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Original article

Preliminary Phytochemical Analysis and Physicochemical Standardization of Vasambu Chooranam (Acoruscalamus)

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ABSTRACT:
The Siddha system of medicine has been widely used for thousands of years in TamilNadu. Siddha is an unique system of medicine as it act both on curative and preventive aspects to achieve the healthy body and mind.Siddha Medicines revitalize and rejunevate the body.Vasambu is the common name for Acorus calamus in Tamil literature.Acorus calamus Linn.(Araceae) commonly known as “Sweet flag”.Vasambu Chooranam is mentioned in the classical siddha literature “Gunapadam Mooligai Vagupu”. Aim: The present study is an attempt to evaluate the phytochemical, physicochemical parameters and HPTLC finger printprofile of VasambuChooranam .Material and Methods: The raw drug was purchased from reputed herbal drug shop, Thackkalay, Kanyakumari district and it is purified as mentioned in the siddha literature. Results: The Phytochemical evaluation showed the presence of Saponins, Alkaloids, Carbohydrates, Terpenoids, Glycosides and Quinones. The Physico chemical analysis showed the values of Loss on drying, total ash,Acid insoluble ash,water soluble ash ,sulphated ash etc. The HPTLC studies of the alcohol extracts of the plant materials were carried out and the chromatograms and fingerprinting profiles at 254nm, 366nm and 575nm after derivatisation were documented. This information will be used for laying down the pharmacopoeial standards of Vasambu Chooranam.

Keywords: Vasambu, phytochemical, Physicochemical, Acoruscalamus,HPTLC.

1. INTRODUCTION

The Siddha system of medicine is one of the oldest medical systems in India. It is assumed that when the normal equilibrium of the three humours (Vatham, Pitham, Kabam) is disturbed, the disease is caused. Vasambu Chooranam is prepared from the plant Acorus calamus Linn. which belongs to the family Araceae [1]. It is a perennial, semiaquatic, aromatic herb with creeping rhizomes .Vasambu Chooranam is indicated for several diseases like Peptic Ulcer, Liver disease, diarrhea, filariasis, Tapeworm infestations etc. which is mentioned in Siddha literature “Gunapadam Mooligai Vagupu”.The present study was carried out to evaluate the phytochemical ,physicochemical parameters and HPTLC fingerprint profile of Vasambu Chooranam and the work was carried out in Siddha Regional Research Institute,Thiruvananthapuram,Kerala.

2. MATERIALS AND METHODS

Collection of raw drugs:

The required drug was purchased from in and around Palayamkottai, Tirunelveli, TamilNadu.

Authentication:

The drug will be identified and authenticated by the Medicinal Botanist and Gunapadam experts at Government Siddha Medical College and Hospital, Palayamkottai-627002.

Purification and preparation of the drug [3]

Vasambu (*Acoruscalamus*): It is burnt up directly into the flame and the ash was collected in a container.

Table 1: Biological source of the plant

DRUG NAME	BOTANICAL NAME	ENGLISH NAME	FAMILY	PARTS USED
Vasambu	*Acorus calamus*	Sweet flag	Araceae	Dried rhizome

Standardisation parameters:

The various standardization parameters used in this studies are preliminary phytochemical analysis, physicochemical analysis and HPTLC analysis [4].

Preliminary phytochemical analysis[1]

Test for Saponins:

To a few mg of extract distilled water is added and shaken well. The formation of foam indicates the presence of saponin.

Test for Tannin:

To Substance in water is added with 5 % alcoholic ferric chloride. Dark blue colour shows presence of tannin.

Test for terpenoids :

To a few mg of extract in chloroform, add conc. H2SO4. Presence of dark brown precipitate indicates the presence of terpenoids.

Test for Phenol:

To Substance in water is added with 5 % alcoholic ferric chloride. Dark blue or green colour shows presence of phenol.

Test for Steroids (Lieberman Burchard Test):

To a few mg of the extract 2 ml of chloroform is added in a dry test tube. Few drops of acetic acid is added, heated and few drops of acetic anhydride and 2 drops of concentrated sulphuric acid are added. The green colour indicates the presence of steroid [5].

Test for Quinones:

To a few mg of extract, add few drops of concentrated sulphuric acid. Appearance of red colour shows the presence of quinone.

