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Research article

Phytochemical Screening of herbo-mineral formulation *Pooranathi Chooranam*

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ABSTRACT

The phytochemical investigation of a siddha medicine ‘’Pooranathi chooranam” which is prescribed to treat Sagalavali (All type of pain), kuthal, kudaichal etc based on Part III of Agathiyar 2000 (*Tamil*) as reference. The chief ingredients such as Saranaiver (*Trianthema portulacastrum*), Poduthalaiver chaaru (*Phyla nodiflora),* Kazharchikaai *(Caesalpinia bondue)* etc are used. The whole powdered compound is mixed and made into aqueous extracted.

The powder extracts were tested for Phytochemical estimation such as alkaloids, coumarins, saponins, tannins, Glycosides, flavanoids, Phenols, triterpenoids, Cyanins and carbohydrates. After the Qualitative assessment of Phytochemicals, it was subjected to TLC & HPTLC profiling under UV at 366nm with integration through CAMAG software.

The results also suggesting that presence of several important phytoconstituents which are essential for maintain the vital body. Chromatographic separation was carried out on the active extracts, and the efficacy of the resulting fractions was tested against the susceptible organism. This result might explain the ethno botanical use of the plant for the treatment of dysentery, gastro internal disorder and constipation based on the literatures. The active principle of many drugs found in plants is phytochemical. The medicinal value of these phytochemicals is because of the presence of chemical substance that produces definite physiological action on the human body. This has been briefly discussed in this research study.

Keywords:

Phytochemicals, Ethnomedicine, Siddha drug, Pooranathi Chooranam.

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INTRODUCTION

Medicinal herbs basically consists of effective sources of flavonoids, tannins, glycosides, anthraquinones, steroids antimicrobial and antioxidant natural products etc. Medicinal herbs are an important source for the have enormous physiological activities in humans and therapeutic remedies of various ailments. These include cancer prevention, antibacterial, immemorial, different parts of medicinal herbs have been antifungal, antioxidative, hormonal action, enzyme used to cure specific ailments(1). Phytochemicals function in plant metabolism is a major constituents of non-nutritive plant chemicals that have protective or phytochemical are consist of carbohydrates, amino acids, disease prevention process(2).

A number of phytochemical in Medicinal herbs are constitute effective sources of flavonoids, tannins, glycosides, anthraquinones, steroids antimicrobial and antioxidant natural products and terpenoids(3). The plants produce proteins and chlorophylls while secondary metabolites. These chemicals are to protect themselves but recent research consist of alkaloids, saponins, steroids, flavonoids and demonstrates that they can protect humans and animals. Apart from this traditional medicinal herbal products are composed of tannins. The tannins and others constituents are playing a significant role in the maintain the tissues(4).

In determining the presence or identification of crude drugs there is a widespread interest in evaluating drugs derived from plant sources. This interest mainly arises from the belief that herbal medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects. Natural medicines have been often derived from plants, animal tissues or microorganisms (5).

The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases. Therefore to meet the increasing demand of manufacturing modern medicines and export, the need of the medicinal plants have enormously increased(6). This demand is generally met with by cultivating uprooted medicinal plants. Before entering into this all, basic phytochemical quantification is essential and need of the hour.

MATERIALS AND METHODS

Sample Collection and Preparation:

In this study, the Pooranathi chooranam drug compounds such as Saranaiver (*Trianthema portulacastrum*), Poduthalaiverchaaru (*Phyla nodiflora),* Kazharchikaai *(Caesalpinia bondue),* Chukku (*Zingiber officinale*), Millagu (*Piper nigrum*), Thippili (*Piper longum*), Perunjchirakam (Pimpinella anisum), Chirakam (*Cuminum cyminum*), Perrungayam (*Ferula asafoetida*), Omam (*Trachyspermum ammi*), KariveppilaiEerku (*Murraya koenigi*), KadukkaiThoal (*Terminalia chebula) and I*nduppu *(Rock salt).* The raw materials were validated from Department of Sirappu Maruthuvam, Chennai. The sample was finely powdered and sealed, stored in air tight container for further works.

