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PHYSICOCHEMICAL, PHYTOCHEMICAL AND GCMS ANALYSIS OF SIDDHA MEDICINE PEENISA CHOORANAM IN THE TREATMENT OF AZHAL THALAINOKKADU

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Abstract

Azhal Thalainokkadu is a disease that can be correlated with the signs and symptoms of rhinitis, headache, pain in the ears and pain over the sinuses. Peenisa Chooranam (PC) is indicated in the dosage of Verukadi alavu (1.5 grams) for 28 days with the anubhanam lukewarm water for the conditions Peenisangal, Nasi naatram and Nasi raththam. To ensure the quality and efficacy, standardisation of the medicines is essential. In such a way that the present study attempts to provide insight into the preliminary analyses.

Place of study: The studies Physico-Chemical analysis, Phytochemical analysis and GCMS analysis of PC were performed at Analytical chemistry lab, Research and Developmental Wing for ISM, AAGHIM campus, Arumbakkam, Chennai - 106.

Methodology: The study medicine Peenisa Chooranam (PC) was prepared as per the literature, study as performed as per PLIM guidelines and results were analysed.

Result: Brown-coloured dried powder shows the correct ingredients as per the literature. Physicochemical analysis shows 06.50% of the total ash value, 03.90% moisture content, and 5.47 pH. Phytochemical analysis shows the presence of alkaloids, flavonoids, glycosides, lignin and other constituents. GCMS analysis shows the presence of piperine with the highest peak area percentage, followed by other compounds.

Conclusion: From this study it is concluded that the study is evidence of quality of formulation and preparation with its active constituents. It can be used to evaluate the authenticity of Peenisa Chooranam to further quality content research with recent analytical tools.

Keywords: Peenisa chooranam, Azhal Thalainokkadu, Sinusitis, Siddha

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INTRODUCTION

The Traditional Siddha System of Medicine has various treatment methods for the disease *Azhal Thalainokkadu*, which can be correlated with sinusitis. *Azhal Thalainokkadu* is a disease which has the signs and symptoms of rhinitis, headache, and pain in the ears and pain over the sinuses (supra and infra orbital region) says the Siddha literature, *Yugi Vaidhiya Sindhamani* [1]. Sinusitis is a common condition of which patients visit OPD often for relief in the clinical practise. Various internal and external Siddha medicines and treatments are available for such condition. One such herbal medicinal formulation is *Peenisa Chooranam* (PC), comprising of *Chukku, Milagu, Thippili, Chenkathari, Kodiveli, Kandangkathiri, Sangam ver, Karunjeeragam, Seeragam* from the reference book *Dhanvandhri Sootchama Vaithiyam 200 Visha Bedhi Vaithiyam*. It is indicated in the dosage of *Verukadi alavu* (1.5 gram) for 28 days with the *anubhanam* luke warm water for the conditions *Peenisangal, Nasi naatram and Nasi raththam* [2]. Since it is a poly herbal formulation, in order to standardize the medicine, preliminary analysis is conducted to identify its physical and chemical properties, detect its contaminants, identifying the bio active compounds and many more. Thus, it is important to perform such tests [3, 4]. This study deals with Physicochemical analysis, Phytochemical analysis and GCMS analysis of Siddha Medicine Peenisa Chooranam in the treatment of Azhal Thalainokkadu.

MATERIALS AND METHODS

The study medicine Peenisa Chooranam (PC) was prepared after proper drug authentication by Botanist, purification and preparation as per the literature. The purified raw drugs were powdered finely followed by *vasthrakayam* individually, mixed together and stored in air tight container [2,5,6].

Table 01 Composition of *Peenisa Chooranam* (PC)

S.no.	Ingredients	Botanical name	Quantity (grams)
1	*Chukku*	*Zingiber officinale*	1 *palam* (35grams)
2	*Milagu*	*Piper nigrum*	1 *palam* (35grams)
3	*Thippili*	*Piper longum*	1 *palam* (35grams)
4	*Chenkathari*	*Capparis aphylla*	1 *palam* (35grams)
5	*Kodiveli*	*Plumbago indica*	1 *palam* (35grams)
6	*Kandangkathiri*	*Solanum surattense*	1 *palam* (35grams)
7	*Sangam ver*	*Azima tetracantha*	1 *palam* (35grams)
8	*Karunjeeragam*	*Nigella sativa*	1 *palam* (35grams)
9	*Seeragam*	*Cuminum cyminum*	1 *palam* (35grams)

The study Physicochemical analysis, Phytochemical analysis, GCMS analysis of PC were performed at Analytical chemistry lab, Research and Developmental Wing for ISM, AAGHIM campus, Arumbakkam, Chennai - 106, as per PLIM (Pharmacopoeia
guidelines of Indian Medicine) guidelines [7].

Powder Microscopy

Fine sample powder of PC was mounted on clean glass slide, clarified with clearing solution and treated with different chemical reagents. Stained samples were then mounted in glycerin water fluid and observed for identification of diagnostic cellular characters.

