characters of TP showed in Table 1, the pH of the parpam was 8.2, which shows the alkaline property of the drug. The adjuvant used is palm jaggeries that possess acidic pH (5.8-6.2). So, the adjuvant used reduced the alkalinity of the TP, which shows that alkaline drugs are absorbed in the alkaline condition that is the intestine. Preliminary phytochemical analysis of TP revealed the presence of flavonoids and phenols (Table 2).

Table 1: Organoleptic characters and physiochemical analysis of Thaalaga parpam

Organoleptic characters	Observation	Physiochemical parameters	Percentage
Color	Ash coloured	Loss on drying	Less than 1%
Odor	Odorless	Total ash value	23.12%
Taste	Tasteless	Acid-insoluble and water-soluble ash	0.54% and 5.46%
State of matter	Solid	pH	8.4%
Consistency	Powder	Solubility	Soluble in acid

Table 2: Qualitative phytochemical analysis of Thaalaga parpam

Phytochemicals	Observation
Alkaloids	-
Flavonoids	+
Phenols	++
Tannins	-

Table 3: Functional groups of Thaalaga parpam

Wavelength(cm^{-1})	Bonds	Functional group
2757.05, 2618.19, 2087.80, 2587.33 and 2271.02	Acid and alkene,	O-H and C=C-H
1965.33 and 1866.97	Aromatics	Aromatics
1800.43	Aromatics	Acids, aldehydes, andRCOOH
1694.35 and 1621.06	Alkene and aromatics	C=C
1557.41, 1518.84, 1288.36 and 1270.04	Aromatic and nitro	C=C
1153.35 and 1130.21	Alcohol and carbonyl	C-O and C-O-C
1044.38, 972.06, 915.16	Carbonyl	C-O-C group
885.27 and 806.19, 775.33	Alkanes	C-H
674.07 and 614.29	Halogen compounds	C-Br and C-S linkage
595.00 and 568.96	Halogen compounds	C-Br

3.2 FTIR analysis

The FTIR spectrum was used to identify the functional groups based on the peak value. On the Infrared radiation, the TP showed the following characteristic absorption spectra (Table 3). In the present investigation, the absorption spectra exhibited a peak at 2757.05 representing the presence of alkene (C=C-H stretch) and carboxylic acid (O-H stretch). The peak at 1965.33 showed the presence of aromatic compounds and the peak at 1694.35 shows the presence of Alkene (C=C) and aromatics. The peak at 1557.84 and the peak at 1044.38 represented the presence of nitro (N-O stretch) and carbonyl (C-O-C) compounds, respectively. The wavelength at 595.00 and 568.96 represents the occurrence of halogen compounds.

3.3 Pharmacological studies

3.3.1 Acute toxicity test

Rats treated with TP at a maximum dose of 2000 mg/kg were not showed mortality or adverse effects after 24 h. There are no behavioral, respiratory, and neurologic changes in low and high-dose treated animals. Hence, $1/10^{th}$ of the dose that is 200 and 400 mg/kg was selected as a therapeutic dose for screening acute anti-inflammatory activity.

3.3.2 Anti-inflammatory studies

The carrageenan treatment produced maximum inflammation confirmed by a significant elevation in rat paw thickness (6.6 ± 0.01 at 3 h after carrageenan administration). The TP (200 and 400 mg/kg) treated rats showed a significant (p<0.01) a reduction in paw edema (Table 4) compared to the carrageenan control. The standard drug indomethacin showed maximum (54.5%) anti-inflammatory effects confirmed by reduction in paw thickness (3.0 ± 0.02) than carrageenan-treated rats. The TP at a dose of 200 and 400 mg/kg treatment showed dose-dependent anti-inflammatory action (37.9% and 45.5% at 3 h).

