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Phytochemical screening and pharmacognostic evaluation of the Siddha poly herbal formulation Madhumega chooranam

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Abstract
To evaluate the physico-chemical, pharmacognostic parameters and to determine qualitative and quantitative phytochemicals in the poly herbal Siddha formulation Madhumega chooranam which being in practice in Tamil Nadu, South India, for controlling blood sugar. The drug was studied for phytochemical analysis like loss on drying, ash value determination and extractive values in water and alcohol. Qualitative phytochemical analysis for carbohydrates, glycosides, alkaloids, tannins, reducing sugars, saponins, steroids, proteins, flavones, triterpenoids, quinones and anthroquinones were performed using standard methods and quantitative analysis for phenols, flavones and tannins were performed adhering to the standard procedures. This exhibited the presence of tannins in high amount, glycosides, steroids, phenols, alkaloids in moderate amount, carbohydrates, reducing sugars, proteins, flavones, saponins, triterpenoids in low amount and absence of quinones and anthroquinones. The present study on phytochemical screening and pharmacognostic parameters will be useful for setting standards for the drug and compilation of a suitable monograph.

Keywords: Siddha, Polyherbal drug, Madhumega chooranam, phytochemical screening, pharmacognostic evaluation

Introduction
Since immemorial, herbs were used for treating many ailments [1]. Morethan 3/4th of the people dwelling in developing countries still rely on herbals for their primary health care [2]. Worldwide acceptance of this herbal medicine and increasing demand creates need for the standardization. Which involves the confirmation of the identity, purity determination and detection of adulterant by various parameters like morphological, microscopical, physical and chemical tests. [3, 4, 5] Madhumega chooranam is a Siddha Polyherbal formulation consisting of seven herbs namely *Terminalia chebula, Phyllanthus emblica, Murraya koenigii, Tinospora cordifolia, Cyperus rotundus, Syzigium cuminii and Phyllanthus amarus* in a specific proportion as mentioned below in the methods. It is being used in treating *Neerizhivu* (Diabetes mellitus) for controlling blood sugar in the government run Siddha hospitals in Tamil Nadu, India. This drug not only controls the blood sugar but also the serum cholesterol level [6]. The preclinical and clinical safety and efficacy will not be possible if authentic drugs are not used. Hence the pharmacognostic characters like macro-microscopic features of herbal drugs forms the first and fundamental step to authenticate. Physico-chemical constants indicate the purity of the formulation and the phytochemical evaluation aids in knowing bioactive secondary metabolites like alkaloids, flavonoids, tannins and many phytochemical constituents which are of great medicinal value [7].

Materials and Methods
The chemicals used in this study were of analytical grade and purchased from M/s.Himedia laboratories, India.

Test drug
Madhumega chooranam was obtained from M/s. Ulaga Tamizh Maruthuva Kazhagam, Tirunelveli and stored at room temperature based on the instructions from the manufacturer.

Composition of MMC

S. No	Botanical Name	Tamil Name	Part used	Proportion
1	*Murraya koenigii* (L.) Spreng.	Kariveppilai	leaf	2 parts
2	*Terminalia chebula*. Retz.	Kadukkai	fruit without seed	2 parts
3	*Phyllanthus emblica*. L	Nelli	fruit without seed	2 parts
4	*Tinospora cordifolia* (Thunb.) Miers	Seethil	stem	1 part
5	*Syzygium cumini* (L.) Skeels	Naaval	seed	1 part
6	*Cyperus rotundus* L.	Koraikizhangu	tuber	1 part
7	*Phyllanthus amarus* Thonn	Kezhanelli	whole plant	1 part

Physico-chemical analysis

The physico-chemical parameters aids in screening the drug purity. The active principle is determined well in dry state, so the percentage of moisture lost is important.

Loss on drying

4gm of the MMC drug is taken in 100ml beaker and heated at 105^0C in an oven for 5 hours. In a desiccator it is then cooled. Till the constant weight is obtained the procedure is repeated. The percentage of loss on weight of the test sample is calculated by the formula,

Loss on drying (%) = weight loss of the test sample X 100 / weight of the test sample in gram

Ash values

Ash contains inorganic substances like carbonates, phosphates and silicates. Inorganic substances are removed by treating with acid and thus acid insoluble ash value is determined revealing the inorganic impurity level.

