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Standardization of Siddha Herbo mineral formulation Chanthirothaya tablet (CHTM) for Rheumatoid Arthritis (RA)

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***Abstract*---**Introduction: Siddha System of Medicine is one of the traditional systems of Indian Medicine. The usage of herbal and mineral medicinal preparations is increasing each day for treating various diseases. Although there are many NSAIDs available nowadays to treat Rheumatoid Arthritis (RA) clinically, the usage of herbomineral formulations comes into existence only after testing the drug for physiochemical and phytochemical analysis inorder to treat RA. Objective: The objective was to analyse Siddha Herbomineral formulation Chanthirothaya Tablet for Rheumatoid Arthritis by screening the physico chemical, phytochemical properties and its organoleptic characters of the tablet. Materials and methods: Chanthirothaya Tablet was prepared as per GMP Guidelines and subjected to analysis for physico chemical, phytochemical screening and its organoleptic characters at Noble research solutions, Chennai as per Pharmacopoeial laboratory for Indian Medicine (PLIM) Guidelines of AYUSH (Ayurveda, yoga, Unani, Siddha, Homeopathy) health system. Result : Physico chemical analysis report showed loss on drying at 105°C is 0.6533±0.293, total ash value 2.933±0.4726, acid insoluble ash 0.4133± 0.05033, water soluble extractive 14.3±1.311and acid soluble extractive 6.667±1.457, while screening of Phytochemicals showed the presence of alkaloid, Coumarin, Saponins, Tanins, Glycosides, phenolic compounds, steroids, triterpenoids and

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carbohydrates. Conclusion: This present study results of physico chemical, phytochemical and organoleptic characters of Chanthirothaya tablet (CHTM) were justified for the nature of its composition and safety profile and standardization of the tablet for therapeutic use in treating Rheumatoid arthritis (RA).

***Keywords*---**Siddha system, herbomineral, physicochemical, phytoconstituents, Chanthirothaya tablet.

Introduction

Siddha system of medicine is one of the oldest system of Indian medicine mainly based on the utilization of natural products that posess great therapeutic importance. Many forms of medicinal formulations had been used by Asian civilizations since decades that are practiced till date. This system aims at getting to the root of the problem with its holistic approach and not just offering symptomatic relief[1].Despite the overwhelming impact of modern medicine and enormous breakthroughs in synthetic drug synthesis, traditional cures (now referred to as herbal pharmaceuticals/herbal treatments in many publications) are supported, suggested, and encouraged by the World Health Organization (WHO). Since these remedies are affordable, healthful, and trustworthy because they have no substantial adverse repercussions, they are not only popular but also a good way to cure the diseases [2, 3]. Hence it is very important to standardize Siddha medicines using scientific techniques to prove its nature of composition, safety and quality that might help in building the confidence for therapeutic medicine usage, among people for global acceptance [4].

Rheumatoid arthritis (RA) is an Auto immune multisystem disease marked with a variety of systemic and extra articular manifestation [5]. The characteristic clinical features were morning stiffness lasting for more than 1 hour, pain (more pronounced at rest), swelling, stiffness and synovitis of minor and major joints causing disabilities and deformities[5,6]. RA affects approximately 0.3-1% of adult population worldwide. The prevalence of RA in India is 0.76%. Females are more affected than male in the ratio of 3:1[7]. Around 40% of RA patients were registered as disabled within 3 years of onset, around 80% were seen moderate to severely disable within 20 years of disease duration. [7] Treatment of RA with NSAIDs has complications on a longer duration. People with inadequate knowledge about the herbomineral medicinal preparations remains as a flaw in the management of rheumatoid and its mortality. Some of the herbal and mineral preparations of Siddha medicine has been validated for their anti-arthritic, anti-inflammatory and immunomodulatory activity through many studies and can be a better option for steroidal drugs usuage with minimal side effects. Medicine associated with few life style modications such as food habit, pattern of sleep and exercises can be a promising path in the management of the disease.

In Siddha medicine, Chanthirothaya tablet (mathirai) is a herbomineral formulation which would be a best choice of drug in treating rheumatoid arthritis. The formulation has been evaluated for its physico-chemical profile such as ash

value, extractive value in water and alcohol and qualitative phytochemical analysis. Therefore the present study is to ensure the standardization of drug.

Material and Methods

Selection of Drug

The drug Chanthirothaya tablet was given in Siddha classical text Veeramamunivar aruli seitha Anuboga Vaithiya Sigamani page. No: 30-31[8].

