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Original article

Physico-chemical and Phytochemical Evaluation of Siddha Medicine Vellarivithai Chooranam

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ABSTRACT:
Background:The Standardisation of Siddha drugs are very essential and needed in this scientific world.Vellarivithai chooranam,a Siddha herbal drug is indicated for kalladaippu (Urolithiasis) noi. Aim:This work was carried out to perform the physico-chemical and preliminary phytochemical screening, high performance thin layer chromatography finger printing of Vellarivithai chooranam. Materials and Methods:The dried seeds of Vellarivithai were ground into fine powder and filtered to get the chooranam.The study was carried out in terms of physicochemical, high performance thin layer chromatography finger printing and preliminary phytochemical parameters were analysed. Results: Preliminary phytochemical screening found, the presence of phytochemicals such as terpenoids, glycosides and alkaloids.The ash value is 5.12%and high performance thin layer chromatography finger printing revealed the presence of many phytochemicals with different Rf values and densitometric scan of the plates showed numerous bands and peaks.Conclusion:The outcome of the study found that, the bioactive components like terpenoids, glycosides and alkaloids are effective in the management of kalladaippu (Urolithiasis).

Keywords: Siddha drug, Vellarivithai chooranam,Physico and phytochemical analysis,High performance thin layer chromatography finger printing.

1. INTRODUCTION

The Siddha system of Medicine is one of the oldest traditions of healthcare in the Indian sub-continent well documented and replete with novel therapeutic inventions and treatment modalities. Started from community practice by Siddhars in ancient times the system has grown immensely in caliber having travelled a journey of many centuries to become institutionalized, regulated and established stream of Traditional Indian Medicine and integral part of national health delivery system [1-3].

Traditional medicines are used by 80% of the world's population. Herbal medicines have been commonly used over the years for treatment and prevention of diseases, health promotion and enhancement of the span and quality of life. The most important things for consumers about medications are purity, safety, potency and efficacy. Standardisation helps to avoid adulteration and improper substitution of medicinal plants [3].

Urolithiasis affects about 12% of the world population at some stage in their lifetime. It affects all ages, sexes and races but occurs more frequently in men than in women within the age of 20-49 years. In Indian population, about 12% of them have urinary stones and out of which 50% may end up with loss of kidney functions [14].

Vellarivithai chooranam is a single herbal drug indicated for Kalladaippu (Urolithiasis) in Ka.Sa. Murugesamudaliar Gunapadam mooligai vaguppu [7, 8]. It shows Anti-urolithiatic, Anti-diarrhoeal, Anti-hypertensive,Anti-inflammatory, proteolytic, Anti-fungal, Anti-oxidant and Anti-panic activities. The seeds are used as diuretic.[13]The results of Vellarivithai Chooranam for physico-chemical, preliminary phytochemical analysis and high performance thin layer chromatographic studies are discussed.

2. MATERIALS AND METHODS

The seeds of Vellarivithai are purchased from a well reputed country shop in Palayamkottai. The raw drug was identified and authenticated by Medicinal Botanist of Govt. Siddha Medical College, Palayamkottai. The drug was purified and was shade dried. This dried seeds were ground into fine powder and filtered to get the chooranam. The chooranam is stored in air tight container and labelled as Vellarivithai chooranam which was used for experimental purposes.

2.1 Physico- chemical analysis

The physico-chemical analysis such as determination of ash value, acid insoluble ash, water soluble ash, sulphated ash,

pH value, extractable matter in water and alcohol, volatile oil and loss on drying at 105°C were carried out by standard methods [4, 16, 19]. The information collected from these tests are used for standardization.

Loss on drying

Loss on drying determines both water and volatile matter. It is carried out by heating to 100-105°C and then the result is noted.

Total Ash

A 3g of the test drug was accurately weighed and incinerated in a crucible dish at a temperature of 500-600°C until it is white, indicating the absence of carbon. It is then cooled in a desiccator for 30 minutes and then weighed. The content of total ash was then calculated.

Acid insoluble ash

To the crucible containing the total ash, 25ml of hydrochloric acid is added and covered with watch-glass and boiled gently for 5 minutes. The watch-glass is rinsed with 5ml of of hot water and this liquid is added to the crucible. The insoluble matter is collected on an ashless filter paper and washed with hot water until the filtrate is neutral. The insoluble matter is dried on the hot plate and the residue is allowed to cool in the dessicator for 30 minutes. The content of acid insoluble ash was then calculated.

