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PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF TRADITIONAL SIDDHA FORMULATION SEENTHIL CHOORANAM IN ACCORDANCE WITH AYUSH GUIDELINES

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ABSTRACT
Siddha is the oldest healing system of medicine and it has fundamental aspects for drug formulation. Major formulations used in Siddha are based on herbs and minerals. Siddha formulations offers tremendous advantage in clinical practice against metabolic and lifestyle disorders including neuro degenerative diseases. Often investigation on siddha preparations attempted on reverse pharmacology basis. Hence nearly 80% of the formulation already have proven track record clinically and now several investigation are being made on its preclinical aspect. Ethnomedicine is a traditional medical practice that concerns the cultural interpretation of health, disease, and illness. The practice of ethnomedicine is a complex multidisciplinary system constituting the use of plants in a spiritual way in the natural environment and has been the source of healing for people for millennia. The primary benefits of using plant-derived medicine are relatively safer than synthetic drugs and offer profound therapeutic benefits. Optimized nutrition through supplementation with plant derived phytochemicals has attracted significant attention to prevent the onset of many chronic diseases. The main aim of the present investigation is to carry out the physicochemical and phytochemical evaluation of the novel siddha formulation Seenthil chooranam (SC). The results obtained from the physiochemical analysis clearly reveals that the loss on drying value of SC was 12.73%, total ash value was 2.7%, in which the water soluble ash is 0.84% and acid insoluble ash is 0.76 %.The alcohol soluble extractive value was 23.03% and water soluble extractive was 18.64%. The result of the phytochemical analysis indicates that the formulation SC shows the presence of flavonoids, steroids, triterpenoids, phenols, tannins, saponins, proteins and carbohydrates. From the data's obtained from the results of the present investigation it was evident that the formulation SC complies with the regulatory standard and also possess significant bioactive phytocomponents which may have a tendency to exert multiple mechanism. Hence from this it was concluded that the formulation SC may be used for treating chronic ailments.

KEYWORDS: Siddha, Seenthil chooranam, Physiochemical, Phytochemical analysis, Ethnomedicine.

1.INTRODUCTION
Siddha formulations offers tremendous advantage in clinical practice against metabolic and lifestyle disorders including neuro degenerative diseases. Often investigation on siddha preparations attempted on reverse pharmacology basis. Hence nearly 80% of the formulation already have proven track record clinically and now several investigation are being made on its preclinical aspect. Siddha pharmacopoeia established in recent times have imposed more on standardization aspect of the formulation. Starting from preparatory phase to storage each and individual step involved in formulating siddha preparation has its own quality check evaluations.

Herbal medicine has long been used to treat several acute and chronic clinical symptoms. Although the precise mechanisms of action of herbal drugs have yet to be determined, some of them have been shown to exert versatile antioxidant effects in a variety of peripheral

systems. Structural diversity of medicinal herbs makes them a valuable source of novel lead compounds against therapeutic targets that are newly discovered by genomics, proteomics, and high-throughput screening.[1] Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine.[2] Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.

The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.[3] The harmful microorganisms can be controlled with drugs and these results in the emergence of multiple drug-resistant bacteria and it has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced a number of new antibiotics; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents.[4]

Plant-based medications are well-accepted by patients and are often preferred over chemically produced therapeutics because of their well-known health-benefitting bio-active ingredients.[5-10] Moreover, plant-extractable compounds have also gained a lot of attention in conventional medicine. For instance, plant-based drugs are now used for therapeutic treatment of diseases such as cancer and various inflammatory disorders.[11,12] Therefore, knowing and assessing the potentials of plant-derived bio-active compounds is important for further drug development. This notion is deducible from the increasing interest of the pharmaceutical industry in gaining the rights to identify and exploit plant-borne compounds from species-rich rainforests in countries of tropical and subtropical regions.[13-15] While there is certainly a great potential in identifying plant-derived medication, the challenges associated with this venture must also be noted. Some of the current discussion revolving around this topic are: the protection of bio-diversity, acceptance of intellectual property rights, as well as biosafety of application.[16,17] The main aim of the present investigation is to carry out the physicochemical and phytochemical evaluation of the novel siddha formulation Seenthil chooranam (SC). Further is attempt will generate the evidence based data on developing a monograph of the preparation SC for future reference.

2. MATERIALS AND METHODS

2.1. Source of raw drugs

Ingredients and other raw materials required for the formulations Seenthil chooranam were purchased from a well reputed indigenous drug shop at Chennai. All raw drugs were properly identified and authenticated by the concerning authority before clinical usage.

2.2. Ingredients

The formulation Seenthil chooranam comprises of the following ingredients

1. Seenthil - Tinospora cordifolia
2. Karisalai - Eclipta prostrate
3. Earthworm - Lumbricus terrestris

2.3. Preparation[18]

Formulations Seenthil chooranam were prepared in accordance with the procedure as mentioned in prescribed text of Agasthiyar Vaidhya kaviyam.

2.1. Standardization and Physicochemical Evaluation[19-20]

2.1.2.Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish .The sample was dried at 105^{o}C for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100

2.1.3.Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.
Total Ash = Weight of Ash/Wt of the Crude drug taken X 100.

2.1.4.Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.
Acid insoluble Ash = Weight of Ash/Wt of the Crude drug taken X 100.

2.1.5.Determination of Water Soluble Ash

The ash obtained by total ash test will be boiled with 25 ml of water for 5 mins. The insoluble matter is collected in crucible and will be washed with hot water, and ignite for 15mins at a temperature not exceeding 450ºC. Weight of the insoluble matter will be subtracted from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.
Water Soluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.1.6.Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to

constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.
Alcohol sol extract = Weight of Extract/ Wt of the Sample taken X 100

2.1.7.Determination of Water Soluble Extractive
Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.
Water soluble extract = Weight of Extract/ Wt of the Sample taken X 100

2.1.8.Determination of pH
Test sample was dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the subjected to pH evaluation.

