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Phytochemical Profiling of *Thumattikai Mezhugu* - A Siddha Formulation

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ABSTRACT

BACKGROUND: Standardization of the Siddha polyherbal formulation *Thumatti Mezhugu,* is crucial to ensure its authenticity, quality, and effectiveness. It is essential to validate their potency and efficacy for global acceptance, especially as public interest in traditional medicine continues to grow. Thumatti Mezhugu, a traditional Siddha formulation used for PCOS, amenorrhea, and dysmenorrhea, was evaluated through qualitative phytochemical analysis using standard methods to assess its therapeutic potential. Phytochemicals are valuable medicinal agents, and there is an urgent need to develop new drugs with novel structures and mechanisms to address the challenges posed by emerging diseases.

Methods: *Thumatti mezhugu* has been standardized as per siddha pharmacopoeia standard protocol guidelines and prepared Mezhugu formulation composed of seven ingredients such as *Citrullus collocynthis, Ferula asafoetida, Borax, Sodium chloride impura, Piper cubeba, Nigella sativa, Picrorhiza kurroa.*

Results: Thumatti mezhugu was found to contain alkaloids, phenols, flavonoids, steroids, glycosides, tannins, and diterpenes. The Siddha medicine Thumatti mezhugu contains various phytochemical compounds that can be potential drugs for human use. The analysis further identified absence of Saponins, Proteins, Anthocyanins and Betacyanins, with further heavy metal testing recommended for standardization.

Keywords: Thumattikai mezhugu, Dysmenorrhoea, PCOS, Phytoconstituents, alkaloids

INTRODUCTION

Medicinal plants possess therapeutic properties owing to their diverse secondary metabolites. Various plant parts, such as bark, leaves, flowers, roots, fruits, and seeds, are used in herbal medicine, as these compounds produce specific physiological effects in the human body [(1)]. *Sodium biborate* and *Sodium chloride impura* are minerals that have been used for a long time in Siddha. It has a wide range of therapeutic applications [(2)]. These phytochemicals show strong antioxidant properties and various biological activities, including antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral effects. They also support gene regulation, boost immunity, enhance cell communication, and protect against certain cancers [(3)]. Ethnobotanic inquiries have recorded the plants used in such a perspective, among which *Citrullus colocynthis* (*C. colocynthis*) is one of the most commonly used species [(4)]. It is a major drug constituent. Asafoetida, a resin from Ferula assa-foetida roots, is traditionally used to treat asthma, digestive

disorders, menstrual disorders and intestinal parasites [5]. Thumattikai Mezhugu is a herbo mineral formulations used in the siddha medicine for conditions like PCOS, Amennorhoea and Dysmennorhoea. Hence, it is important to purify and standadrdize the drug it for therapeutic use. Despite its extensive use in traditional medicine, there is insufficient scientific data on its phytochemical profiles. The analysis was carried out following the PLIM guidelines outlined in the quality control manual for ASU drugs. Therefore, this study focused on assessing the preliminary phytochemical constituents of Thumattikai Mezhugu to establish a scientific foundation for its therapeutic use.

MATERIALS & METHODS:

Preparation of THUMATTIKAI MEZHUGU:

Ingredients:

- Thumattikai (*Citrullus colocynthis*)
- Perungayam(*Ferula asafoetida*)
- Vengaram(*Sodium biborate*)
- Indhuppu(*Sodium chloride impura*)
- Vaal milagu (*Piper cubeba*)
- Kadugurohani (*Picrorhiza kurroa*)
- Karunjeeragam (*Nigella sativa*)

Fresh juice of *Thummattikkai* is taken in the required quantity. Separately, fried *asafoetida*, roasted *borax*, rock salt, mustard, *Kadugu Rogani*, black cumin, and cubeb pepper were powdered finely and sieved through a clean cloth (Vasthirakayam) to obtain a smooth powder.

The Thummattikkai juice is then poured into a well-seasoned earthen vessel and heated on a low flame. It is allowed to boil gently until the volume reduces to more than half and reaches a semi-thick (gravy-like) consistency. At this stage, the previously prepared fine powder was added slowly with continuous stirring, and the mixture was further heated and stirred until it attained a wax-like consistency (Mezhugu padham). Finally, it was removed from the heat, allowed to cool, and then stored in an appropriate container for use.

Dosage:

Kundri alavu (130mg) with Panai vellam-once in the morning

Indications:

Suthaga sikkal, Suthaga vayu, Suthaga vayutru vali

Reference:

"Anubava Vaithiya Deva Ragasiyam". First edition 1926, Pg. No. 294,295

Phytochemical Analysis:

This study was conducted to detect the major bioactive compounds present in Thumattikai Mezhugu, offering insights into its chemical composition and possible therapeutic effects. Standard qualitative techniques, including color change and precipitation reactions were used for the analysis. These methods are commonly used to identify various groups of phytochemicals, including as alkaloids, flavonoids, tannins, phenols, steroids, triterpenoids, glycosides, saponins, proteins, and carbohydrates. All procedures were performed following established pharmacognostical protocols to ensure consistent and reliable results.

Test for alkaloids:

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test
Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:
Alkaline reagent test. Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

Test for phenols:
Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:
To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turned into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids
Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube; appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins
Aanthocyanin:
To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100^{o}C. Formation of bluish green colour indicates the presence of anthocyanin.

