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Physico-chemical properties and Phytochemical screening of Murukkan Vithai Mathirai, Siddha Polyherbal formulations

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Abstract

Objective: The main objective of this research paper is to evaluate the Physico-chemical properties and Phytochemical screening of Murukkan Vithai Mathirai, Siddha Polyherbal formulations.
Materials and Methods: Murukkan Vithai Mathirai was prepared as per the siddha literature and investigated for Physico-chemical properties and Phyto-chemical screening by authenticated methods.
Results: The values of loss on Drying at 105ºC / moisture content, pH were calculated as 7.028%,5.07 respectively. Total ash, Acid insoluble Ash, Water Soluble ash values were found to be 5.21%, 1.52%, 3.98%, respectively. Water soluble extraction and alcohol soluble extraction were 10.08%, 35.11% respectively. Uniformity of weight was 90.35to110.4%.Friability, Hardness, Distintegration Time were found to be 0.2282%, 2Kg/cm2, more than 1 hour. Bulk density, Tapped density, Hausner 's ratio, carr index were found to be 0.3636 gm/ml,0.5657 gm/ml, 1.56,35.71 respectively. Preliminary phytochemical screening indicated the presence of Glycosides, Saponins, Flavanoids, Diterpenes, Gum, Mucilage and Quininones.
Conclusion: The results obtained from this study will be a reference data for further research and standardization of siddha formulations in perspective days.

Keywords: Siddha, Murukkan Vithai Maathirai, Physico- chemical properties, Phyto chemical screening.

Introduction

Since mankind Siddha system of medicine is existing in the world. Fossil records indicate the use of natural products, especially the plants as medicine since Middle Paleolithic (approximately 60,000 years) age[1]. Enormous therapeutic Siddha preparations were widely available in literature. Therapeutic preparations take account of Herbs, Metals, Minerals. They may be a single drug or complex drug. Synthetic principles like active ingredients and their mechanism of action is not notorious in Siddha system of Medicine. In this system herbs were treated as a whole material not as a single active ingredient. The synthesized drugs are single active chemical molecules and target one specific pathway, whereas herbal medicines contain pleiotropic molecules that work on an orchestral approach which are able to target many elements of the complex cellular pathway[2]. This complex therapeutics produces a great challenge in standardization for universal acceptance. Hence the basic scientific and quality parameters have to be evaluated.

Murukkan Vithai Mathirai [3] is one of the polyherbal formulation mentioned in Siddha text. Murukkan Vithai Mathirai comprises of *Piper nigrum, Zingiber officinalis, Piper longum, Cuminum syminum, Coptis teeta, Butea monosperma* and *Croton tiglium*. It is indicated for worm infestations, indigestion, muscle cramps, Abdominal distension, ascites. Generally it is given for purgation therapy particularly with the patients having worm infestations. It is one among the most commonly and widely used medicine by Siddha practitioners and Government Siddha hospitals and it is being used for many years. The formulation has never subjected for scientific assessment and documentation. In this study this formulation was evaluated for its physico- chemical properties and Phyto-chemical screening which are prerequisite and mandatory for the further evaluation of this formulation.

Materials and Methods

The ingredients of Murukkan Vithai Mathirai are *Piper nigrum, Zingiber officinalis, Piper longum, Cuminum syminum, Coptis teeta, Butea monosperma* and *Croton tiglium*. All ingredients were procured from raw drug store in Chennai. All were authenticated by competent authority.

Preparation of Murukkan Vithai Mathirai[3]:

Piper nigrum, Zingiber officinalis, Piper longum, Cuminum syminum, Coptis teeta, Butea monosperma were taken 35 grams each. 180 grams of *Croton tiglium* was taken. All the ingredients were powdered and ground with water to make karkam. The karkam was then made in to tablet of Sundai (*Solamum torvum*) fruit size.

Physico-chemical properties:

Loss on drying at 105 °C /Moisture content[4]:

This parameter was determined by Moisture balance method. A sample of 5 g was accurately weighed, and the material was spread homogeneously on dishes provided with the instrument. The dishes were placed in the instrument and the instrument was adjusted accordingly at zero, temperature was set at 105° C. When the reading became constant in the circular scale for 15 minutes the readings had been taken. The percentage loss was directly determined.

pH (10% w/v solution)[5]:

The pH of a given solution can be measured with the help of an apparatus called pH meter, consists of a voltmeter connected with two electrodes comprising of standard electrode of known potential and a special electrodes (the probe)enclosed in a glass membrane that allows migration of H^+ ions. The glass case contained a reference solution of dilute hydrochloric acid.

The two electrodes were dipped in the sample solution to be tested. Since the sample solution had a different pH from the solution in the probe, an electrical potential resulted .Thus the potential between the standard electrode and the glass electrode varied with the pH of the solution under test. This potential was recorded by an inbuilt potentiometer of pH meter. The potentiometer reading was automatically converted electrically to a direct reading of the pH of the sample solution.