Phytochemical Screening of Crude Extracts Procedure:

The raw herbs were powdered and it was subjected to take the Aqueous extract and used for the qualitative phytochemical estimation studies.

Preparation of drug

Saranaiver is boiled in the goat's milk and podudhalai charu, and dried in the sunshade. Then kalarchikai will be placed in mud plate and closed with mud plate and seal it with a mud pasted cloth (sellai). It is subjected to heating puda process with 25 cow dunk cake. Then the balance raw drugs such as *Trianthemaportulacastrum, Phyla nodiflora, Zingiber officinale, Piper nigrum, Piper longum, Pimpinella anisum, Cuminum cyminum, Ferula asafoetida, Trachyspermum ammi*, *Murraya koenigi,* Terminalia chebula will be slightly fried and ground separately using a stone mortar and sieved by white cloth and to be mixed with above prepared powder and used for the analysis.

Test for Alkaloids

Two millitre of aqueous sample extract was measured using a measuring cylinder and equal volume of ethanol containing 3 % tartaric acid was added and shaken. Then few drops of marquin's reagent were added into the mixture. The formation of precipitate any form indicated the presence of alkaloids. The same procedure was repeated for ethanol extract. Absence of saponins, 0.5 g of the dried extract was placed in a test tube and 3ml of distilled water added and boiled for fifteen minutes. The content was filtered and the filtrate shaken vigorously. The same procedure was repeated for the other dried extracts of the eight selected herbs(7).

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

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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

Aanthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. 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.

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro litre by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm(8).

High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition it is a reliable method for the quantitation of nano grams level of samples. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials(9).

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.

HPTLC Chromatographic condition

Sample : *Pooranathi Chooranam*

Instrument: CAMAG TLC SCANNER III

Stationary phase : Aluminium Coated Silica Gel – Merck

Mobile phase : Chloroform: n-butanol: methanol: water: Acetic acid (4:1:1:0.5:0.5)

Scanning wavelength : 366 nm

Sample concentration : 10 mg/ml

Applied volume : 5 µl

Application mode : CAMAG HPTLC

RESULTS

Table 1. Phytochemcial analysis report of Pooranathi chooranam

Test	Inference
Test for alkaloids	+ve
Test for coumarins	+ve
Test for saponins	+ve
Test for tannins	+ve
Test for glycosides- Borntrager's Test	+ve
Test for flavonoids:	+ve
Test for phenols:	+ve
Test for steroids:	+ve
Triterpenoids	+ve
Test for Cyanins	+ve
Test for Carbohydrates - Benedict's test	+ve
Proteins (Biuret Test)	+ve

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The medicinal values of the herbal secondary metabolites are the very important for the therapeutic managements. Glycosides (based on borntrager's test) are known to metabolites is due to the presence of chemical substances hamper the Na+ /K+ pump. This results in an increase that produces a definite physiological action on the human body. The most important of these secondary for the Aqueous extracts for *Pooranathi chooranam* are tannins, saponins, tannins, steroids and flavonoids etc.

The alkaloids are having pharmacological properties including antiprotozoal, cytotoxic, antidiabetic and anti-inflammatory, and presence of properties exhibits various therapeutic benefits(10). Plant steroids are known to be important for their cardiotonic activities, insecticidal, anti-inflammatory, analgesic properties, central nervous system activities.

The Presence of tannin exhibits antidiabetic, anti-inflammatory, antibacterial and antitumor activities. It was reported that certain tannins were able to inhibit HIV replication selectively besides use as diuretics.