Test for glycosides:

Substance is treated with anthrone and concentrated sulphuric acid. On heating over a water bath, the appearance of green colour shows the presence of glycoside [6].

Test for carbohydrates :

To the sample solution, added few drops of α-naphthol and 2-3 ml conc. H2SO4. The appearance of reddish violet or purple ring at the junction of two liquids indicates the presence of Carbohydrates.

Test for Alkaloids (Dragendorff's Test):

Few mg of extract in separate test tube was warmed with 2% Sulphuric acid for 2 minutes and it was filtered in separate test tube and few drops of Dragendorff's reagent were added. The presence of orange red precipitates indicates the presence of alkaloids [7].

Test for Flavonoids:

To the substance in alcohol add 10% NaOH or ammonia. A dark yellow colour indicates the presence of flavonoid

Test for Proteins (Biuret test):

To the sample solution in a test tube, add sodium hydroxide solution and then add a few drops of very dilute (1 %) copper II sulphate solution and mix gently. Appearance of purple colour indicates the presence of protein.

Physicochemical evaluation [8]

The physicochemical analysis of Vasambu Chooranam was carried out as per WHO guidelines. The test procedures were done at Siddha Research Regional Institute, Thiruvananthapuram, Kerala

Loss on drying at 105°c:

A 100 ml beaker is accurately weighed. Take about 4 g of powdered drug (about 3 mm in thickness) in the beaker and accurately weighed. Place the beaker in an oven and dry at 105°C for 5 hours. Cooled in a desiccator and weighed. Repeat the process until constant weight is obtained [9].

Percentage of loss on drying at 105°C = Loss in weight of the sample ×100/ Weight of the sample taken …(Equation 1)

Total ash:

A silica crucible is ignited, cooled and weighed. Take about 2 g of powdered drug in the crucible and accurately weighed. Incinerate the drug until free from carbon in a muffle furnace, cool and weigh [10].

Percentage of total ash= Loss in weight of the sample ×100/ Weight of the sample taken …(Equation 2)

Acid insoluble ash:

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter.

Percentage of acid insoluble ash = Weight of acid insoluble residue×100/Weight of the sample taken ..(Equation 3)

Water soluble ash:

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Percentage of water soluble ash = Weight of water soluble ash ×100/Weight of sample taken …(Equation 4)

Sulphated ash:

Take total ash in the crucible. Moisten the ash with small amount (1ml) of sulphuric acid. Heat it in a Muffle furnace at 600°C till the charred ash is completely incinerated. Weigh the cooled crucible and calculate the residue percentage.

Percentage of sulphated ash = Weight of sulphated ash ×100/Weight of sample taken.…(Equation 5)

Alcohol soluble extractive:

Take about 4 g of coarsely powdered drug and accurately weighed. Transfer it in a glass stoppered conical flask and add 100 ml alcohol (approximately 95%). Allow to stand for 18 hrs. Filter rapidly. Take a 50 ml beaker and weigh. Pipette out 25 ml of the filtrate to the beaker. Evaporate to dryness on water bath and keep it in air oven at 105°C for 6 hours. Cooled in desiccator and weighed [11].

Percentage of solubility in alcohol = Weight of extract×100×100/25×Weight of sample taken ..(Equation 6)

Water soluble extractive:

Take about 4 g of coarsely powdered drug and accurately weighed. Transfer it in a glass stoppered conical flask and add 100 ml distilled water. Allow to stand for 18 hrs. Filter rapidly. Take a 50 ml beaker and weigh. Pipette out 25 ml of the filtrate to the beaker. Evaporate to dryness on water bath and keep it in air oven at 105°C for 6 hours. Cool in desiccator and weigh.

Percentage of solubility in water = Weight of extract× 100× 100/25×Weight of sample taken. …(Equation 7)

pH of water extract :

The pH value of a liquid can be determined potentiometrically by means of the glass electrode, a reference electrode and a pH meter of the digital type

Volatile oil:

Take 20 g coarsely powdered drug in a 1 litre R. B. flask. Add 300 ml of water and a few porous pieces. The flask is connected to a volatile oil apparatus (Clevenger apparatus). The contents of the flask are now heated and boiled for 2 hours or until distillation is completed. The flask is rotated occasionally to wash down any material that adheres to its sides. The apparatus is allowed to cool for 10 minutes and the volume is read. The % Volatile oil is calculated [12].

