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PHYSIOCHEMICAL, PHYTOCHEMICAL, BIO-CHEMICAL ANALYSIS OF NEURO PROTECTIVE TRADITIONAL SIDDHA MEDICINE CHITRAMUTTI KUDINEER

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ABSTRACT

Siddha system is one of the oldest systems of medicine in India, particularly in Tamilnadu. Based on the principles of panchaboothangal (Five types of elements), Arusuvaigal (Six variety of tastes), Mutthathukkal (three life forces like Vaatham, Pitham, Kabam). Most of the traditional systems of medicine are effective but they are lack in characterization and standardization. Worldwide consumption of Siddha and Ayurvedha medicine also tremendously increased. There is a need to develop the characterization of Indian medicine. In Indian system of medicine the herbs are enormously used. Standardization of Siddha formulations is an essential factor in order to assess the quality, purity, safety and efficacy of drugs based on the concentration of their active principles. In this view, this study is aimed to characterize the Siddha herbal preparation Chitramutti kudineer Chooranam which is used for 80 types of Vaatha diseases. In this study the preliminary phytochemicals, physico-chemical and bio- chemical analysis were performed. The physiochemical analysis showed 5.37% on Loss on drying, 4.32% on Total Ash value, less than 1% on Acid insoluble ash, 1.96% on Water soluble ash, 10.63% on Water soluble extraction, 16.4% on Alcohol soluble extraction. The results of preliminary phytochemical analysis showed the presence of Carbohydrates, Saponins, Flavanoids,

Diterpenes, Quinones. The organoleptic characters like colour, odour, solubility and bio-chemical basic radicles like potassium, ferrous iron. And Acid radicals like chlorides and phosphate, Alkaloids, Tannic acid. This study explored the natural photochemical present in Chitramutti kudineer chooranam.

KEYWORDS: 80 types of Vaatha disease, Neuro protective, physio-chemical, phyto-chemical, bio-chemical, Siddha medicine.

INTRODUCTION

The brain, spinal cord, and nerves constitute the nervous system. Together they control all the functions of the body. Structural, biochemical or electrical abnormalities in the brain, spinal cord or other nerves can result in a range of symptoms like trouble in moving, speaking, swallowing, breathing, or learning, and also have problems with memory, senses, or mood, Paralysis, muscle weakness, poor coordination, loss of sensation, seizures, confusion, pain and altered levels of consciousness. The neurological disorder is any disorder of the nervous system. The specific cause of neurological problems vary, but can include genetic disorders, congenital abnormalities or disorders, infections, lifestyle or environmental health problems including malnutrition, and brain injury, spinal cord injury or nerve injury. There are more than 600 neurological diseases. Major types include Huntington's disease and muscular dystrophy, spina bifida, Degenerative disease such as Parkinson's disease and Alzheimer's disease, stroke, injuries to the spinal cord and brain, epilepsy, brain tumors, meningitis[1] more than 50 medical conditions drugs, and toxins are known to cause nerve damage including Diabetes, HIV, Celiac disease, Amyloidosis, Fabry's disease, medications, including B6 Ipyridoxine), isoniazid, HIV drugs, or chemotherapy, toxins such as heavy alcohol use, auto immune conditions such as lupus and vasculitis, vitamin B12 deficiency, some cancers, such as lymphoma or myeloma, lyme diaseas.[2] The problem may start in another body system that interact with nervous system. Eg. Cerebrovascular disorders involve brain injury due to problems with blood vessels (cardiovascular system) supplying the brain. Autoimmune disorders involve damage lysosomal storage diseases such as Niemann-Pick disease are lead to neurological deterioration. The National Institute of Health recommend considering the evaluation of an underlying celiac disease and people with unexplained neurological symptoms, particularly peripheral neuropathy or ataxia. In a substantial minority of cases of neurological symptoms, no neural cause can be identified using current testing procedures, such as idiopathic condition.

Brain damage according to cerebral lobe-lower brain areas such as basal ganglia, cerebellum, brainstem.