Figure.1. Phytochemical estimation

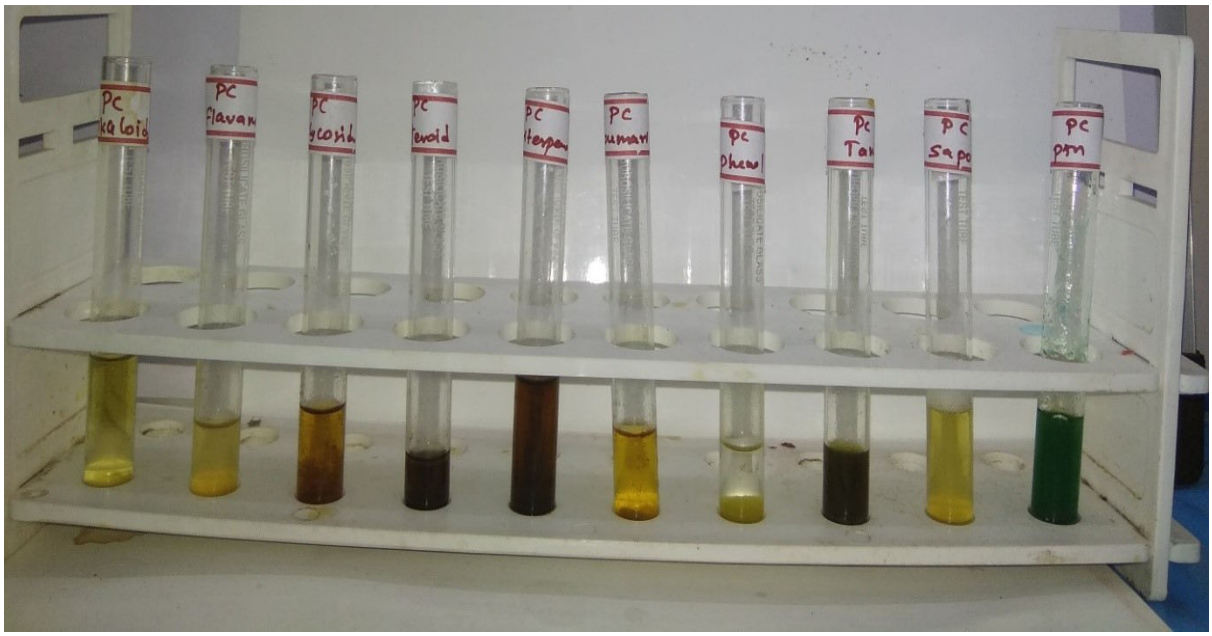

Figure 2. TLC Analysis at 366nm

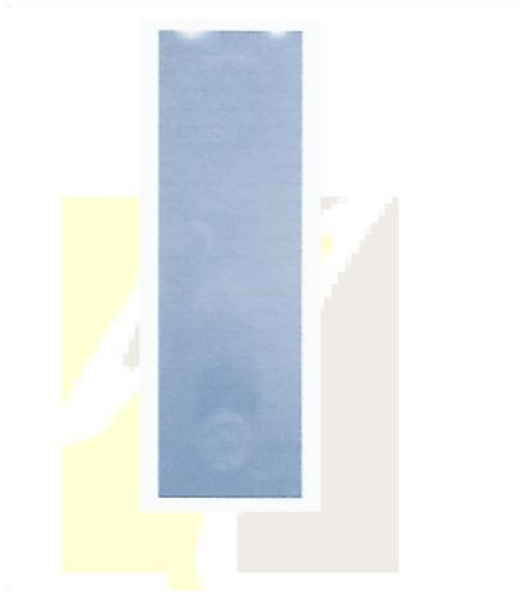

Figure 3. Track of all wavelength observed.

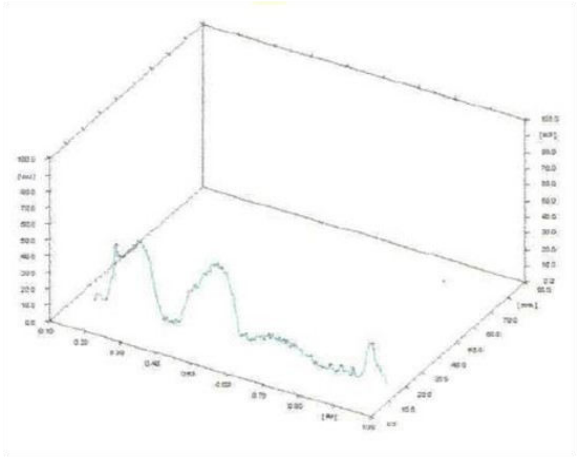

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Figure 4. HPTLC finger printing of Siddha drug Pooranathi chooranam

