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Research Article

Phytochemical and Biochemical analysis of Siddha formulation Meni Lavana Chooranam.

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ABSTRACT

BACKGROUND:

The *Meni lavana chooranam* is a herbomineral combination used for treating severe abdominal pain, *Gunmam*, *Soothaga vayu*[1].

AIM & OBJECTIVE:

The aim was to establish a fingerprint to ensure the quality and safety of *Meni lavana chooranam* by phytochemical and biochemical analysis.

MATERIALS & METHODS:

The drug *Meni lavana chooranam* was prepared as per Siddha literature *Kannusaamy parambarai vaidhiyam*, ingredients such as *Chukku, Indhuppu, Kariuppu, Perungayam, Omam and Kuppaimeni*[1] . The drug was subjected to Phytochemical & Biochemical analysis.

RESULTS & DISCUSSION:

In the present study shows that The qualitative analysis of sample through phytochemical and biochemical analysis reveals the presence of alkaloids, flavonoids, phenols, steroids, tannins, terpenoids, Calcium, sulphate, chloride, starch, unsaturated compounds and amino acids.

CONCLUSION

Thus the above studies concluded that the Siddha Herbo mineral formulation "*MENI LAVANA CHOORANAM*" was subjected to phytochemical and biochemical analysis to provide footprints for further clinical studies.

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INTRODUCTION

Siddha system of medicine is one among the Indian system of medicines has been practicing Tamil Nadu, Kerala, Malaysia, Singapore, Srilanka and other Indian Ocean countries. In Siddha system of medicine, the understanding of the tri-humours status is very essential. The humours *Vadha, pitha* and *kabha* exist in the ratio1:1/2:1/4 is normal physiological status in man, any imbalance (or) deviation from this state leads to disease Siddha system is also base on *arusuvai*, theory of *panchabootham*, concept of *naadi* and so on though it is considered that the herbomineral formulation are always safe, scientific validation is essential now a days because of our changing environment.

In view of this issue, we validated the safety and efficacy of Siddha drug "*Meni lavana chooranam*" which is indicated for severe abdominal pain, *Gunmam* and *soothaga vayu* by analyzing the phyto chemical and bio chemical analysis. But there is no scientific validation of safety profile behind this formulation. Now we have established the fingerprint for this drug pave the way towards quality standard.

AIM AND OBJECTIVE:

The aim of this study is to do phytochemical and Bio-chemical analysis for fingerprinting the drug" *Meni lavana chooranam* ".

MATERIALS & METHODS:

In the present study, herbomineral preparation "*Meni lavana chooranam*" has been selected to establish its phytochemical and biochemical analysis from classical Siddha literature *kannusaamy parambarai vaidhiyam*.

INGREDIENTS OF THE FORMULATION

1.*Chukku* (Zingiber officinale) – 10 palam (350 gms)
2.*Indhuppu* (Sodium chloride impure) – 10 palam (350 gms)
3.*Kariuppu* (Sodium chloride Linn) – 10 palam (350 gms)
4.*Perungayam* (Ferula asafetida) – 5 palam (175 gms)
5.*Omam* (Trachyspermum ammi) – 10 palam (350 gms)
6.*Kuppaimeni* (Acalypha indica) – 10 palam (350 gms)

PURIFICATION OF INGREDIENTS:

1.*Chukku* (Dried ginger)

Soak dried ginger in lime stone water for 3 hours. Then remove the skin and dry it[2],[5].

2.*Indhuppu* (Rock salt)

Soaked in vinegar for 3 days and kept in sunlight for drying[3].

3.*Kariuppu* (Common Salt)

Common salt dissolved in sea water or rain water and filtered. The filter is boiled till it reaches semi consistency state. It is dried in daylight till it attains the solid status as purified salt[3],[5].

4.*Perungayam* (Asafoetida)[2]

It was fried in ghee and purified.

5.*Omam* (The Bishops weed)[2]

Soak the *omam* in lime stone water for 3 hours and dry it in sunlight.

METHOD OF PREPARATION:

The dried, scraped ginger rhizomes were taken in a vessel and soaked with the leaf juice of Acalypha indica for a day. Next day, this juice was poured out and ginger rhizomes were soaked with fresh Acalypha indica leaf juice. This process was repeated for 18 days. Then the rhizomes were dried in sunlight for 3 days. *Indhuppu* and *kariuppu* were grind with Acalypha indica leaf juice and made into a paste. The ginger rhizome pieces (2 – 3 at a time) were pierced with a thin steel rod at its sharp end. It was immersed in the above salt paste and burnt till the salt started sparkling in the fire. Trachyspermum ammi and ferula asafetida were fried. All these were powdered, sieved and stored in airtight container[1].

SHELF LIFE :

3 months

METHODS OF APPLICATIONS:

Adjuvants :Water
Dosage :*Thirigadi pramanam (Mooviral Alavu)* – 800 to 1000mg

INDICATION:

Ethagaiya kodiya vayitru vali (Severe abdominal pain)

Gunmam (Acute abdomen)

Soothaga vayu (PCOD)

ANALYTICAL PARAMETERS:

The analytical parameters such as Phytochemical analysis and Biochemical analysis were studied.

PHYTOCHEMICAL AND BIOCHEMICAL ANALYSIS OF AYA CHENDHOORAM

The siddha preparation *Meni lavana chooranam* was prepared and used for phytochemical and Biochemical analysis.

PHYTOCHEMICAL ANALYSIS

Phytochemicals, chemical compounds that occur naturally in plants (phyto means "plant" in Greek), are responsible for color and biological properties. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients.