Physicochemical analysis of PC

Percentage of Loss on Drying

The test drug PC was accurately weighed 2grams and taken in 100ml beaker, dried at
110^{0}C for 5 hours, weighed and the percentage was calculated.

Determination of Total Ash

The test drug PC was accurately weighed 2grams in a crucible and incinerated in the furnace at a temperature of 500ºC for 7 hours until it turns white which indicates the absence of carbon. The percentage of total ash was calculated.

Determination of Acid-Insoluble Ash

The crucible ash obtained by the total ash test, mixed with 25 ml of dilute hydrochloric acid was transferred into conical flask and heated for 5 minutes. Then the insoluble matter was filtered, collected in the crucible and heated in the furnace for 6 hours at 650^{0}C, cooled, weighed. The percentage of acid-insoluble ash was
calculated.

Determination of Water-Soluble Extractive

5 grams of the test sample PC, taken in 250ml iodine flask was mixed with 100ml distilled water, kept in the shaker for about 6 hours. It is kept rested for the whole night and filtered. Taking 10 ml of the filtrate in 250ml beaker, was kept in the oven at 110^{0}C for one hour, later allowed to cooled and weighed. The percentage of watersoluble extractive was calculated.

Determination of Alcohol Soluble Extractive

2.5 grams of the test sample PC, was macerated with 50ml of ethanol in a 250ml iodine flask for about 6 hours and allowing resting for the whole night. Filter rapidly, taking 10 ml of the filtrate in 250ml beaker, was kept in the oven at 110^{0}C for one hour, later allowed to cooled and weighed. The percentage of the alcohol-soluble extractive was calculated.

Phytochemical analysis of PC

1 gram of PC was dissolved in 100ml of distilled water and filtered.

Test for alkaloids

Dragendroff's Test: Filtrate was treated with 2ml of Dragendroff's reagent and formation of reddish-brown precipitate was observed.

Test for carbohydrates

Resorcinol test: 2ml of aqueous solution was mixed and heated with few crystals of resorcinol and equal volume of conc. Hydrochloric acid. Presence and absence of rose colour was observed.

Test for flavanoids

Ferric chloride test: Extract of aqueous solution was treated with 3-4 drops of 10% ferric chloride solution. Formation of green color precipitate was noted.

Test for glycosides

Legal test: Dissolve 30gram of plant extract in pyridine with sodium nitroprusside and 10% sodium hydroxide. Test was observed for its red color changes and noted.

Test for steroids

Hesse's response: 5ml of aqueous extract of the sample PC was mixed with 2ml chloroform and 2ml conc.H_2SO_4. Presence of pink or red colour ring in the lower chloroform layer was observed and noted.

Test for lignins

Furfuraldehyde test: extract solution was mixed with 2% furfuraldehyde solution and was observed for its red color changes.

Test for Gums and Mucilage: To 1ml of extract of the sample PC, 2.5ml of absolute alcohol was added and stirred constantly. Formation of white or curdy precipitate was observed and then it was dried in air and examined for its swelling properties and noted.

Test for terpenoids

2ml of chloroform was mixed with 5ml of the extract (evaporated on water bath) and 3ml of conc. H_2SO_4 (boiled on water bath). The test was observed for the formation of grey coloured solution and noted.

Test for saponnins

Foam test: 0.5 gm of extract was shaken with 2 ml of water. The foam produced persisted for ten minutes was observed and noted.

Test for tannins

Gelatin test: The extract was dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing10% NaCl was added to it. Presence and absence of formation of white precipitate was observed and noted.

Test for phenolic compounds

Ferric chloride test: Extract of the sample PC was treated with 3-4 drops of 5% ferric chloride solution. Formation of bluish black or dark green color was observed and noted.

Gas Chromatography-Mass Spectrometry (GCMS) Analysis of Pc

The extract of the sample *Peenisa Chooranam* (PC) was subjected to GC-MS analysis by the standard procedure of relative retention index with peaks and the compounds were separated and identified by comparing the retention time with GC-MS Library (NIST and WILEY).

RESULT

Figure 1 *Peenisa Chooranam* (PC)

Table 2 Powder microscopy analysis of PC

Parameters	Result of analysis	API specification
Description	Brown colored dried fine powder	-
Foreign matter	NIL	Not more than 2%
Powder microscopy	The powder microscopic studies show the presence of lignified and non-lignified trichomes, xylem vessels, fibres, unicellular warty trichomes, stone cells, fragments of thick cells with oil globules. Microrosete crystals and raphides shape calcium oxalate crystals. Fibres along with medullar rays, compound and oval shaped simple starch grains so the sample conforms to characteristics cell features of *Zingiber officinale, Piper nigrum,* *Piper longum, Capparis aphylla,* *Plumbago indica, Solanum surattense, Azima tetracantha, Nigella sativa, Cuminum cyminum.*	*Zingiber officinale,* *Piper nigrum,* *Piper longum,* *Capparis aphylla,* *Plumbago indica,* *Solanum surattense, Azima tetracantha,* *Nigella sativa,* *Cuminum cyminum.*