Determination of ash values[6]:

Total ash:

4 g of the sample was accurately weighed, powdered, incinerated and ground in a tared silica dish. The crucible was kept in a muffle–furnace at a temperature not exceeding 600°C until free from carbon (white ash). Then it was cooled and weighed until it was white, indicating the absence of carbon. The material was cooled in a desiccator and weighed. The content of total ash was calculated in mg/g of air-dried material. The percentage of total ash was calculated with reference to the air-dried drug.

Acid-insoluble ash:

The total ash obtained was placed in a 250 ml beaker without loss of ash and 100ml of dilute hydrochloric acid was added. The crucible was washed with 10 ml of acid and the washings of the beaker were transferred. The beaker was heated till the liquid was boiled. The solution was filtered and the insoluble matter was collected on an ashless filterpaper (What man no, 41) and washed with hot water until the filtrate was neutral. The insoluble matter left on the filter paper was transferred to the original crucible and dried on a hot plate, ignited at 600° C in a muffle furnace (until it became white ash). The residue was allowed to cool in suitable desiccators for 30 minutes and was weighed without delay. The process was repeated until constant weight was obtained. The acid insoluble ash was calculated with reference to the air dried drug.

Water Soluble ash:

The total ash obtained was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected in a Gooch crucible. Then the insoluble ash was washed with hot water and ignited for 15 minutes at a temperature not exceeding 600° C. The weight of the insoluble matter was subtracted from the weight of the ash, The difference in weight represented the water-

soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Uniformity of Weight [7]

20 tablets of Swasa Kudori Mathirai were selected at random and average weight was calculated. Individual weight of the tablets were also calculated. Not more than two of the individual weights should be deviated from the prescribed average weight by more than the percentage .None should be deviated by more than twice of that percentage.

Friability, Hardness, Disintegration Time [8]:

Friability:

It was determined by means of apparatus for the friability test. The tablets should be carefully de dusted prior to testing.10 tablets were weighed accurately and rotated it for 100 times in the drum. The loose dust was removed from the tablets as before, and accurately weighed. Friability was then calculated.

Hardness:

The hardness of 10 tablets were selected randomly was determined by Monsanto hardness tester. In this test the tablet is placed between two anvils, force is applied to the anvils, and the crushing strength that just causes the tablet to break is recorded.

Distintegration Time:

It was calculated by means of disintegration apparatus. The tank of the disintegration apparatus was filled with distilled water up to the mark. 750ml of distilled water was filled in each of the 1000 ml beaker. The instrument was set for 90 minutes and the temperature of water in beaker was set to 37c± 0.5C. One tablet was introduced into each tube of apparatus and a disk was added to each tube. The apparatus was operated and the time duration at which the tablet disintegrated was noted.

Bulk density[9]:

A weighed quantity of 15g (m) of sample powder was gently introduced in to a 50ml dry graduated cylinder without compacting. Carefully the powder was leveled up without compacting, and the unsettled apparent volume (V0) to the nearest graduated unit was read. The bulk density was calculated in (g/ml) using the formula m/V0.

Tap density [9]:

15 g of sample powder was filled in 50 ml of dry graduated measuring cylinder. It was then placed on a mechanical tapper apparatus which operates for a fixed number of tap (approximately 100) until the powder has reached to its minimum level. Volume was measured to determine its tapped density.

Hausner's ratio[10]:

It was calculated by the following formula, where VO, the unsettled apparent volume, and Vf, the final tapped volume, of the powder after tapping the material until no further volume changes occurred.

$$H = V0 / Vf.$$

Carr index[10]:

It was calculated by the following formula where VO, the unsettled apparent volume, and Vf, the final tapped volume, of the powder after tapping the material until no further volume changes occurred.

$$\text{Compressibility index} = 100 \times (V0 - Vf.) / Vf.$$

Determination of Water Soluble Extractive[11]:

5grams of air dried drug Murukkan Vithai Maathirai was macerated with 100ml of distilled water in a closed flask for 24 hours shaking frequently. Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100° C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drugs.

Determination of Water Soluble Extractive[11]:

5grams of air dried drug Murukkan Vithai Maathirai was macerated with 50ml of alcohol in a closed flask for 24 hours shaking frequently. Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100° C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drugs.

Phyto chemical screening[12]:

Tests of Alkaloids:

The extracts were mixed with ammonia and then extracted with chloroform solution. To this dilute hydrochloride acid was added. The acid layer was used for chemical tests for alkaloids.

Mayer's test (Potassium Mercuric Iodide):

The acid layer with few drops of Mayer's reagent gives a creamy white precipitate.