Percentage of volatile oil= Volume of volatile oil ×100/Weight of drug taken ….(Equation 8)

Swelling index:

Take 1 g powdered drug in a 25 ml glass stoppered measuring cylinder. Add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature. Measure the volume in ml occupied by the plant material, including any sticky mucilage [11].

Foaming index:

The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

Foaming index = 1000/a….. (Equation 9)

HPTLC analysis:

High Performance Thin Layer Chromatography analysis , this is a modern sophisticated and automated separation technique derived from TLC.HPTLC is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics [2].

PROCEDURE:

Developing solvent system

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 solvent system: Toluene: Ethyl acetate: (5:2)

Sample application

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F254 pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4) [12].

Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation

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 [9].

Densitometry

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 CATS software associated with the scanner.

Post chromatographic derivatisation

The plate was derivatized using vanillin-sulphuric acid reagent, heated at 105^0 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

Table 2: Preliminary phytochemical analysis of vasambu chooranam:

S.NO	PHYTOCHEMICALS	TEST	RESULTS
1	Saponin	Froth test	Present
2	Tannin	Ferric chloride test	Absent
3	Phenol	Alcoholic ferric chloride test	Absent
4	Terpenoids	Libermann test	Present
5	Alkaloids	Dragendorff's test	Present
6	Flavonoids	Alkaline reagent test	Absent
7	Steroids	Libermann Burchard test	Absent
8	Glycosides	Anthrone test	Present
9	Carbohydrates	Molisch's test	Present
10	Quinone	Sulphuric acid test	Present
11	Proteins	Biuret test	Absent

As per table 2,

The qualitative analysis of the ethanolic extracts from Vasambu Choornam showed the presence of Saponins, Alkaloids, Carbohydrates, Terpenoids, Glycosides and Quinones.

Saponins:

Saponins decrease blood lipids,lower cancer risks and lower blood glucose response. Saponin posess antiviral, anticancer, anti inflammatory activities

Carbohydrates:

Carbohydrates play an important role in homeostasis of glucose and fatty acids in Liver.

Alkaloids:

Alkaloids are the active principles producing many essential effects in protecting the body. These are made up of nitrogenous compounds. They possess antibacterial, antivirus, anti cancer and antioxidant activities.

Glycosides:

Glycosides possess anticancerous, analgesic ,anti inflammatory activities. There are different types of Glycosides depending upon their effects such as alcoholic glycosides, Anthroquinone glycosides, Coumarin glycosides, Flavanoid glycosides, cardiac glycosides etc.

Terpenoids:

Terpenoids have been found to be useful in prevention and theraphy of several diseases including cancer and also possess antimicrobial, antifungal and Anti inflammatory activities.

Quinones:

Quinones by their anti oxidant activity improve general health conditions. As Vitamins, they help in preventing and treating illnesses such as Osteoporosis, Cardio vascular diseases.

Table 3: Physicochemical analysis of vasambu chooranam

S.NO	PARAMETERS	RESULT
1	LOD at 105ºC	4.2%
2	Total ash	31.19%
3	Acid insoluble ash	2.16%
4	Water soluble ash	17.92%
5	Sulphated ash	39.17%
6	pH of water extract	5.27
7	Volatile oil	Nil
8	Alcohol soluble extractives	7.17%
9	Water soluble extractives	4.64%
10	Swelling index	2.5
11	Foaming index	< 100

As per table 3

LOD at 105°C: It measures 4.2% which determines the moisture content and volatile oil present in the drug.

Total Ash: It measures about 31.19% which tells about the total amount of material remaining after ignition

Acid insoluble ash: It values about 2.16% which measures the amount of silica present, especially as sand and siliceous earth

Water soluble ash: It values 17.92% which is the difference in weight between total Ash and the residue after treatment of the total ash with water.

Sulphated ash: It values 39.17% which is usually used for determining the content of inorganic impurities in an organic substance.

Alcohol Soluble Extractives: It measures about 7.17% which indicates the presence of polar, non polar and secondary metabolites present in the plant.

Water Soluble Extractives: It values about 4.64% indicating the presence of sugar, acids and inorganic compounds

pH of water extract: It values about 5.27,indicating the water extract of the drug is acidic.

Swelling Index: It is used to determine the quality and purity of crude drug. It measures about 2.5.