Determination of total ash

4gm of the drug sample is weighed in a silica dish. The sample is spread evenly and ignited in a muffle furnace at 600^0C till it is white, specifying the absence of carbon. The dish containing the test drug is cooled and it is weighed to calculate the percentage of total ash using the formula,

$$\text{Total ash (\%)} = \frac{\text{Weight of ash *100}}{\text{Weight of the drug sample}}$$

Acid insoluble ash determination

The total ash is taken in a silica dish and 45ml of 1.5 hydrochloric acid is added in three portions of 15ml each time. Gently boiled for 5 minutes and filtered. The insoluble content present on the filter paper (What man no.41) is collected and rinsed with distilled water till the residue is free from acid. Then the content is transferred to the silica dish, dried and it is ignited to obtain the constant weight. Before weighing the dish is cooled in a desiccator. The acid insoluble ash is determined by the formula,

$$\text{Acid-insoluble ash (\%)} = \frac{\text{Weight of the acid insoluble residue *100}}{\text{Weight of the sample}}$$

Determination of extractive values

It is a useful process in the evaluation and estimation of chemical constituents present in the drug.

Preparations of the extracts

Cold Maceration

4g of the drug MMC is taken in each of two glass stoppered flask. To one flask 100ml of distilled water and to the other flask 100ml of 95% alcohol is added, shaken for six hours occasionally and for 18 hours to standstill. It is filtered carefully so as to prevent any loss of solvent. 25ml of the filtrate is pipetted to 100ml beaker. It is evaporated on a water bath and placed in an air oven at 105^oC for six hours. In the desiccator it is cooled and weighed. The process is repeated twice and the average value is taken. The water soluble extractive and alcohol soluble extractive is calculated using the formula,

$$\text{water soluble extractive (\%)} = \frac{\text{Weight of the extract *100*100}}{25 * \text{weight of the sample}}$$

$$\text{Alcohol soluble extractive(\%)} = \frac{\text{Weight of the extract *100*100}}{25 * \text{weight of the sample}}$$

Determination of the pH value

10gm of MMC drug sample is taken in a beaker and 100ml distilled water is added to it, stirred well and filtered. The filtrate is used for the experiment. pH meter (Digital) Elico, Model L1- 120 is switched on for 30 minutes for warming up. pH 4 solution is first introduced and the pH meter is adjusted using the knob to 4.00 for 20^0C, 4.01 for room temperature 25^0C, 4.02 for room temperature 30^0C. Then pH7 solution is introduced and adjusted the pH meter to 7 using the knob. Then pH 9.2 solution is introduced and pH reading is checked without adjusting the knob. Then the sample solution is introduced and the reading is noted. It is repeated for four times and the average reading is taken.

Preliminary qualitative phytochemical analysis

A. Preparation of Extract

About 2 g of Madhumega chooranam was weighed and soaked in 10 mL of 80% methanol for 1-2 h and filtered to get 20% solution. Filtrate was used for subsequent analysis.

B. Qualitative phytochemical analysis

Phyto constituents such as alkaloids, phenolic compounds, tannins, flavones, glycosides, saponins, anthraquinones, quinones, reducing sugars and proteins were analysed qualitatively using standard methods [8].

Sample preparation

Approximately 5mL of filtrate was diluted into 1: 10 with methanol and used for preliminary phytochemical analysis. The standard methods are as follows:

1. Test for Carbohydrates

Molisch's Test: To the 0.5 ml of filtrate, 1 ml of alpha naphthol solution in alcohol was added and well shaken. Then few drops of sulphuric acid (concentrated) was added from the sides of the test tube. At the junction of two solutions, appearance of violet ring indicates the carbohydrates presence.

2. Test for reducing sugars

Fehling test: To1 ml of filtrate equal volume of Fehling's solution I and II is added and heated; the appearance of red colour precipitate shows the presence of reducing sugars.

Benedict's test: 1 ml of filtrate was mixed with Benedict's reagent and heated; the formation of orange red colour precipitate specifies the presence of reducing sugars.

3. Test for Glycosides

In a watch glass, 0.2 ml of filtrate was mixed with a pinch of anthrone; one drop of conc. sulphuric acid was added and warmed gently over the water bath. Presence of dark green colour reveals glycoside presence.

4. Test for Steroids

Libermann-Burchard Test: Few ml of filtrate was further extracted with few drops of chloroform. To the chloroform layer, 3 ml of acetic anhydride was added then concentrated sulphuric acid is added in drops. Bluish green color appearance indicates the presence of steroids.