Physico-Chemical analysis

Table 3 Physico-Chemical analysis of PC

Parameters	Result of analysis
Description	Brown color fine powder
Total ash	06.50%
Acid insoluble ash	00.90%
Loss on drying at 110^{0}C	03.90%
pH (5% aqueous solution)	5.47

Water soluble extractive	13.54%
Alcohol soluble extractive	10.56%

Phytochemical analysis

Table 4 Phytochemical analysis of PC

Phytoconstituents	Aqueous extract of PC
alkaloids	+
flavonoids	+
glycosides	+
lignin	+
saponnins	-
steroids	+
terepenoids	+
tannins	+
phenolic compounds	+
Proteins and amino acids	-
Carbohydrates	-
Gums and mucilage	-

GCMS analysis

Table 5 Peak report TIC of *Peenisa Chooranam* (PC)

Peak#	R.Time	Area	Area%	Compound name
1	9.847	906520	4.89	Benzaldehyde
2	9.918	269196	1.45	3,7,7-Trimethylbicyclo [4.1.0] hept-3-ene-2,5-dione
3	12.362	1061465	5.72	Caryophyllene
4	18.810	1484589	8.00	2,4-Decadienamide
5	19.219	2089128	11.26	n-Hexadecanoic acid
6	23.662	892500	4.81	1H-Indene
7	24.274	5317715	28.66	Retrofractamide-A
8	27.409	1004942	5.42	E, E, Z-1,3,12-Nonadecatriene-5,14-diol
9	29.741	5525296	29.78	Piperine

Discussion

The brown-coloured dried fine powder, *Peenisa Chooranam* (PC), revealed the presence of the ingredients *Zingiber officinale, Piper nigrum, Piper longum, Capparis aphylla, Plumbago indica, Solanum surattense, Azima tetracantha, Nigella sativa, and Cuminum cyminum* from the powder microscopy. The physicochemical analysis of the Siddha medicine *Peenisa Chooranam* (PC) test report shows that the pH, moisture content, ash value and extractive value were within the acceptable limits. The total ash value of 6.5% indicates the presence of an acceptable limit of inorganic matter in the medicine PC. 0.90% of acid insoluble ash indicates the presence of a very small percentage of any earthy materials in the preparation. Moisture content in the medicine, depicted by loss on drying at 110^{0}C is 3.90%, which is low and represents the good shelf life of the medicine. 13.54% of water-soluble extractive and 10.56% alcohol-soluble extractive of the medicine show the amount of active constituents that are soluble in water and alcohol, respectively. These active constituents were further depicted by the results of phytochemical and GCMS analysis of the same study. The pH value of 5.47 in the 5% solution shows that the medicine is acidic in nature. This also helps in maintaining nasal pH, as the nasal mucosal pH is between 5.5 and 6.5[8]. The results of the physico chemical analysis show the good quality and proper preparation of the study drug PC, and it is safe for

consumption. Phytochemical analysis of Siddha formulation PC shows the presence of alkaloids, flavonoids, glycosides, lignin, steroids, terepenoids, tannins and phenolic compounds.

GCMS analysis of Siddha formulation PC shows the presence of Benzaldehyde, 3,7,7 - Trimethylbicyclo [4.1.0] hept-3-ene-2,5-dione, Caryophyllene, 2,4-Decadienamide, n-Hexadecanoic acid, 1H-Indene, Retrofractamide-A,E,E,Z-1,3,12-Nonadecatriene5,14-diol and Piperine. Out of which Piperine (29.78%) is the more dominant compound with higher peak area % followed by Retrofractamide-A (28.66%) and nHexadecanoic acid (11.26%). The compound Piperine has hepatoprotective, antiallergic, anti-inflammatory, and neuroprotective properties [9]. The compound Caryophyllene has anticancer and analgesic properties as well. These pharmacological activities help in alleviating the symptoms of rhinitis, headache and pain [10]. Also, the individual ingredients of *Peenisa chooranam* (PC) *Zingiber officinale, Piper nigrum, Piper longum, Capparis aphylla, Plumbago indica, Solanum surattense, Azima tetracantha, Nigella sativa, and Cuminum cyminum* helps in reducing the symptoms, with its taste, potency, post digestive transformation and Siddha pharmacological action says the Siddha system of medicine [11].

Conclusion

Yet the present study's physico-chemical analysis, phytochemical analysis and GCMS analysis of PC are preliminary analyses which are all part of standardisation and need further evaluation and clinical studies to emphasise the results and strengthen the knowledge of Siddha medicine in the treatment of *Azhal Thalainokkadu*. This study shows the quality of formulation, preparation and presence of phytocompounds present in the *Peenisa Chooranam* (PC).

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Conflict of Interest

Authors have declared that no competing interests exist.

Informed Consent

Not applicable

Ethical Statement

Not applicable

Author Contribution

Sujatha R, conducted the study and prepared the manuscript, Sudhamathi pushaparaj K, guided the study and approved the manuscript.

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