Brain dysfunction according to type Aphasia (language), Dysgraphia (writing), Dysarthria (speech), Apraxia (patterns or sequences of movements), Agnosia (identifying things or people), Amnesia (memory).

Spinal cord disorders (injury, inflammation). peripheral neuropathy and other peripheral nervous system disorders. Cranial nerves disorder such as Trigeminal neuralgia. Autonomic nervous system disorders such as dysautonomia, Multiple system atrophy. Seizure disorders such as epilepsy. Movement disorders of the central and peripheral nervous system such as Parkinson's disease, Essential tremor, Amyotrophic lateral sclerosis and various types of peripheral Neuropathy. Sleep disorders such as Narcolepsy. Migraines and other types of Headache such as cluster head ache and tension head ache. Low back ache and neck pain, central neuropathy Neuropsychiatric illnesses- attention deficit hyper activity disorder, Autism, Tourette's syndrome. Neurobehavioral associated symptoms of degenerative of the nervous system such as Parkinson's, essential tremor, Huntington's disease, Alzheimer's disease, multiple sclerosis and organic psychosis.

Siddha system is one of the oldest systems of medicine in India. The term Siddha means achievements and Siddhars were saintly persons, mastered in preparing medicines from herbal, metal, mineral and animal products. Even though the traditional systems of medicine are effective, to globalise the wealth of the Siddha system, there is a need to develop standardization technique.[3]

AYUSH has given preliminary guidelines for standardization / characterization of the conventionally used formulations. It is very important to establish a system of standardization for every herbal medicine in the market.[4]

The world Health Organization defines herbal medicine as those containing plant parts or plant materials in raw state or processed from containing active principles.[5]

Chitramutti kudineer is a siddha herbal traditional formulation in which five different herbs rhizomes of Zingiber officinale and Alpinia officinarum, bulb of Allium sativum , root of Pavonia zeylanica , seeds of Vigna mungo are used as ingredient. (Table 1). The decoction made from this siddha formulation has been used for all (80) types of vaatha diseases.[6]

Table 1: Ingredients ofChitramutti Kudineer.

S. No	Siddha Name	English Name/Chemical Name	Quantity
1.	Chukku	Zingiber officinale	12.5gm
2.	Arathai	Alpinia officinarum	12.5gm
3.	Chitramutti	Pavonia zeylanica	12.5gm
4.	Poondu	Allium sativum	12.5gm
5.	Ulunthu	Vigna mungo	12.5gm

Since there is no single study in analytical / characterization of this formulation Chittramutti kudineer. This study is aimed to analyze the analytical parameters including preliminary phytochemical screening.

Siddiqui et al. Organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste and texture etc. (Table. No: 2).

Materials and Methods Preparation of Chittramutti kudineer

Chittramutti kudineer is a herbal preparation in which 5 ingredients are used. The name of the ingredients is listed in Table no.1.

Method of Preparation

All the ingredients were procured from authenticated country raw drug store in Chennai. The raw drugs were authenticated by the competent Authority of Medicinal Botany department and authenticated by the Faculties. Then the medicines were purified well and dried, and prepared in Gunapadam Laboratory of National Institute of Siddha. After proper purification, the prepared medicines were also authenticated by the guide and concern head of the department for its completeness. At the time o administration the decoction is prepared.

Analytical Parameters

Physico chemical studies like total ash, water soluble ash, acid insoluble ash, water and alcohol soluble extract, loss on drying at 105°C were carried out as per the WHO guide lines[7] and Indian Pharmacopoeia.[8] Preliminary phytochemical tests were performed as per the standard methods.

Organoleptic Evaluation[9,10]

The organoleptic characters of Chittramutti kudineer were evaluated based on the method described by An overview of advances in the standardization of herbal drugs.

Physico-chemical investigations[11,12]

Physico-chemical parameters like Loss on drying, Total ash, Acid insoluble ash, water soluble ash, water soluble extraction, Alcohol soluble extraction values were performed the for study drug Chitramutti kudineer as per the standard method. (Table. No: 3).