Ferric chloride - Acetic acid Test: Few ml of filtrate was extracted with few drops of chloroform. To this chloroform fraction, 2 ml of ferric chloride acetic acid reagent was added first and then 1 ml of concentrated sulphuric acid. Reddish pink coloration reveals the presence of steroids.

5. Test for Proteins

Biuret Test: To 0.5 ml filtrate, 2.5 ml of diluted Biuret reagent was added. Development of purple color indicates protein presence.

Million's test: To 0.5 ml filtrate, 2.5 ml of Million's reagent was added. The white precipitate formed is warmed and if it turns brick red, the presence of protein is confirmed.

6. Test for Total Phenols

Ferric Chloride test: To 0.5 ml filtrate, 1 ml of alcoholic ferric chloride solution was added. Appearance of bluish green or bluish black manifests the existence of phenol.

7. Test for Tannins

Lead Acetate solution test: To 0.25 ml filtrate, 1 ml of basic lead acetate solution was added. Formation of orange red precipitate specifies the existence of tannins.

8. Test for Quinones

Alkali test: To 0.5 ml filtrate, 1 ml of 10 % sodium hydroxide was added. Quinones presence is manifested by the appearance of blue, green or red colour.

9. Test for Flavones

Shinoda Test: To 0.5 ml filtrate, few mg of magnesium turnings and concentrated hydrochloric acid few drops was added and boiled in a water bath for five minutes. Appearance of red color demonstrates the presence of flavones.

Alkaline Reagent Test: To 0.5 ml filtrate, 1 ml of 1% ammonia was added. Dark yellow color indicates flavones presence.

Ferric Chloride Test: To 0.5 ml filtrate, ferric chloride solution few drops was added. Intense green color exhibits the presence of flavones.

Lead Acetate Solution Test: To 0.5 ml filtrate, when few drops of lead acetate solution (10%) was added, the appearance of yellow coloured precipitate reveals the presence of flavones.

10. Test for Saponins

Foam Test: 0.5 ml of filtrate along with 5 ml water was shaken well; profuse lather formation exhibits the presence of saponins.

11. Test for Alkaloids

Draggendroff Test: To 0.5 ml of filtrate, 0.2 ml of acetic acid and Draggendroff's reagent added was shaken well. Development of orange red coloured precipitate reveals the presence of alkaloids.

Meyer's Test: To 0.5 ml of filtrate, little amount of dilute hydrochloric acid and 1.0 ml of Meyer's reagent was added. Development of white precipitate reveals the presence of alkaloids.

Wagnor's Test: 0.5ml of filtrate was mixed with 1.0 ml of Wagnor's reagent. Development of yellowish orange color reveals the presence of alkaloids.

12. Test for Anthraquinones

Borntrager's Test: 1 ml of filtrate was macerated with ether and filtered. To this, 1 ml of aqueous ammonia was added. After shaking, appearance of pink or red or violet color demonstrates the presence of anthraquinones.

13. Test for Triterpenoids

Salkowski test: Few ml of filtrate was extracted with few drops of chloroform. To this solution, few drops of concentrated sulphuric acid was added. Development of brown ring reveals the presence of phytosterols - Triterpenoids.

C. Quantitative analysis

Determination of total phenols

Total phenols were determined by Folin Ciocalteau reagent [9]. The test drug MMC was diluted with distilled water in the ratio 1:10 was mixed with 5ml Folin Ciocalteau reagent and 4ml of 7.5% aqueous Na_2CO_3 and for 15 minutes it was allowed to standstill. Using the spectrophotometer at 765 nm the phenols were quantified. The standard curve using 0, 50, 100, 150, 200, 250 mg L-1 solutions of gallic acid in methanol: water (50:50, v/v) was prepared. Total phenol values in terms of gallic acid equivalent were expressed %w/w.

Total flavonoids determination

Flavonoids determination was performed by Aluminum chloride colorimetric method [10]. MMC extract (10 mg/ml) in 70:30 % methanol was prepared and mixed with 4.5 ml of methanol, 0.1 ml of 1 M sodium acetate and 0.1 ml of 10% aluminum chloride. Kept in room temperature for half an hour; the absorbance of the reaction mixture was measured using spectrophotometer at 415 nm (Perkin Elmer UV/Visible, USA). Flavonoids content was expressed in %w/w /.