Water soluble Ash

To the crucible containing the total ash,25ml of water is added and allowed to boil for 5 minutes. The insoluble matter is collected in an ashless filter paper. It is washed with hot water and allowed to heat in a crucible for 15 minutes at a temperature not exceeding 450°C.The content of water soluble ash is determined by subtracting the weight of this residue from the weight of total ash.

Sulphated Ash

An 1g of the powdered substance into a platinum crucible and moisten with sulphuric acid. It is heated gently to remove the excess of acid and ignited at about 800°C until all the black particles disappeared. It is again moistened with sulfuric acid and reignited. To it added a small of ammonium carbonate and ignited to constant weight.

pH value

pH value was determined by a glass electrode and suitable p^H meter.

Alcohol Soluble Extractive Value

3g of test drug powder was weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The solution was shaken continuously for 24 hours. It was then allowed to stand and soak for 18 hours. The solution is filtered and allowed to evaporate in a flat bottomed shallow dish and dried at 105°C.The content was then cooled and weighed.

Water Soluble Extractive Value

3g of the powdered drug was weighed and macerated with chloroform and water respectively at 80°C for 24 hours. The solution was shaken continuously for 6 hours and allowed to stand and soak for 24 hours and filtered. The solution from both chloroform and water respectively was filtered and the filtrate was allowed to evaporate in a flat bottomed shallow dish. It was dried at 105°C and then cooled and weighed.

2.2 Preliminary Phytochemical Analysis

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedures [5, 11].

2.3 High Performance Thin Layer Chromatography condition

High Performance Thin Layer Chromatography is a popular method for the quality control of herbal products and the analysis of herbal medicines. It is widely used for separation qualitative and quantitative estimation of marker compounds present in herbal drugs. HPTLC fingerprint profile is suitable for standardization of components followed by determination of specific bio-active phytoconstituents from plant materials [1].

Preparation of the alcoholic extract of the drug for HPTLC analysis

Five gram of the powdered sample is taken and reflux with 200ml of alcohol using a soxhlet apparatus on a water bath for 30 minutes. Filter the extract and concentrate to 5ml then the sample extract obtained is used for further experimental studies [18].

Chromatographic conditions

Instrument	:CAMAG (Switzerland)
Sample Applicator	: Automatic TLC Sampler 4 (ATS4).
Photo documentation system	: CAMAG Visualizer
Scanner	: TLC Scanner 4 and Cats software
Development chamber	: CAMAG developing chamber (10 cm × 10 cm)
Quantity applied	: 5, 10µl for extracts and 5 µl for standards
Stationary phase	: Aluminium plate pre-coated with silica gel 60
Mobile phase	: For Alcohol extract-Toluene: Ethyl acetate (5:2)
Scanning wavelength	: 254nm, 366nm, 575nm

A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the specified solvent system. The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F 254 pre-coated aluminium sheets through CAMAG micro liter syringe using Automatic TLC Sampler 4 (ATS4). After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected. The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm. The plate was scanned at 254 nm and 366 nm using TLC

Scanner 4 and the finger print profiles were documented. The Rf values and finger print data were recorded with win CATS software associated with the scanner. The plate was derivatized using vanillin-sulphuric acid reagent, heated at 105^{o}C by Placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the Rf values and finger print data were documented.

3. RESULTS AND DISCUSSION

3.1Physico-chemical analysis

Table1: Physico-chemical Analysis of Vellarivithai chooranam

Sl. No.	Tests	Result%
1	LOD at 105^{0}C	7.04
2	Total Ash	5.12
3	Acid insoluble ash	0.41
4	Water soluble ash	3.12
5	Sulphated ash	4.79
6	pH (4% water extract)	5.6
7	Volatile oil	Nil
8	Alcohol soluble extractives	11.74
9	Water soluble extractives	9.55

Interpretation

Physico-chemical Analysis

The moisture content of the drug reveals the stability and shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life. Loss on drying is 7.04%. Ash constitutes the inorganic residues residues obtained after complete combustion of a drug. Total ash value is 5.12% which determines the absence of inorganic content. The acid insoluble ash value of the drug denotes the amount of siliceous matter present in it. The quality of the drug is better if the acid insoluble value is low. Acid insoluble ash is 0.42%.Water soluble ash is the part of total ash content which is soluble in water. Water soluble ash is 3.12%. Sulfated ash is used for determining the content of inorganic impurities in an organic substance. Sulfated ash is 4.79%**.** The pH is a measure of hydrogen ion concentration .It is the measure of the acidic or basic in nature. The pH of the drug is 5.6 which are acidic in nature. Alcohol soluble extractives indicated the presence of polar constituents like alkaloids, glycosides, terpenoids. The alcohol soluble extractive was found to be 11.74**.**Water soluble extractive value is 9.55 which indicate the presence of sugar, acids and inorganic compounds present in the drug.