Preliminary Phytochemical Analysis[13]

The preliminary phytochemical screening test was carried out for Chitramutti kudineer as per the standard conventional protocols (Table. No: 4).

Detection of alkaloids

Extract was dissolved in diluted hydrochloric acid and filtered.

Mayer's test

2 ml of extract was treated with few drops of Mayers' reagent; formation of yellow colored precipitate indicates the presence of alkaloids.

Wagner's test

2 ml of filtrate was treated with Wagner's reagent. Formation of brown /reddish precipitate indicates the presence of alkaloids. Detection of carbohydrate Extract was dissolved in 5 ml distilled water and filtered. The filtrates was used to test for presence of carbohydrates.

Molisch's test

2 ml of filtrate was treated with few drops of alcoholic Alpha naphthol solution in a test tube. Formation of the violet ring at the junction indicates presence of Carbohydrates.

Benedict's test

Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of Glycosides Liebermann's test

2ml of extract was treated with 2ml chloroform and 2ml of acetic acid, Violet colour change into blue and green indicates presence of Glycosides.

Detection of Saponins

Froth test

Extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 centimetre layer of foam indicates the presence of Saponins.

Foam test

0.5-gram extract was shaken with 2 ml of water. If foam produced persists for 10 minutes, it indicates the presence of Saponins. Detection of phytosterols Salkowski's test.

Extract was treated with chloroform and filtered; the filtrate was treated with few drops of concentrated sulphuric acid, shaken well and allowed to stand for few minutes. Golden yellow colour indicates the presence of triterpenes.

Detection of phenols

Ferric Chloride test

2 ml of extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin test

To the extract, 1% of gelatin solution containing sodium chloride was added, formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline reagent test

The extract was treated with few drops of 10% sodium hydroxide, formation of intense yellow colour then on addition of diluted hydrochloric acid it becomes colourless, and it indicates the presence of flavonoids.

Lead acetate test

Extract was treated with few drops of lead acetate solution; yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and amino acids

Xanthoproteic Test

The extract was treated with few drops of Conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of diterpenes

Copper Acetate test

Extract was dissolved in water and treated with 3-4 drops of copper Acetate solution; formation of emerald green colour indicates the presence of diterpenes.

Test for gum and mucilage

The extract was dissolved in 10 ml of distilled water and to this 2ml of absolute alcohol with the constant stirring white cloudy precipitate indicates the presence of gum and mucilage.

Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

Biochemical Analysis

Experimental procedure

5 g of Chitra Mutti Kudineer Chooranam was taken in a 250 ml of clean beaker and 50ml of distilled water was added to it. Then it was boiled well for about 10 min. Then it is allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation is used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Preparation of extract

5gm of Chitra Mutti Kudineer Chooranam is weighed accurately and placed in a 250ml clean beaker and 50ml of distilled water was added with it. Then it was boiled well for about 10 minutes. Then it was allowed to cool and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. The bio-chemical analysis of Chitra Mutti Kudineer Chooranam was done at Biochemistry lab, National Institute of siddha, Chennai47.

Preliminary test for Copper, Sodium, Silicate and Carbonate.

Test for Silicate

A little (500mg) of the sample is shaken well with distilled water. A little (500mg) of the sample is shaken well with con. HCl/Con. H2So4.

Action of Heat

A small amount (500mg) of the sample is taken in a dry test tube and heated gently at first and then strong.

Flame Test

A small amount (500mg) of the sample is made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.

Ash Test

A filter paper is soaked into a mixture of sample and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.

Test For Acid Radicals

Test For Sulphate

2ml of the above prepared extract was taken in a test tube and 2ml of 4% dil. ammonium oxalate solution was added.

Test For Chloride

2ml of the above prepared extracts was added with 2ml of dilHNO3 until the effervescence ceases off. Then 2 ml of silver nitrate solution was added.