Total Tannins determination

The estimation of total tannins content was performed by Schanderl method [11]. 1ml with duplicates of MMC extract of concentration (500 µg/ml) in test tube is pipetted out. Using distilled water the volume was made up to 1 ml which serves as the blank. To this 0.5 ml Folins phenol reagent (1:2) and 5 ml of 35% sodium carbonate was added and placed in room temperature for 5 minutes. Blue colour formation was seen and at 640 nm the intensity of the color was read. Tannin

content was determined using the standard graph and the results are expressed as %w/w.

Powder microscopy
A pinch of the sample was sift through sieve number 60 and mounted on a microscopic slide with a drop of glycerin-water. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Nikon COOLPIX 5400 digital camera under bright field light. Photomicrographs of diagnostic characters were captured and segregated to identify characters of different ingredients.

Analysis
The phytochemical analysis was performed in duplicates. Interpretation was done with standard values using Graph Pad prism 5.0 Software. The results of are furnished in table 2 and 3.

Results and Discussion
The quantitative analysis of pharmacognostic parameters were useful in setting standards for Polyherbal formulation. The purity of the drug depends on the inorganic matter which is demonstrated by various ash values

Macroscopy
Madhumega chooranam is a greenish brown fine powder with aromatic odour and astringent taste

Microscopy
Powder microscopy features of Madhumega chooranam was documented. Characters of all the ingredients of the formulations have been observed and recorded namely, *Terminalia chebula* – pericarp: epidermal fragments, fibres, group of sclereids, vessels with simple pits, *Phyllanthus emblica* – pericarp: epicarp, parenchyma of the mesocarp, fibres, sclereids of the endocarp, *Muraya koengi* – Leaf: Epidermis of rachis, two layered palisade, unicellular trichome, prismatic crystals, *Tinospora cordifolia* – Stem: cork cells, pitted fibre, fragment of vessel, simple starch grains, *Cyperus rotundus* – Tuber: parenchyma with starch grains, cells with contents, vessel elements, group of fibres from scale leaves, *Syzigium cuminii* – Seed: sclereids, parenchyma of cotyledon with starch, scalariform tracheids and *Phyllanthus amarus* – Whole plant: parenchyma of the stem, fibres, vessel, cells of the seed testa. (Figure 1-7)