3.2 Preliminary Phytochemical analysis

Table 2: Phytochemical analysis of Vellarivithai Chooranam

Tests	Result
Saponins	-
Tannins	-
Terpenoids	+
Phenols	-
Steroids	-
Quinones	-
Antraquinones	-
Glycosides	+
Carbohydrates	-
Alkaloids	+
Lignans	-
Flavanoids	-
Proteins	-

Interpetation

Terpenoides

Terpenoides are phenolic compounds that exhibit the antimicrobial activity. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds [17].

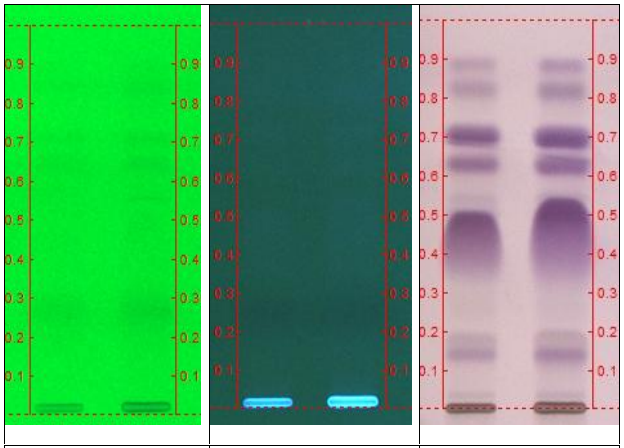
Glycosides

Glycosides are a heterogenous and diverse group of secondary metabolites with significant bioactivity, which include phenol, alcohol or sulfur –related compounds [10]. Triterpenoid glycoside present in cucumber seeds may be responsible for the antioxidant and anti-ulcer activity [6].

Alkaloides

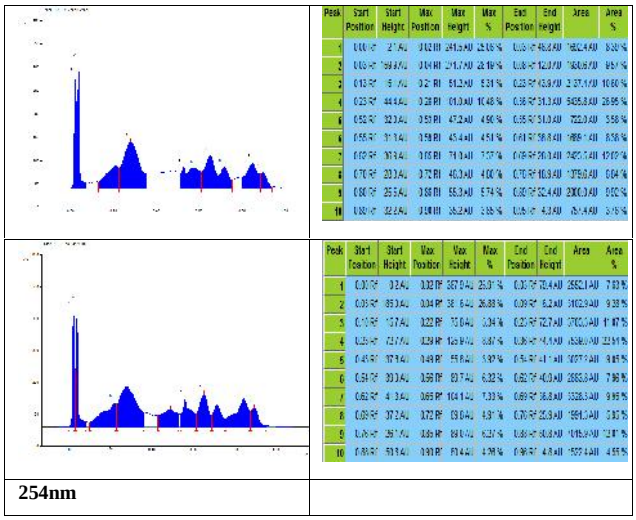
Alkaloids are large group of plant secondary metabolites. They are pharmacologically active organic compound present in plant kingdom synthesized from aminoacid having basic or cationic property due to the presence of positively charged α-nitrogen in their heterocyclic ring [2, 12]. It reveals, the broad spectrum antibacterial property [16] and analgesic property [9], also they possess anti-spasmodic, anti-fungal, anti-fibrogenic effects [15].