Ingredients

The composition of Chanthirothaya tablet (CHTM) was made up of with nine ingredients of which six were herbal and three are mineral compounds. Drug composition- *curcuma longa, and Citrus lemon,Hydragyrum subchloride (Calomel), Sodium biborate (Borax)* [9,10]. Adjuvant composition- *Zingiber officinale, Piper Nigrum, Piper longum, Cuminum cyminum,and Sodium Chloride impure (rocksalt)*[9,10].

Collection of raw material

The indigeneous raw materials were procured from the local drug store at parys, Chennai. They were identified and authenticated by the Botanist of Government Siddha Medical College, Chennai, Tamilnadu - 106(Voucher number GSMC/MB-490/22 – 494/22)

Sample preparation

The Herbomineral siddha formulation Chanthirothaya Tablet (CHTM) was prepared as per classical Siddha text. The ingredients were purified as per Siddha text Sikitcha rathna deepam saraku suthi muraigal[11]. After purification the ingredients were grounded separately in to powder as per the ratio given in the text. All the powdered drugs were mixed and grounded for 6 hours in the stone mortar by adding sufficient quantity of lime juice and made in to 360 mg tablets.The tablets were dried well in shade and stored in a clean dry air-tight porcelain jar.

Adjuvant
The adjuvant drug was also taken as per the given ratio and purified, powdered and grounded in the stone mortar for about half an hour by adding sufficient quantity of honey. The sample of both the drug and its adjuvant were taken together for the standardization of CHTM. The phyto chemical screening, presence of Organoleptic characters and physico chemical analysis of CHTM was carried out at NABH accredited lab, Noble research solutions, kolathur, Chennai, Project ID - NRS/AS/0701/05/2021, ISO 9001: 2015.

Organoleptic characters

The organoleptic characters like state, nature, odour, touch, flow property, appearance were observed [12,13] . The results were showed in Table1.

Physico-Chemical Parameters

The solubility profiles using various solvents were executed on CHTM and its end results were depicted in Table 2. The different extracts obtained from the formulation were prepared for the study and the physico chemical screening such as percentage of loss on drying, total ash, acid-insoluble ash,water soluble extractive and alcohol soluble extractive were carried out for CHTM[12,13] based on the AYUSH PLIM Guidelines and presented in Table 3.

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish and the sample was dried for 5 hours at 105°C and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and has been ignited to the temperature 400 °C at the furnace until it turns white in color. This indicated the absence of carbon. Percentage of total ash was calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test was boiled for 6 mins by adding 25 ml of dilute hydrochloric acid to it. The insoluble matter formed was collected in crucible and washed with hot water and then incinerated to get a constant weight. Percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

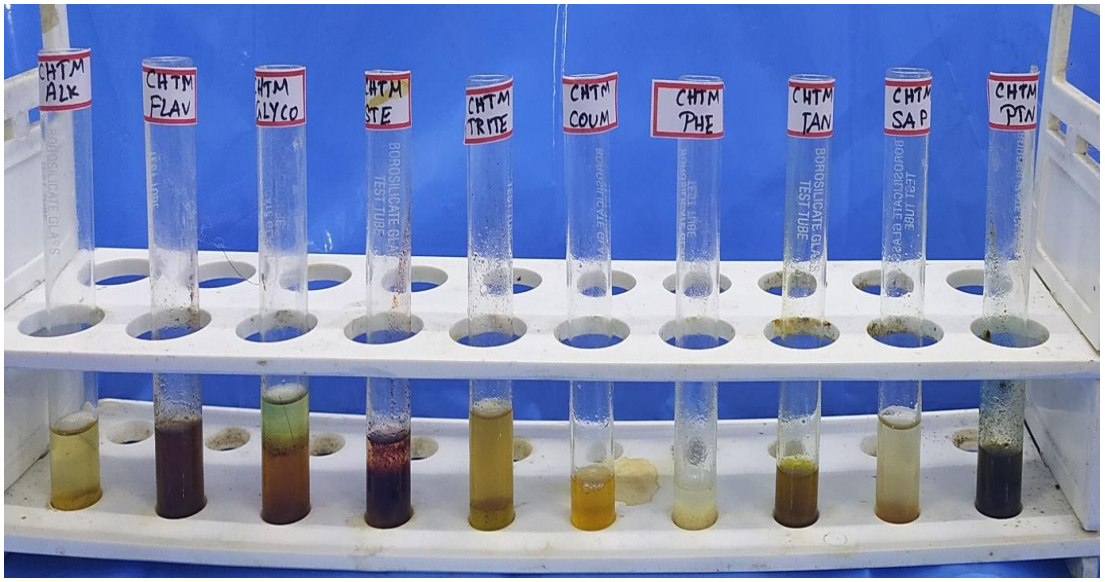
The test sample was macerated for about twenty-four hours using 100 ml of Alcohol taken in closed flask. It was shaken frequently during six hours and then allowed to stand for eighteen hours. Precaution measures were taken and filtered rapidly to prevent the loss of solvent; the 25 ml of filtrate was then evaporated for dryness at 105°C on a tarred flat bottomed shallow dish, to get an constant weight and then weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Determination of Water Soluble Extractive