Test For Phosphate

2ml of the extract was treated with 2ml of con.HNo3 and 2ml of dil. ammonium molybdate solution.

Test For Carbonate

2ml of the extract was treated with 2ml dil. magnesium sulphate solution.

Test For Nitrate: 1gm of the substance was heated with copper turning and concentrated H2SO4 and viewed the test tube vertically down.

Test For Sulphide: 1gm of the substance was treated with 2ml of con. HCL.

Test For Fluoride & Oxalate: 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil. calcium chloride solution and heated.

Test For Nitrite: 3drops of the extract was placed on a filter paper, on that-2 drops of dil. acetic acid and 2 drops of dil. Benzidine solution were placed.

Test For Basic Radicals

Test For Lead: 2ml of the extract was added with 2ml of dil. potassium iodine solution.

Test For Copper: One pinch (50mg) of substance was made into paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame.

***Test For* Aluminium:** In the 2ml of extract dil. sodium hydroxide was added in 5 drops to excess.

Test For Iron: a. To the 2ml of extract add 2ml of dil. ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added.

Test For Zinc: In 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. ammonium chloride was added.

Test For Calcium: 2ml of the extract was added with 2ml of 4% dil. ammonium oxalate solution.

Test For Magnesium: In 2ml of extract dil. sodium hydroxide solution was added in drops to excess.

Test For Ammonium

In 2ml of extract 1 ml of Nessler's reagent and excess of dil. sodium hydroxide solution were added.

Test For Potassium: A pinch (25mg) of substance was treated with 2ml of dil. sodium nitrite solution and then treated with 2ml of dil. cobalt nitrate in 30% dil. glacial acetic acid.

Test For Sodium: 2 pinches (50mg) of the substance was made into paste by using HCl and introduced into the blue flame of Bunsen burner.

Test For Mercury: 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.

Test For Arsenic: 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.
Other constituents

Test For Starch: 2ml of extract was treated with weak dil. iodine solution.

Test For Reducing Sugar: 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes.

Test For The Alkaloids

a) 2ml of the extract is treated with 2ml of dil. potassium iodide solution.
b) 2ml of the extract is treated with 2ml of dil. picric acid.

Test For Tannic Acid: 2ml of extract was treated with 2ml of dil. ferric chloride solution.

Test For Unsaturated Compound: In the 2ml of extract 2ml of dil. Potassium permanganate solution was added.

Test For Amino Acid: 2 drops of the extract was placed on a filter paper and dried well, and then 20ml of Burette reagent was added in it.

RESULTS AND CONCLUSION

As a part of standardization procedure, the sample was tested for relevant physicochemical parameters, and also subjected to Preliminary phyto-chemical analysis through quality control measures. Organoleptic parameters revealed that the kudineer is in yellowish green in colour, with an omam odour, complete soluble in nature and powder texture (Table. No: 2).

Table 2 Organoleptic characters of Chitramutti Kudineer chooranam.

S. no.	Parameters	Results	Method of Testing
1.	Colour	Yellowish green	By visual
2.	Odour	Odour(Omam Smell)	Olfactory examination
3.	Solublity	Completely soluble	Qualitative
4.	Nature	Powder	By visual

Results of physico-chemical parameters such as Total ash, Acid insoluble ash, Alcohol soluble extractives, Water soluble extractive, Loss on drying at 105º C were analyzed and the results were tabulated (Table. No: 3). Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. Percent weight loss on drying or moisture content was found to be 5.37% w/w. The less value of moisture content could prevent bacterial, fungal or yeast growth

Table 3: Physiochemical Evaluation of Chitramutti kudineer chooranam.

S. No.	Parameters	Percentage
1	Loss on drying	5.37%
2	Total ash value	4.32%
3	Acid insoluble ash	Less than 1%
4	Water soluble ash	1.96%
5	Water soluble extraction	10.63%
5	Alcohol soluble extraction	16.4%

Preliminary phytochemical analysis revealed the presence of Carbohydrates, Saponins, Flavonoids, Diterpenes, Quinone (Table. No: 4). This study concluded that, the effect of Chitramutti kudineer may be due to the presence of bioactive compounds particularly flavonoids, Quinons and Diterpenes which may enhance the therapeutic effect.