Wagner's Tests (Solution of Iodine in Potassium Iodide): The acid layer with few drops of Wagner's reagent gives reddish brown coloured precipitate

Tests for carbohydrates

Molisch's solution test:

Shake 2 ml of Molisch's solution with crude plant extract then add 2 ml of concentrated H_2SO_4 and poured carefully along the side of the test tube. A violet ring appeared at the inter phase of the test tube indicated the presence of carbohydrates.

Benedict's reagent test:

Boil 2 ml of Benedict's reagent with a crude extract, a reddish brown color indicated the presence of the carbohydrates

Tests for Glycosides

Liebermann's Test. We added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added H_2SO_4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Test for Saponins:

Foam test

Five milliliter of distilled water was added to crude plant extract in a test tube and it was shaken vigorously. The foam formation indicated the presence of saponins.

Test for phytosterols

Salkowski Tests:

Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing yields red colour.

Test for phenols:

Two milliliter of 2% solution of $FeCl_3$ mixed with crude extract. Black or blue-green color indicates the presence of phenols.

Test for tannins:

Extracts mixed with few drops of 1% solution of gelatin containing 10% sodium chloride gives white precipitate.

Tests for Flavonoids

Alkaline Reagent Test. 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color was produced, which became colorless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Lead acetate test:

Alcoholic solution of the extracts mixed with few drops of 10 % lead acetate gives yellow precipitate.

Test for proteins and amino acids:

The extracts were treated with few drops of conc .Nitri acid. formation of yellow colour indicates the presence of proteins.

Detection of diterpenes:

Copper acetate test:

Extracts were 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 gums and mucilages

About 10 ml of the extracts was added to 25 ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for quinones:

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

Results and Discussion

Organoleptic charcters:

It was Light brown colour round shaped tablets with characteristic odor.

Loss on Drying at 105ºC / moisture content:

This parameter determines the amount of volatile matter. (I.e. water drying off from the drug). It was shown in the Table 1

The maximum moisture content limit is 8% /g of herbal preparations are satisfactory according to National Agency for Food and Drug Administration and Control [13]. Excess moisture content can result in the breakdown of important constituents by enzymatic activity leads to growth of yeast and fungi so that cannot be stored for a long period eventually may lead to rejection of the drug

pH (10% w/v solution):

This parameter provides a useful practical means for the quantitative indication of the acidity and alkalinity of the sample. The pH (10% w/v solution) of Murukkan Vithai Mathirai was shown in the Table 1. So the drug was acidic in nature.

Table 1: Loss on Drying at 105ºC/moisture content, pH (10% w/v solution) of Murukkan Vithai Mathirai

Parameters	Values
Loss on Drying at 105ºC/Moisture content.	7.028%
pH(10% w/v solution)	5.07

Total ash, Acid insoluble Ash, Water Soluble ash:

Ash values are important parameter regarding adulteration, identification and purity which were shown in the Table 2.

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Table 2: Ash values of Murukkan Vithai Mathirai

Ash values	Percentages
Total ash	5.21%
Acid insoluble ash	1.52%
Water soluble ash	3.98%

Total ash usually consists of carbonates, phosphates, silicates and silica which includes both physiological ash-which is derived from the plant tissue itself and non physiological ash –which is the residue of the adhering material to the plant surface[14] e.g sand, soil

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Standard references of ash value for Siddha polyherbal formulations has not been documented so far. But according to the European Pharmacopoeia maximum acceptable limit of total ash is 14 % (w/w) and Acid insoluble Ash is 2% [15]

Total ash was within the limit. Acid insoluble ash particularly indicates contamination with sillicious material [16]e.g earth and sand. There was a slight increase in acid insoluble ash. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Water soluble ash is that part of the total ash content which is soluble in water.

Uniformity of weight [17]:

This parameter is applicable to tablets and capsules. This parameter is not applicable to ayureveda and siddha formulations consisting of vegetable parts. It is not easier to control the weight of vati/gutti. Nevertheless this test had been performed. The tablets comply with the test if not more than one of the individual values thus obtained is outside the limits 85 to 115% of the average value and none is outside the limits 75 to 125% of the average value.It was within the acceptable range.

Friability, Hardness, Distintegration Time[8]

Friability, Hardness, Distintegration Time were also obtained and mentioned in the Table 3.

Table 3: Friability, Hardness, Distintegration Time of Murukkan Vithai Mathirai.

Parameters	Values
Friability	0.2282%
Hardness	2kg/cm^2
Disintegration time	More than 1 hour

Friability:

This test was intended to determine the physical strength of tablets. A maximum weight loss (obtained from a single test or from the mean of three tests) of not more than 1.0% is considered acceptable for most products [18]. So the friability was within the acceptable range.