Test sample was macerated for twenty-four hours using 100 ml of chloroform water taken in a closed flask; It was shaken frequently during six hours and then allowed to stand for eighteen hours. Precaution measures were taken to prevent the loss of solvent and filtered rapidly; the 25 ml of filtrate was then evaporated for dryness at 105°C on a tarred flat bottomed shallow dish, to get an constant weight and then weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Phytochemical Screening

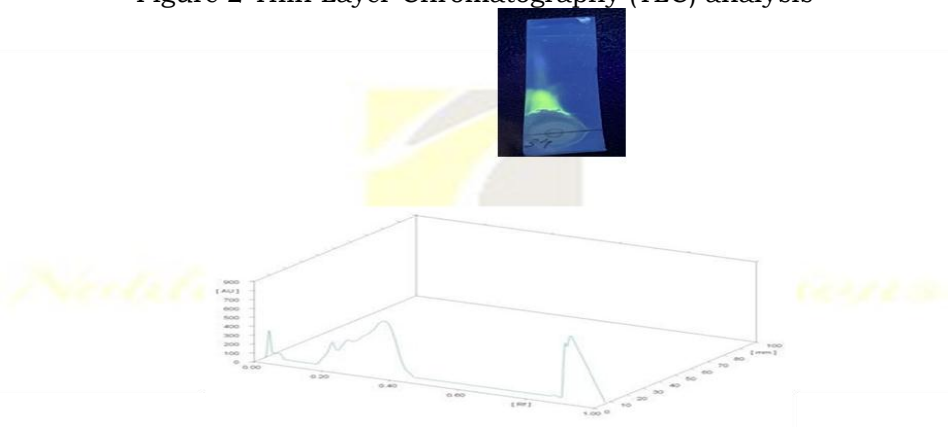
The qualitative phytoconstituents tests were performed on CHTM for the presence of alkaloids, flavonoids, phenols, steroids, Triterpenoids, Cyanins, carbohydrates, coumarins, saponins, tannins, glycosides and proteins [14]. The results obtained on each test were given in Table 4. Figure 1 Qualitative phytochemical investigation of CHTM.



Thin Layer Chromatography (TLC) Analysis

Test sample was been subjected to thin layer chromatography (TLC) as per one dimensional conventional ascending method by using silica gel 60F254, 7X6 cm (Merck) were cut with normal household scissors. Soft pencil was used for the plate markings. To the TLC applied sample volume 10-micro liter, Micro pipette were used to spot the sample at a distance of 1 cm at 5 tracks [15]. The specified solvent system of twin trough chamber with after the run plates were dried and observed using visible Short-wave UV light 254nm and long-wave UV light 365 nm and the illustration is shown in figure 2.

Figure 2 Thin Layer Chromatography (TLC) analysis



High Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC method is a highly developed automated separation technique derived from TLC. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials accurately and cost effectively. Pre-coated graded plates of HPTLC and auto sampler were used to obtain accuracy and sensitized to significant separation by both qualitative and quantitative. This method offers high degree of promptness, selectivity, and sensitivity combined with the single-step of sample preparation. Thus routine quality control analysis can be done using this method [16].The chromatographic fingerprint of phytochemicals required for confirmation of identity and purity of phytotherapeutics was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried. The Plates were scanned under UV at 366nm; data obtained from scanning were fed into CAMAG software for integration [16]. Detection of phytoconstituents in each sample was done by Chromatographic finger print method and their respective Rf values were tabulated in table 5.