Carbohydrates: the nervous system mainly dependent on the glucose as its main energy source, the nervous system and many of its functions are influenced by electrophysiological and metabolic consequence of carbohydrate ingestion.[14]

Saponins- helps in Neuroprotective effects on attenuation of central nervoud system disorders

Flavanoids: glial cells as key elements in the formation maintenance and refinement of synapses, natural compounds are capable of modulating nervous system, modulating in the injured brain. The cellular and molecular mechanisms underlying the actions of flavanoids in the nervous system.[15]

Diterpenes: it helps the myelin sheet formation.[16]

Quinones: adrenergic nervous system and hemostatic action.[17]

Table 4: Phytochemical parameters of Chittramutti kudineer Chooranam.

S. No.	Phytochemicals	Test Name	H2O Extract
1.	Alkaloids	Mayer's Test	Absent
		Wagner's Test	Absent
		Dragendroff's Test	Absent
		Hager's Test	Absent
2.	Carbohydrates	Molish's Test	Present
		Benedict's Test	Present
3.	Glycoside	Modified Borntrager's test	Absent
		Keller killiani	Absent
4.	Saponin	Forth test	Present
		Foam Test	Absent
5.	Phytosterol	Salkowski's Test	Absent
6.	Phenols	Ferric Chloride Test	Absent
7.	Tannins	Gelatin test	Absent
8.	Flavnoids	Alkaline Reagent Test	Present
		Lead acetate Test	Present
9.	Protenis and amino acids	Xanthoprotetic Test	Absent
10.	Diterpenes	Copper Acetate test	Present
11.	Gum & mucilage	Extract + Alc0hol	Absent
12.	Fat & Fixed oil	Spot Test	Absent
13.	Quinones	NAOH + Extract	Present

Table 5: Test for Basic radicals in Chitramutti kudineer chooranam.

S. no.	Procedures	Chitramutti kudineer Chooranam
1.	Test for Ammonium	Absent
2.	Test for Sodium	Absent
3.	Test for Magnesium	Absent
4.	Test for Aluminium	Absent
5.	Test for Potassium	Present
6.	Test for Calcium	Absent
7.	Test for Ferrous iron	Present
8.	Test for Copper	Absent
9.	Test for Zinc	Absent
10.	Test for Arsenic	Absent
11.	Test for Mercury	Absent
12.	Test for Lead	Absent

Table 6: Test for Acid radicel in Chitramutti Kudineer chooranam.

S. no.	Procedures	ChitraMuttiKudineer chooranam
1.	Test for Sulphate	Absent
2.	Test for Chloride	Present
3.	Test for Phosphate	Present
4.	Test for Flouride & Oxalate	Absent
5.	Test for Nitrate	Absent

Table 7: Test for Acid radicel in Chitramutti Kudineer chooranam.

S. no.	Procedures	Chittramutti kudineer Chooranam
1.	Test for Starch	Absent
2.	Test for Reducing sugar	Absent
3.	Test for Alkaloids	Present
4.	Test for Amino acids	Absent
5.	Test for Tannic acids	Present
6.	Test for type of compounds	No Change

Bio-chemical analysis revealed the presence of basic radicles like potassium, Ferrous iron (Table. No: 5). Potassium helps regulate muscle and heart contrations. However altered blood potassium levels can affect nerve signals in the nervous system, weakening muscle contractions. Both low and high blood levels can affect nerve impulses by altering the voltage of nerve cells. Ferrous iron Acid radicles are (Table No. 6, 7) chlorides and phosphate and Alkaloids and Tannic acid are present This study concluded that, the effect of Chitramutti kudineer may be due to the presence of bioactive compounds particularly flavonoids, Quinons and Diterpenes which may enhance the therapeutic effect.

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