Foaming Index: It is used to express the quality of the crude drug containing saponins. It measures about <100

HPTLC analysis of vasambu chooranam:

The HPTLC fingerprinting patterns of alcoholic extract of Vasambu Chooranam was developed at 254 nm, 366 nm and after derivatisation with vanillin sulphuric acid at 575 nm. The solvent system, Toluene: Ethyl acetate (5:2) efficiently resolved the components (Figure 1).

HPTLC photo documentation profile of the Vasambu Chooranam at 254 nm, 366nm and after derivatisation is given in figure 1,2,3. The finger print profile and the Rf value and percentage area of the peaks are shown in corresponding figures.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 (Figure 2).

III. HPTLC Analysis

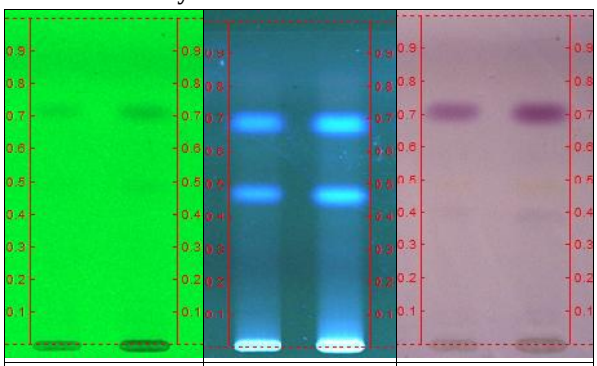


Fig 1: HPTLC profile of alcohol extract of Vasambu Chooranam viewed in UV short; UV long; White light after derivatisation using vanillin-sulphuric acid ; Solvent system –Toluene: Ethyl acetate –(5:2); volume applied; Track 1-5 µl: Track 2-10 µl

254 nm

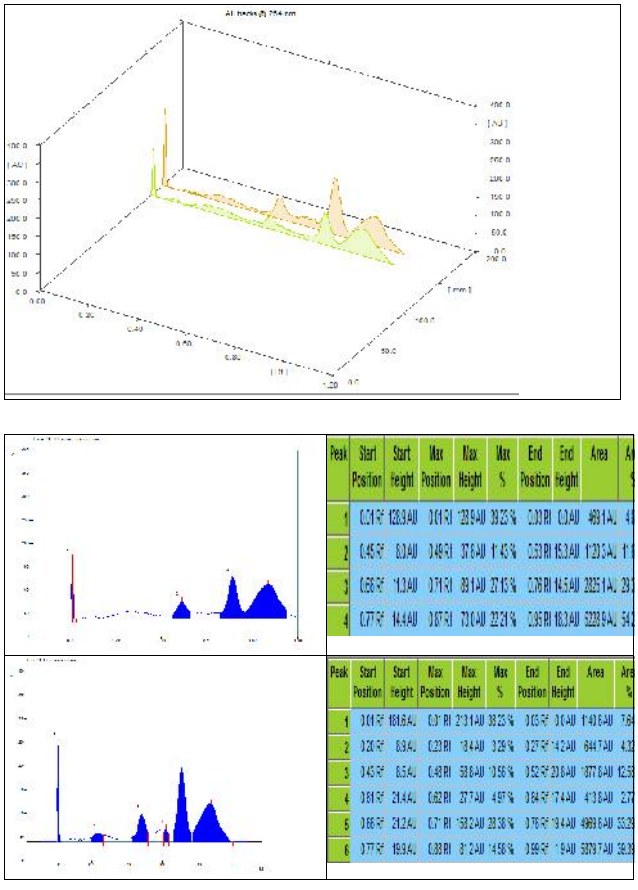
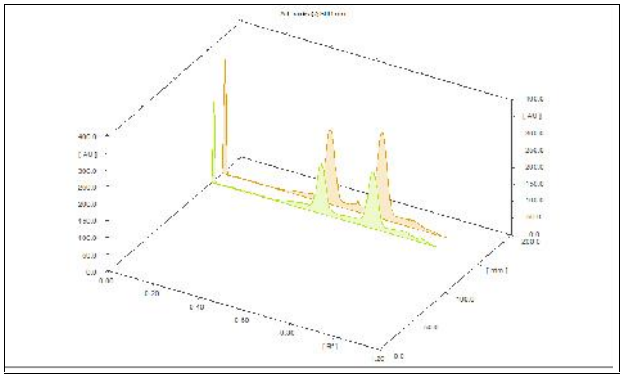
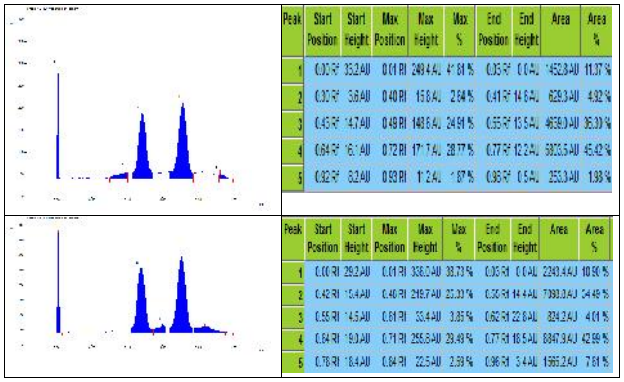