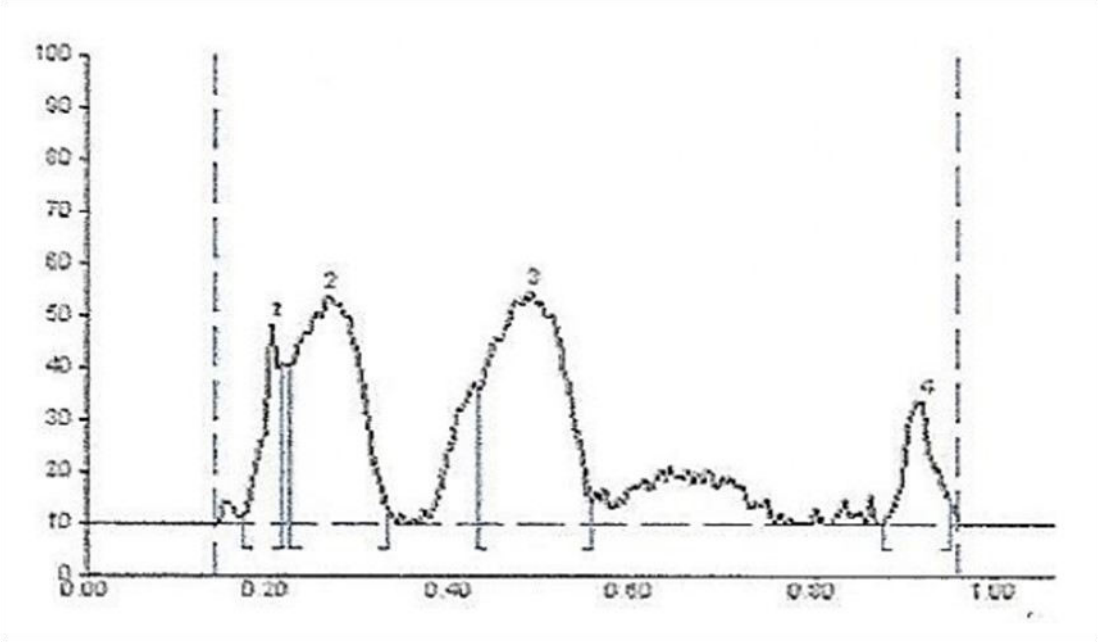

Table 2. Peak table of HPTLC finger printing of *Pooranathi chooranam*

Peak	Start	Start	Max Rf	Max	Max %	End Rf	End	Area	Area %
1	0.17	22	0.21	38.3	25.39	0.22	31.1	632.5	9.16
2	0.23	310	0.27	43.9	26.06	0.33	27	2503.0	36.26
3	0.43	258	0.49	45.0	29.80	0.56	38	3114.2	45.11
4	0.88	01	0.92	23.8	15.79	0.95	50	654.0	9.47

HPTLC finger printing analysis of the Pooranathi Chooranam reveals the presence of four prominent peaks corresponds to presence of four versatile phytocomponents present within it. Rf value of the peaks ranges from 0.17 to 0.88. Further the peak 2 and 3 occupies the major percentage of area of 36.26 and 45.11% which denotes the abundant existence of such compound. Followed by this peak 1 and 4 occupies the percentage area of 9.16 and 9.47%.

CONCLUSION

The present study on the pharmacognostic standardization and evaluation of the *Pooranathi chooranam* which might be useful to contribute information with regard to its identification parameters, which are very essential and significant for the acceptability of herbal drugs in the current drug market to ensure the quality and safety of herbal drugs. Further studies are needed with these poly combination drugs to isolate, characterize and elucidate the structure of the bioactive compounds of the individual herbs which are responsible for the medicinal value are to be analysed.

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CONFLICT OF INTEREST : None declared

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