The following tests are used for the analysis of phytochemicals as described by **Harborne** and **Onwukaemeand coworkers, 1999** were carried on alcoholic extract of plant.

1. Alkaloids

Dragandroff's test

8gm of $Bi(No_3)_3$. $5H_2O$ was dissolved in 20 ml HNO_3 and 2.72g of potassium iodide in 50 ml H_2O.These were mixed and allowed to stand. When KNO_3 crystals out, the supernatant was discarded off and made up to 100 ml with distilled water. The alkaloids were regenerated from the precipitate by treating with Na_2CO_3 followed by extraction of the liberated base with ether.

To 0.5ml of alcoholic solution of extract added to 2.0 ml of HCl. To this acidic medium 1.0 ml of reagent was added. An orange red precipitate produced immediately indicates the presence of alkaloids.

2. Flavanoids

To 0.5ml of alcoholic solution of extract, 5-10 drops of dilute HCl and a pinch of Magnesium chloride were added and solution was boiled for a few minutes. Presence of reddish pink or dirty brown colour indicates the presence of flavanoids.

3. Saponins

In a test tube containing 0.5 ml of aqueous extract, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like froth was formed and it showed the presence of Saponins.

4. Phenol

Ferric chloride test

To 2 ml of alcoholic solution of extract, 2 ml of distilled water followed by drops of 10% aqueous solution of $FeCl_3$ solution were added. Formation of blue or green colour indicates the presence of phenols**.**

5. **Steroids**

Salkowski test

To 2ml of chloroform extract 1ml of concentrated H_2SO_4 was added carefully along the sides of test tube. A red ring was produced in the chloroform layer in the presence of steroids.

6. Glycosides

A small amount of alcoholic extract was dissolved in 1 ml of H_2O and the aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides.

7. Tannins

Ferric chloride test

To 1-2 ml of aqueous extract, few drops of 5% ferric chloride solution were added. A bluish black colour which disappears in addition of a few ml of sulfuric acid, there is no formation of yellowish brown precipitate.

8. Terpenoids

To 2ml of chloroform extract 1ml of conc.H_2SO_4 was added carefully along the sides of the test tube. In presence of terpenoids , red colour was produced in chloroform layer.

BIOCHEMICAL ANALYSIS

5gm of the drug was weighted accurately and placed in a 250ml clean beaker then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is made to 100ml with distilled water. This fluid is taken for analysis.

QUALITATIVE ANALYSIS:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Carbonate:

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for Ferric iron

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

Test for Ferrous iron

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

Test for albumin:

The extract is treated with Esbach`s reagent. Formation of yellow precipitate indicates the presence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannic acid.

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

Test for amino acid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

Test for Zinc:

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc[5].

RESULT AND DISCUSSION:

PHYTOCHEMICAL ANALYSIS

Table:1 Phytochemical screening of *Meni lavana chooranam*

TEST	OBSERVATION	INFERENCE
ALKALOIDS	An orange red precipitate produced	Presence of Alkaloids (+ + +)
FLAVANOIDS	A reddish pink or dirty brown colour was formed	Presence of Flavanoids(+)

PHENOLS	A blue or green colour was formed	Presence of Phenols (+)
GLYCOSIDES	No characteristic change was observed	Absence of Glycosides (-)
SAPONINS	No characteristic change was observed	Absence of Saponins (-)
STEROIDS	A red color was produced in the chloroform layer	Presence of Steroids (+ + +)
TANNINS	The bluish black colour was disappeared in the addition of sulphuic acid, no formation of yellowish brown precipitate	Presence of Tannins (+)
TERPENOIDS	A red ring was produced in the chloroform layer	Presence of Terpenoids (+ + +)

The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins and flavonoids . [Edeogal, et.al, 2005]

The Phytochemical screening of the alcohol and aqueous extracts of plant samples are revealed the presence of alkaloids, flavonoids, phenols, steroids, terpenoids and tannins in all the plant extract.

Table:2 Biochemical screening of *Meni lavana chooranam*

Expements	**Observation**	**Inference**
Test for calcium	A white precipitate is formed	Present
Test for sulphate	A white precipitate is formed	Present
Test for chloride	A white precipitate is formed	Present
Test for carbonate	No brisk effervessence is formed	Absent
Test for starch	Blue colour is formed	Present
Test for ferric iron	No blue colour is formed	Absent
Test for ferrous iron	No blood red colour is formed	Absent
Test for phosphate	No yellow precipitate is formed	Absent
Test for Albumin	No yellow precipitate is formed	Absent
Test for Tannic acid	No blue black precipitate is formed	Absent
Test for unsaturation	It gets decolourised	Present
Test for the reducing sugar	No colour change occurs	Absent
Test for amino acid	Violet colour is formed	Present
Test for zinc	No white precipitate is formed	Absent

The Biochemical analysis shows the presence of Calcium, Sulphate, Chloride, Starch, Unsaturated compound and Amino acids. Calcium is essential for bone health[4]. Sulphate has anti bacterial activity and it is one of the macronutrient of cells[4]. Amino acids also help support the expansion and contraction of the arteries with each heartbeat[4].

CONCLUSION

Thus the above studies concluded that the Siddha Herbo mineral formulation "*MENI LAVANA CHOORANAM*" was subjected to phytochemical and biochemical analysis shows the presence of alkaloids, flavonoids, phenols, steroids, terpenoids, tannins, Calcium, Sulphate, Chloride, Starch, Unsaturated compound and Amino acids will provide footprints for further clinical studies.

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