Fig 2: HPTLC fingerprint profile of 5µl and 10 µl of alcohol extract of Vasambu Chooranam of 254 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 254 nm, the sample reveals the presence of 6 prominent peaks corresponds to the presence of 6 versatile phytocomponents present within it . Rf value of the peaks ranges from 0.01Rf – 0.77Rf.Further the peak 5 and 6 occupies the major percentage of area of 33.29% and 39.39% which denotes the abundant existence of such compound (Figure 3).

366 nm





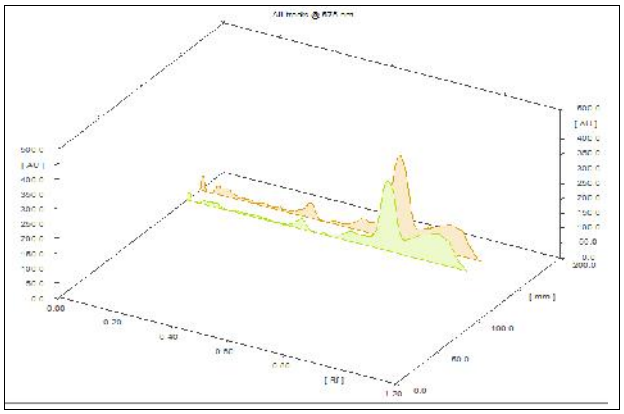
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	35.2 AU	0.01 Rf	249.4 AU	4.61 %	0.05 Rf	0.0 AU	1452.8 AU	11.37 %
2	0.05 Rf	3.6 AU	0.10 Rf	15.8 AU	2.64 %	0.41 Rf	14.6 AU	629.3 AU	4.92 %
3	0.45 Rf	14.7 AU	0.49 Rf	148.6 AU	24.61 %	0.55 Rf	10.5 AU	4639.0 AU	36.30 %
4	0.64 Rf	16.1 AU	0.72 Rf	177.7 AU	28.77 %	0.77 Rf	12.2 AU	5805.5 AU	45.42 %
5	0.82 Rf	6.2 AU	0.83 Rf	11.2 AU	1.87 %	0.86 Rf	0.5 AU	253.3 AU	1.98 %

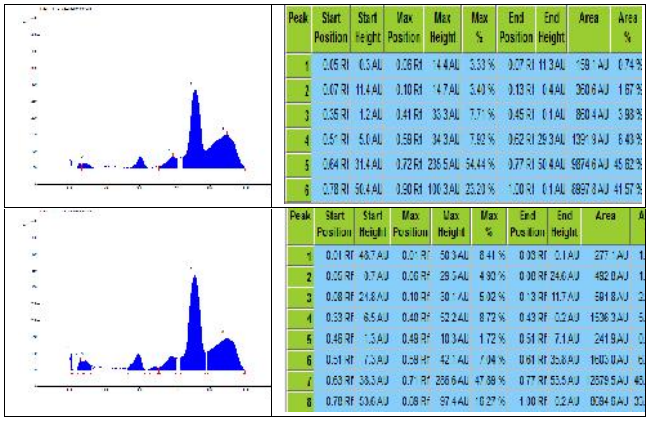
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	29.2 AU	0.01 Rf	336.0 AU	38.73 %	0.05 Rf	0.0 AU	2240.4 AU	10.56 %
2	0.42 Rf	15.4 AU	0.49 Rf	216.7 AU	25.00 %	0.56 Rf	14.4 AU	7390.8 AU	34.49 %
3	0.55 Rf	14.5 AU	0.61 Rf	33.4 AU	3.85 %	0.62 Rf	22.6 AU	824.2 AU	4.01 %
4	0.64 Rf	19.0 AU	0.71 Rf	255.6 AU	29.49 %	0.77 Rf	18.5 AU	8347.3 AU	42.99 %
5	0.78 Rf	18.4 AU	0.84 Rf	22.5 AU	2.59 %	0.96 Rf	3.4 AU	1365.2 AU	7.81 %