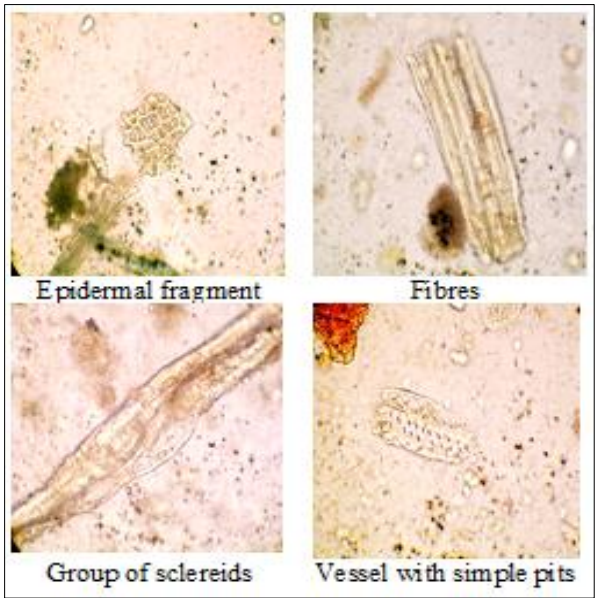

Fig 1: Characters of *Terminalia chebula* - Pericarp

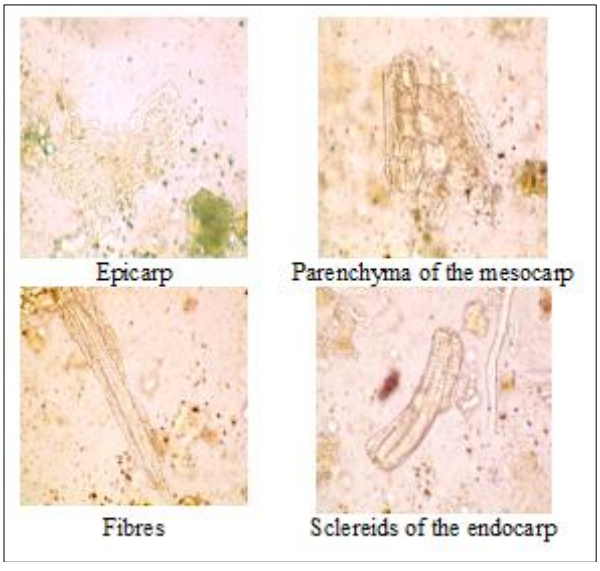

Fig 2: Characters of *Phyllanthus emblica* - Pericarp

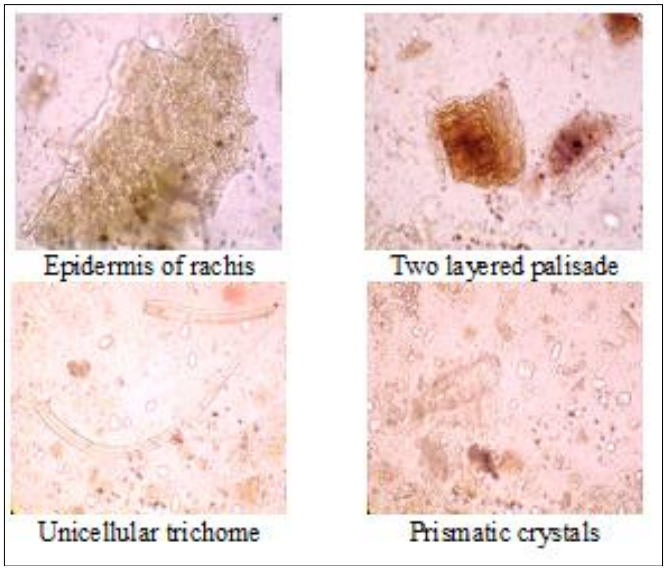

Fig 3: Characters of *Muraya koengi* – Leaf

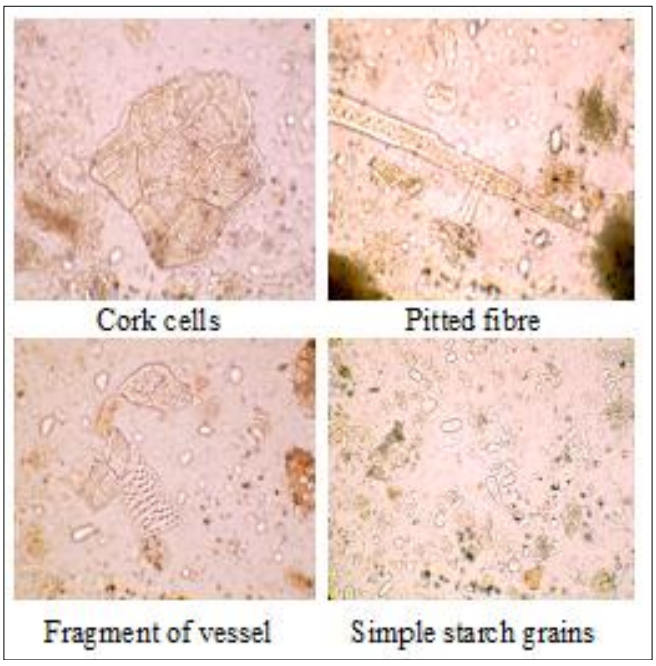

Fig 4: Characters of *Tinospora cordifolia* – Stem

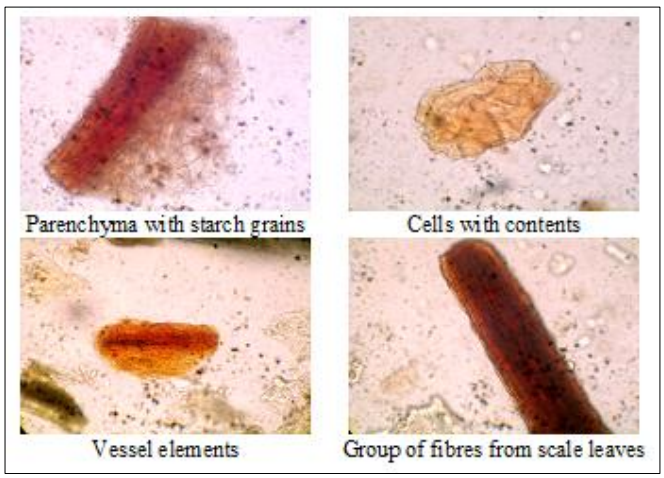

Fig 5: Characters of *Cyperus rotundus* – Tuber

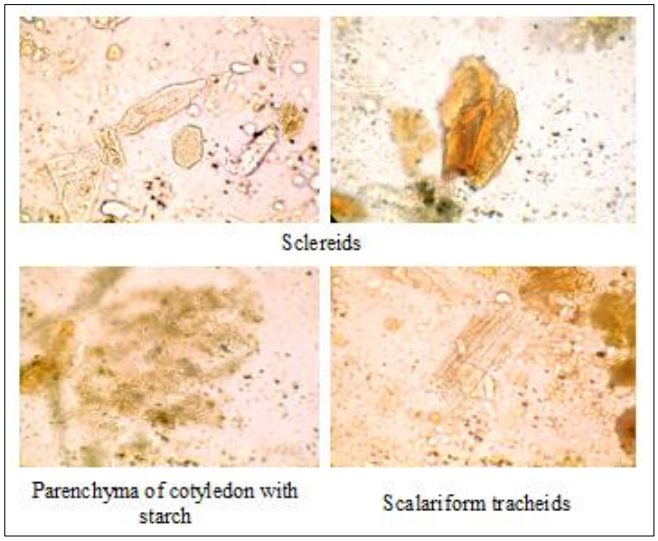

Fig 6: Characters of *Syzigium cuminii* – Seed

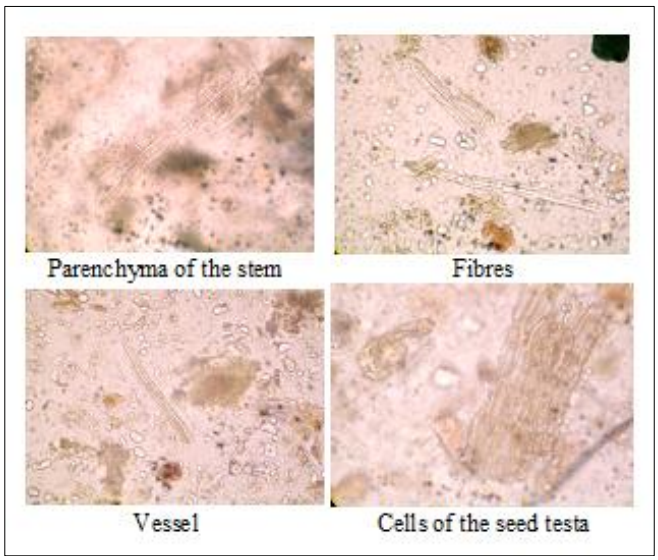

Fig 7: Characters of *Phyllanthus amarus* – Whole plant

Physico-chemical analysis

Physico-chemical analysis of MMC, shows 8.435% of moisture content. Ash content of the drug was 6.39% and 2.785% of acid in-soluble ash shows the siliceous matter in the plant. Alcohol soluble extractives 24.885% represent the extraction of polar constituents like phenols, tannins, glycosides, alkaloids and flavonoids. The water soluble extractive 25.385% denotes the presence of inorganic contents (Table 1).

Table 1: Physico-chemical parameters of Madhumega chooranam

S. No	Parameters	First value	Second value	Mean (In %)
1	Loss on drying	8.59	8.28	8.435
2	Total ash	6.47	6.31	6.39
3	Water soluble ash	1.91	1.41	1.66