Hardness:

If the hardness is too high it may delay the disintegration time and if it is too soft it will not withstand the packing conditions. Minimum satisfactory hardness is 4 kg for a compressed tablet. Since the tablets were prepared manually, was not compressed it may not met the minimum limit. Excipients like binding agents may be added to this formulation to increase the hardness.

Disintegration Time:

Disintegration is defined as that state in which no residue of the unit under test remains on the screen of the apparatus, if a residue remains or it consists of fragments of disintegrated parts of the tablets, vattis, gutika and pills component parts such as insoluble coatings. This test determines whether dosage forms such as tablets, vatti, gutika and pills etc. disintegrate within a prescribed time when placed in a liquid medium(water) under the prescribed experimental conditions. Disintegration time is more than 1 hour.

Bulk density and Tap density:

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the inter particulate void volume. Hence the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample [9]

The tap density of a material can be used to predict both its flow properties and its compressibility. The tapped density is measured for two primary purposes, the tapped value is more reproducibly measured than the bulk value, and the "flowability" of a powder is inferred from the ratio of these two measured densities [18].

Bulk density and Tapped density of Murukkan Vithai Mathirai granules were obtained and mentioned in the Table 4.

Table 4: Bulk density and Tapped density of Murukkan Vithai Mathirai granules

Parameters	Values
Bulk density	0.3636gm/ml
Tap density	0.5657gm/ml

In a free-flowing powder, inter particulate interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticulate interactions, and a greater difference between the Bulk and tapped densities will be observed. Since Bulk density and Tapped density of Murukkan Vithai Mathirai powder were in a closer value indicates that it has a good flowing property.

Hausner 's ratio, carr index:

In Siddha system of medicine mathirai (tablet) will be prepared manually is now changing to modern methods for a large scale manufacturing like a rotary multi-station tablet press method. Thus, the flow of powder from the hopper into the dies often determines weight, hardness, and content uniformity of tablets. In case of capsules manufacturing, similar volume filling of powders or granules is widely used [19].

Regarding this understanding the flow property of granules is one of the important parameter in tablet manufacturing process. Powders flow properties are measured using a number of parameters such as angle of repose, compressibility index (Carr's index) and Hausner ratio[20]. Hence the flow properties of Murukkan Vithai Mathirai were evaluated and showed in the Table 5.

Table 5: Values for the flow properties of Murukkan Vithai Mathirai Granules.

Parameters	Values
Carr index	35.71%
Hausners ratio	1.55

Flow character is rated based on compressibility index and Hausner ratio. Lower CI or lower Hausner ratios of a material indicate better flow properties than higher ones. A Carr's CI of <10 or HR of <1.11 is considered 'excellent' flow where as CI > 38 or HR > 1.60 is considered 'very very poor' flow. There are intermediate scales for CI between 11–15 or HR between 1.12–1.18 is considered 'good' flow, CI between 16–20 or HR between 1.19–1.25 is considered 'fair' flow, CI between 21–25 or HR between 1.26–1.34 is considered passable flow, CI between 26–31 or HR between 1.35–1.45 is considered 'poor' flow, and CI between 32–37 or HR between 1.46–1.59 is considered 'very poor' flow . Hausner's ratio is within the range but Carr index did not meet the challenge.

Table 6: Values for water and alcohol soluble extraction of Murukkan Vithai Granules.

Parameters	Values
Water soluble extraction	10.08%
Alcohol soluble extraction	35.11%

Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as purity of the drug. Thus, alcohol and water soluble extractive values were determined[21].

Preliminary Phytochemical Screening of Murukkan Vithai Mathirai:

Results of Preliminary Phytochemical Screening of Murukkan Vithai Mathirai were shown in the table 7.

Table 7: Results of Preliminary Phytochemical Screening of Murukkan Vithai Mathirai

S.no	Phytochemicals	Test name	Result
1.	Alkaloids	Mayer's test	Negative
		Wagners test	Negative
2	Carbohydrates	Molischs test	Negative
		Benedicts test	Negative
3	Glycosides	Libermann burchards test	Positive
4.	Saponins	Froth test	Negative
		Foam test	Positive
5.	Phytosterols	Salkowski's test	Negative
6.	Phenols	Ferric chloride test	Negative
7	Tannins	Gelatine test	Negative
8.	Flavanoids	Alkaline reagent test	Positive
		Lead acetate test	Positive
9.	Proteins and Amino acids	Xanthoproteic test	Negative
10.	Diterpenes	Copper acetate test	Positive
11	Gum & Mucilage	Extract +alcohol	Positive
12	Quinones	Naoh+extract	Positive

Preliminary phytochemical screening indicated the presence of Glycosides, Saponins, Flavanoids, Diterpenes, Gum, Mucilage and Quininones.

Conclusion

Physicochemical evaluation and Phytochemical properties screening may pave the way for further evaluation of the formulation Murukkan vithai mathirai in animal model and clinical trial.

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