3.3 High Performance Thin Layer Chromatography



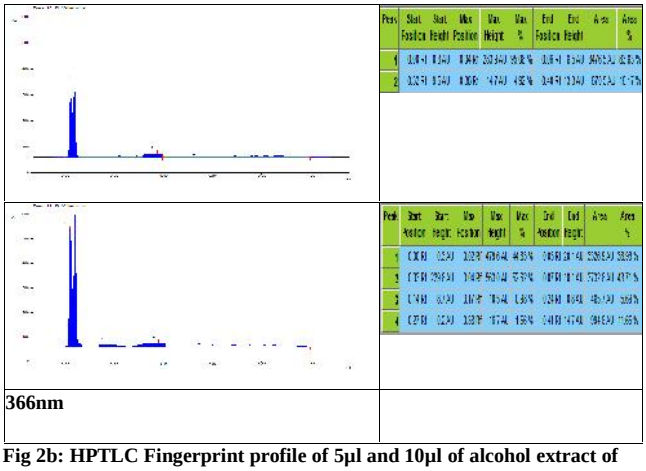
Under UV short	Under UV long	Under white light after derivatisation

Fig 1: HPTLC profile of alcohol extract of Vellarivithai Chooranam viewed in UV short;UV long;White light after derivatisation using vanillin-sulphuric acid; Solvent system-Toluene: Ethyl acetate-(5:2);Volume applied; Track 1- 5 µl: Track 2 – 10 µl



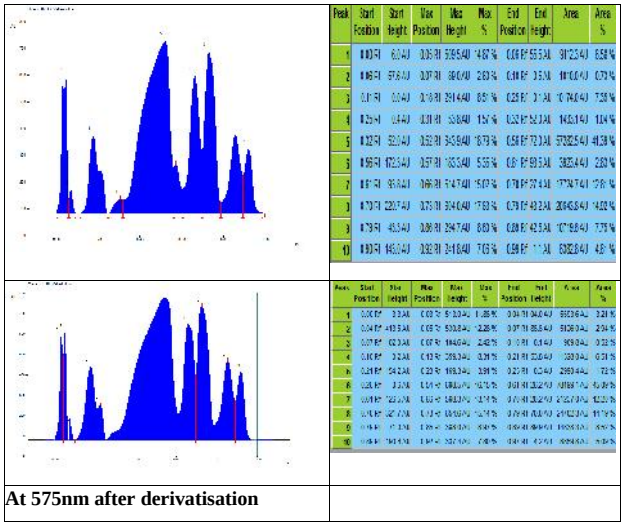
254nm

Fig 2 a.HPTLC fingerprint profile of 5µl and 10µl of alcohol extract of Vellarivithai chooranam at 366nm.



366nm

Fig 2b: HPTLC Fingerprint profile of 5µl and 10µl of alcohol extract of Vellarivithai chooranam at 366nm.



At 575nm after derivatisation

Fig 2c: HPTLC fingerprint profile of 5µl and 10µl of alcohol extract of Vellarivithai chooranam at 575nm after derivatisation.

The HPTLC fingerprinting patterns of alcohol extract of Vellarivithai chooranam was developed at 254nm, 366nm and after derivatisation with vanillin sulphuric acid at 575nm.The solvent system, Toluene: Ethyl acetate-(5:2) efficiently resolved the components. HPTLC photo documentation profile of the Vellarivithai chooranam at 254nm, 366nm and after derivatisation is given in Figure.1.The fingerprint profile and the R_f value and percentage area of the peaks are shown in Figure.2a,2b,2c. High Performance Thin Layer Chromatography shows the presence of many phytochemicals with different Rf values and densitometric scan of the plates showed numerous bands and peaks. On observation of Figure 2a, 10 bands were appeared under UV short with R_f 0.02,0.04,0.22,0.29,0.49,0.56,0.65,0.72,0.85 and0.90. Out of which R_f value at 0.29 has the maximum area 22.54% indicating the presence of highest concentration of phytoconstituents. HPTLC pattern at 366nm mentioned in Figure.2b showed the peak at R_f 0.04 having the maximum area of 43.71%. HPTLC pattern after derivatisation mentioned in Figure.2c showed 10 bands with R_f 0.03,0.05,0.07,0.18,0.23,0.54,0.66,0.73,0.85 and 0.92.Out of which R_f value at 0.54 has the maximum area 45.09%.Each band indicates the presence of phytoconstituent present in the extract.

4. CONCLUSION

The different physicochemical parameters, phytochemical parameters and HPTLC fingerprint helps in the correct identification, standardization and quality control of Vellarivithai chooranam. The result obtained from this study helps in the correct identification and authentication and may help to prevent adulteration. The bioactive constituents like terpenoids, glycosides and alkaloids are responsible for its therapeutic activity. Thus it can be assumed that it will be

effective in the management of Kalladaippu (Urolithiasis) in Siddha system.

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