4	Acid soluble ash	2.81	2.76	2.785
5	Water soluble extractive	25.03	25.74	25.385
6	Alcohol soluble extractive	25.53	24.24	24.885
7	pH	3.59		

The qualitative phytochemical analysis of the Polyherbal formulation exhibited the presence of high amount of tannins, glycosides, steroids, phenols, alkaloids in moderate amount, carbohydrates, reducing sugars, proteins, flavones, saponins, triterpenoids in low amount and absence of quinones and anthroquinones. (Table 2)

Table 2: Preliminary Qualitative Phytochemical Analysis

Chemical test	MMC
Carbohydrates	+
Reducing sugars	+
Glycosides	++
Steroids	++
Proteins	+
Phenols	++
Flavones	+
Tannins	+++
Quinones	-
Saponins	+
Alkaloids	++
Anthroquinones	-
Triterpenoids	+

Inference: + indicate Low; ++ indicate moderate; +++ indicate high; - indicate absent

Table 3: Quantification of Secondary Metabolites

Test material	% w/w		
	Total phenols	Flavanoids	Tannins
MMC (Traditional)	2.69±0.02	0.45±0.003	0.85±0.12

Conclusion

The phytochemical screening and pharmacognostic parameters will be useful for setting standards for the Siddha Polyherbal formulation Madhumega chooranam and compilation of a suitable monograph.

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Conflict of Interest: Nil

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