Table 1. Organoleptic characters of Chanthirothaya Tablet (CHTM)

State	Solid
Nature	Moderately fine
Odour	Aromatic
Touch	Soft
Flow Property	Non Free flowing
Appearance	Brownish

Table 2. Solubility profile of Chanthirothaya Tablet- CHTM

S.No	Solvent Used	Solubility / Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble

4	Ethyl acetate	Insoluble
5	DMSO	Soluble

Table 3. Physico-Chemical Analysis of Siddha formulation Chanthirothaya Tablet

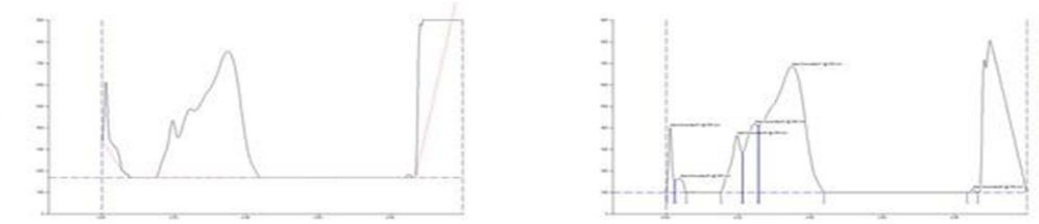
S.No	Parameter	Mean (n=3) SD
1.	*Loss on Drying at 105 °C (%)*	0.6533 ± 0.293
2.	*Total Ash (%)*	2.933 ± 0.4726
3.	*Acid insoluble Ash (%)*	0.4133 ± 0.05033
4.	*Water soluble Extractive (%)*	14.3 ± 1.311
5.	*Alcohol Soluble Extractive (%)*	6.667 ± 1.457

Table 4. Phytoconstituents analysis of Siddha formulation Chanthirothaya Tablet

S.no	Phytoconstituents	Tests Used	CHTM
1	ALKALOIDS	Mayer's test	+
2	FLAVANOIDS	Alkaline reagent test	-
3	GLYCOSIDES	Borntrager's test	+
4	STEROIDS	Salkowski test	+
5	TRITERPENOIDS	Liebermann- Burchard test	+
6	COUMARIN	Sodium hydroxide test	+
7	PHENOL	Lead Acetate test	+
8	TANIN	Ferric chloride test	+
9	PROTEIN	Biuret test	-
10	SAPONINS	Foam test	+
11	SUGAR	Benedicts test	+
10	ANTHOCYANIN	Sodium hydroxide test	-
11	BETACYANIN	Sodium hydroxide test	-

+ → Indicates positive and - → Indicates negative

Table 5. Analysis of High Performance Thin Layer Chromatography (HPTLC) of Siddha Formulation Chanthirothaya tablet – CHTM



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	8.9	0.01	304.3	19.52	0.02	56.4	2062.4	3.73
2	0.02	57.1	0.04	66.6	4.28	0.06	1.4	996.3	1.80
3	0.15	2.0	0.20	267.1	17.14	0.21	185.8	5328.8	9.64
4	0.21	187.2	0.25	319.5	20.50	0.26	311.4	7404.7	13.39
5	0.26	311.3	0.35	585.4	37.55	0.44	1.5	39403.2	71.25
6	0.83	0.0	0.85	15.8	1.02	0.86	0.0	109.8	0.20

Discussion

The Purpose of phytochemical and HPTLC analysis is to prove the efficacy of any formulation in establishing the bioactive components of the drug that has therapeutic importance whereas the physico chemical analysis is to assess the macro and microscopic properties of the formulation. There were previous studies done on the anti-arthritic and anti-inflammatory activity of certain ingredients of this herbomineral formulation to denote their therapeutic importance [17, 18,19]. High performance thin layer chromatography (HPTLC) analysis of the sample revealed the presence of six versatile phytocomponents present with Rf value of the six prominent peaks ranges from 0.02 to 0.83[15]. Hence the above Siddha Siddha formulation Chanthirothaya tablet (CHTM) was standardized for its quality and the beneficial of phytoconstiuents present in it.

Conclusion

Physico chemical analysis of the current study concluded loss on drying at 105°C was 0.6533 ± 0.293, total ash value 2.933 ± 0.4726 , acid insoluble ash 0.4133 ± 0.05033, water soluble extractive 14.3 ± 1.311 and acid soluble extractive 6.667 ± 1.457 . Phytochemical screening showed the presence of bioactive components such as alkaloids, coumarins, saponins, tannins, glycosides, phenols, steroids, Triterpenoids and carbohydrates which suggests that it can be a better drug of choice for Rheumatoid arthritis. But Invivo studies and clinical studies in large samples have to be done in future to prove its efficacy.

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Author's contribution
The author DR. P.V. Sudhaa Devi executed the study and written the draft, edited and approved by other authors.

Conflict of interest
The authors declared no conflict of interest.

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None

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