Table 4: Anti-inflammatory activities of Thaalaga parpam in rats by carrageenan-induced paw edema

Group	Paw thickness in mm					% Inhibition at 3 h
	0 h	1 h	2 h	3 h	4 h	
Group I Carrageenan	1.4 ± 0.02	3.2 ± 0.01	5.4 ± 0.02	6.6 ± 0.01	5.3 ± 0.03	-
Group II Indomethacin	1.6 ± 0.02	2.2 ± 0.02**	2.3 ± 0.01**	3.0 ± 0.02**	2.3 ± 0.01**	54.5
Group III 200 mg/kg TP	1.9 ± 0.02	3.1 ± 0.02**	3.6 ± 0.01**	4.1 ± 0.03**	3.4 ± 0.02**	37.9
Group IV 400 mg/kg TP	1.6 ± 0.01	3.4 ± 0.01**	3.2 ± 0.02**	3.6 ± 0.03**	2.4 ± 0.01**	45.5

Values were mean ± SEM, n=6, **p<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

4. Discussion

The traditional system of medicine is a peculiar system that gives excellent cures for all chronic diseases like respiratory diseases, dermatological diseases, cancerous diseases, diabetes, tuberculosis, syphilis, *etc*. (Yuan *et al*., 2016). TP a herbomineral formulation is commonly used in the Siddha system as a drug of choice for the treatment of tuberculosis and asthma (Balaman *et al.*, 2021). The above results correlated with Siddha's authorization and characterization of TP that organoleptic characters showed the accuracy of raw drugs and formulations about their color, shape, texture, pH and identity. Another study also suggested that there is no change in color and odor indicating that it does not possess any toxic effect (Kalaivanan and Manjari, 2019; Kabilan, 2017). Any medicines with less moisture content were extremely valued as they may have maximum stability (Vijayalakshmi *et al*., 2021). The drug TP possesses very low (>1%) moisture content. The shelf-life of parpam stated in the Siddha literature is around 100 years. As TP has a very lesser moisture content, and hence the stability of the drug is increased. So, it possesses higher shelf-life and the growths of microbes are prevented. Another study suggested that loss of moisture on drying is very less, which is similar to the present study (Akila *et al.*, 2014).

Ash is an inorganic filtrate that remains after heating. The quantity of ash remains after heating gives an idea of the mineral constituent present in the drug. The amount of ash was found to be high which shows that it is an inorganic material and the acid-insoluble ash was very low in TP (Nalini *et al*., 2016). The above result gives an interpretation that the medicine was prepared without any contamination as this may disturb the absorption of the drug.

The present study is correlated with the study on Poonaga parpam (Elakkiya *et al*., 2018) as it is absorbed in alkaline conditions. The TP is not soluble in water and soluble in acids (HCl and H_2So_4). As *T. indicus*, is the main ingredient used for the preparation of TP, the drug possesses anticancer, anti-inflammatory and antimicrobial activities (Moncrieffe *et al*., 2019). The above results were also suggested in Kara Sooda Sathu parpam, Velvanga parpam, Chandra prabhava chendooram and Senna lavana parpam (Bakyalakshmi C, Kingsly, 2019; Aparnaa and Thiruthani, 2018; Packia Sri and Kingsly, 2021; Suntharalingam and Antony Duraichi, 2019).

The results of acute toxicity studies indicated that produced TP is safe and does not produce any untoward action. The anti-inflammatory evaluation of TP showed a dose-dependent anti-inflammatory action. The herbomineral formulation can be considered safe and efficacious, act as a mineral source and reduced side effects and acts as an important traditional therapeutic drug in the treatment of inflammation. The assessment of TP was correlated with the anti-inflammatory effect of *Inula cuspidata* (Sarvesh Kumar *et al.*, 2016). The present study revealed the organoleptic character, physicochemical properties, phytochemical analysis, FTIR spectral analysis, acute toxicity study and anti-inflammatory analysis of TP as a process of standardization to use as a drug for the welfare of the community.

5. Conclusion

Traditional medicines are more advantageous than any other system of medicine because of their milder side effects. But, there is a lack in standardization of traditional medicine. Nowadays, sophisticated modernized investigation methods are available for the evaluation of ancient medicines. Organoleptic, physicochemical properties,

phytochemical, FTIR instrumental analysis and anti-inflammatory study of the above drug indicate the presence of major groups of the compounds. This finding supports that the therapeutic efficacy of TP does not affect it, as it contains permissible limits of heavy metal. So, the drug was considered safe and can be used as internal medicine. Henceforth, the research to be needed phytochemical quantification and human use of TP for various inflammatory diseases.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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