Fig 3: HPTLC fingerprint profile of 5 µl and 10 µl of alcohol extract of Vasambu chooranam at 366 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 366 nm,the sample reveals the presence of 5 prominent peaks corresponds to presence of 5 versatile phytocomponents present within it.Rf value of the peak ranges from 0.00Rf – 0.78Rf. Further the peak 2 and 4 occupies the major percentage of area of 34.49% and 42.99%, which denotes the abundant existence of such compound (Figure 4).

575 nm





Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.3 AU	0.26 Rf	14.4 AU	3.53 %	0.27 Rf	11.3 AU	159.1 AU	0.74 %
2	0.07 Rf	11.4 AU	0.10 Rf	14.7 AU	3.40 %	0.13 Rf	0.4 AU	360.6 AU	1.67 %
3	0.35 Rf	1.2 AU	0.41 Rf	33.3 AU	7.71 %	0.45 Rf	0.1 AU	850.4 AU	3.93 %
4	0.5* Rf	5.0 AU	0.59 Rf	34.3 AU	7.92 %	0.62 Rf	29.3 AU	1397.3 AU	6.43 %
5	0.64 Rf	31.4 AU	0.72 Rf	236.5 AU	54.44 %	0.77 Rf	50.4 AU	9874.6 AU	45.62 %
6	0.78 Rf	50.4 AU	0.90 Rf	100.3 AU	23.20 %	1.00 Rf	0.1 AU	8957.8 AU	41.57 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	A
1	0.01 Rf	48.7 AU	0.01 Rf	50.3 AU	8.41 %	0.03 Rf	0.1 AU	277.1 AU	1.
2	0.05 Rf	0.7 AU	0.08 Rf	29.5 AU	4.93 %	0.10 Rf	24.6 AU	482.0 AU	1.
3	0.08 Rf	24.8 AU	0.10 Rf	58.1 AU	9.72 %	0.13 Rf	11.7 AU	584.8 AU	2.
4	0.33 Rf	6.5 AU	0.40 Rf	52.2 AU	8.73 %	0.43 Rf	0.2 AU	1536.3 AU	6.
5	0.48 Rf	1.3 AU	0.49 Rf	10.3 AU	1.72 %	0.51 Rf	7.1 AU	241.9 AU	1.
6	0.51 Rf	7.5 AU	0.58 Rf	42.1 AU	7.04 %	0.61 Rf	35.8 AU	1600.0 AU	6.
7	0.63 Rf	38.3 AU	0.7* Rf	256.6 AU	47.88 %	0.77 Rf	53.5 AU	2579.5 AU	45.
8	0.78 Rf	53.6 AU	0.88 Rf	97.4 AU	18.27 %	1.00 Rf	0.2 AU	8084.0 AU	33.

Fig 4: HPTLC fingerprint profile of 5 µl and 10 µl of alcohol extract of Vasambu chooranam at 575 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 575 nm,the sample reveals the presence of 8 prominent peaks corresponds to the presence of 8 prominent peaks corresponds to presence of 8 versatile phytocomponents present within it.Rf value of the peak ranges from 0.01Rf – 0.78 Rf .Further the peak 7 and 8 occupies the major percentage of area of 48.94% and 33.04% , which denotes the abundant existence of such compound.

4. CONCLUSION

Preliminary Phytochemical analysis of Vasambu Choornam showed the presence of Saponins, Alkaloids, Carbohydrates, Terpenoids, Glycosides and quinones.Physicochemical evaluation of Vasambu Chooranam showed the values of total ash, sulphated ash, water soluble ash ,swelling index, foaming index etc. HPTLC finger printing revealed the evidence of many phytochemicals with different Rf values and densitometric scan of the plates showed numerous bands and peaks.The present study on phytochemical and physicochemical parameters, HPTLC analysis provides important information which can be used as a fingerprint for further clinical studies.

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