2.2. Qualitative Phytochemical Analysis[21]
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
To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.
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 turns 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
A. Aanthocyanin
To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100ºC. 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 Benedic'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.

3. RESULTS
3.1. Physicochemical Evaluation of SC
The results obtained from the physiochemical analysis clearly reveals that the loss on drying value of SC was 12.73%, total ash value was 2.7%, in which the water soluble ash is 0.84% and acid insoluble ash is 0.76 %.The alcohol soluble extractive value was 23.03% and water soluble extractive was 18.64%. pH plays an vital role in drug absorption and penetration in the present study the pH of the formulation SC was found to be 4.The results were tabulated in Table 1.

Table 1: Physicochemical Analysis of the test drug SC.

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	12.73 ± 2.60
2.	Total Ash (%)	2.7 ± 0.45
3.	Acid insoluble Ash (%)	0.76 ± 0.12
4.	Water Soluble Ash (%)	0.84 ± 0.05
5.	Alcohol Soluble Extractive (%)	23.03 ± 1.11
6.	Water soluble Extractive (%)	18.64 ± 0.38
7.	pH	4

The results were represented in triplicate mean± SD

3.2. Qualitative Phytochemical analysis of SC

It is evident that the medicinal activities of the formulation SC are due to the presence of various active principles or phytoconstituents. The results of the preliminary phytochemical analysis of the sample SC reveals the presence of bioactive phytocomponents such as flavonoids, steroids, triterpenoids, phenols, tannins, saponins ,proteins and carbohydrates. The results were tabulated in Table 2 and illustrated in figure 1.

Figure 1: Phytochemical Analysis study report of SC.

Table 2: Preliminary Phytochemical Analysis of SC.

S.NO	TEST	OBSERVATION
1	ALKALOIDS	-
2	FLAVANOIDS	+
3	GLYCOSIDES	-
4	STEROIDS	+
5	TRITERPENOIDS	+
6	COUMARIN	-
7	PHENOL	+
8	TANIN	+
9	PROTEIN	+
10	SAPONINS	+
11	SUGAR	+
12	ANTHOCYANIN	-
13	BETACYANIN	-

Note: +-> Indicates Presence and - -> Indicates Absence of the Phytocomponents.

4. DISCUSSION

Quality assurance of herbal medicinal products is the shared responsibility of manufacturers and regulatory bodies. National drug regulatory authorities have to establish guidelines on all elements of quality assurance, evaluate dossiers and data submitted by the producers, and check post-marketing compliance of products with the specifications set out by the producers as well as

compliance with Good Manufacturing Practices (GMP). The results obtained from the physiochemical analysis clearly reveals that the loss on drying value of SC was 12.73%, total ash value was 2.7%, in which the water soluble ash is 0.84% and acid insoluble ash is 0.76 %.The alcohol soluble extractive value was 23.03% and water soluble extractive was 18.64%.

Phytochemicals, also referred to as phytonutrients, are found in fruits, vegetables, whole grains, legumes, beans, herbs, spices, nuts, and seeds and are classified according to their chemical structures and functional properties. The terminology used to describe phytochemicals (flavonoids, flavonols, flavanols, proanthocyanidins, procyanidins) can be confusing. Phytochemicals include compounds such as salicylates, phytosterols, saponins, glucosinolates, polyphenols, protease inhibitors, monoterpenes, phytoestrogens, sulphides, terpenes, lectins, and many more.[22]
Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function.[23]

Triterpenoids are used for medicinal purposes in many Asian countries for antiinflammatory, analgesic, antipyretic, hepatoprotective, cardiotonic, sedative and tonic effects.[24,25] Recent studies have not only confirmed some of the aforementioned pharmacological properties of several triterpenoids, but also identified a variety of additional biological activities including antioxidant, antimicrobial, antiviral, antiallergic, antipruritic, antiangiogenic and spasmolytic activity.[26,27]

Tannins are water-soluble polyphenolic compounds of variable molecular weights abundantly found in nature which have the ability to precipitate proteins.[28] Many studies of phenolic compounds (resveratrol, quercetin, rutin, catechin, proanthocyanidins) have been present in the last few years, most of these works were directed to improvements of human health and they demonstrate that tannins have multiple biological activities, including cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral, and antibacterial properties attributed mainly to their antioxidant and antiradical activity.[29]

Saponins are naturally occurring structurally and functionally diverse phytochemicals that are widely distributed in plants. They are a complex and chemically varied group of compounds consisting of triterpenoid or steroidal aglycones linked to oligosaccharide moieties. They are generally considered to have important roles in defense of plants against pathogens, pests and herbivores due to their antimicrobial, antifungal, antiparasitic, insecticidal and anti-feedant properties.[30,31] It is evident that the medicinal activities of the formulation SC are due to the presence of various active principles or phytoconstituents. The results of the preliminary phytochemical analysis of the sample SC reveals the presence of bioactive phytocomponents such as flavonoids, steroids, triterpenoids, phenols, tannins, saponins, proteins and carbohydrates.

5.CONCLUSION

According to the WHO, herbs or herbal products are used by the large number of populations for basic healthcare needs. Herbal medicine includes herbs, herbal materials (like plant parts) or preparations, processed and finished herbal products, active ingredients. It was evident from the present study that the siddha formulation SC complies with the recommended standard. Bioactive phytocomponents like flavonoids, steroids, triterpenoids, phenols, tannins, saponins, proteins and carbohydrates present in preparations like SC have unique advantage of multiple mode of action. Synergy of using combined phytomedicines has well established in traditional medicine like siddha.

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