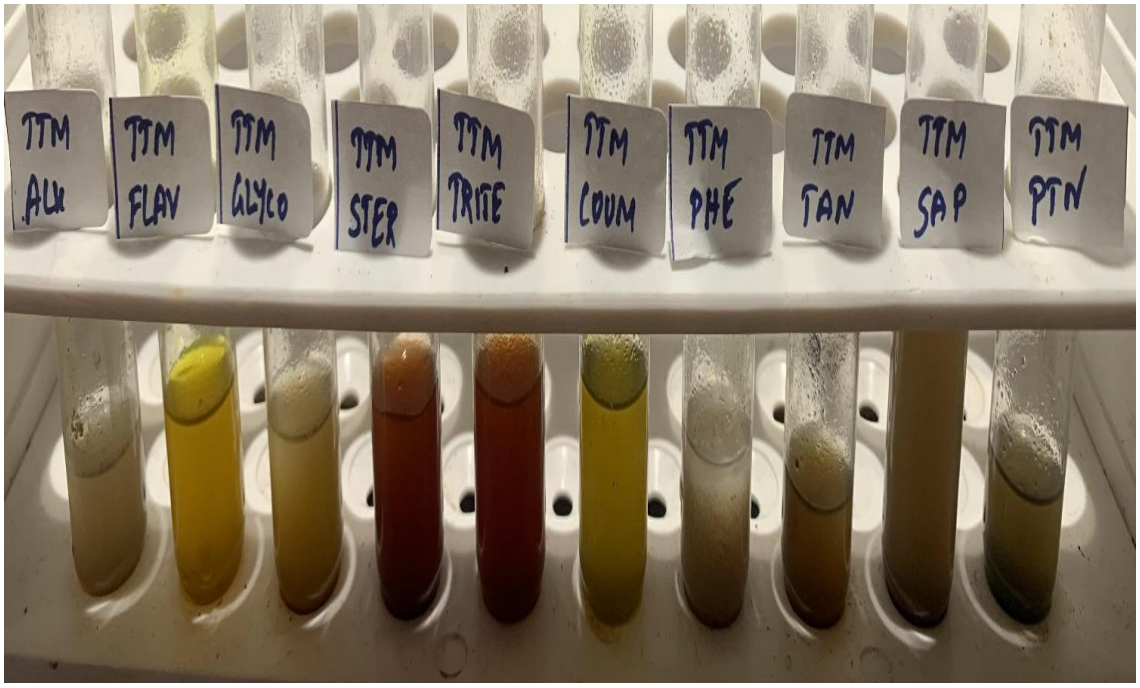
Test for Carbohydrates - Benedict's test
To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic-coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)
To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

RESULTS

Qualitative Phytochemical Investigation

SNO	PHYTOCHEMICAL	OBSERVATION
1	ALKALOIDS	+
2	FLAVINOIDS	+
3	GLYCOSIDES	+
4	STEROIDS	+
5	TRITERPENOIDS	+
6	COUMARINS	+
7	PHENOL	+
8	TANIN	+
9	SAPONINS	-
10	PROTEINS	-
11	SUGAR	+
12	ANTHOCYANINS	-
13	BETACYANINS	-



DISCUSSION

Phytochemical analysis of Thumatikai Mezhugu revealed a rich profile of bioactive compounds that substantiate its traditional application in Siddha medicine. The presence of Alkaloids, Flavonoids, Glycosides, Steroids, Triterpenoids, Coumarin, Phenol, tannin and sugar distinct phytochemical classes provides a biochemical rationale for the medicinal properties of this medicine. Phenolic compounds are widespread plant metabolites known for their antioxidant, anti-inflammatory, anticancer, antiaging, and cardioprotective properties, along with their role in inhibiting angiogenesis and cell proliferation [6]. Flavonoids are plant-derived phenolic compounds with antimicrobial, antioxidant, and anticancer properties, produced in response to microbial infections [7] The presence of flavonoids further supports the antioxidant and anti-inflammatory potential of Thumattikai Mezhugu, as flavonoids are well-established free radical scavengers and contribute significantly to reducing oxidative stress. Alkaloids were also strongly present in Citrullus colocynthis. The presence of alkaloids in most of these samples supports the reports of various authors [8]. These nitrogen-containing compounds are known to exhibit diverse pharmacological activities including anti-inflammatory, antimicrobial, and analgesic properties. Sterols help reduce plasma and LDL cholesterol, thereby lowering cardiovascular risk, while glycosides are reported to possess blood pressure–lowering properties [9] which align with traditional uses of Ayurvedic and Siddha formulations. The presence of coumarin, and tannins further reinforces the antimicrobial and astringent properties of Thumattikai Mezhugu. The primary active ingredient in the fruits of *C. colocynthis*, colocynthin or cucurbitacin E-2-O-glucoside, has cathartic, antihistaminic, anticholinergic, negative chronotropic, and negative inotropic properties [10] The detection of sugars indicates the presence of carbohydrate components, which may contribute to the formulation's demulcent and energy-providing properties. The absence of saponins and proteins suggests that Thumattikai Mezhugu does not rely on these constituents for its therapeutic action, which may be relevant for formulation stability and specific therapeutic applications.

CONCLUSION

The phytochemical profile of Thumattikai Mezhugu supports its traditional therapeutic claims and the synergistic action of the herbal and mineral components may contribute to

its therapeutic efficacy. It contains alkaloids, flavonoids, glycosides, Steroids, Triterpenoids, Coumarin, Phenol, tanin and sugar. Absence of phytochemicals such as saponin, proteins, anthocyanin and betacyanin. The findings of this study provide a scientific basis for the traditional use of Thumattikai Mezhugu in the management of PCOS, dysmenorrhoea, amenorrhoea. This study provides a scientific basis for further pharmacological and clinical investigations.

Declaration by Authors

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