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An evaluation of physico – chemical and phytochemical analysis of *Siddha* poly herbal formulation "*Siringipaerathi chooranam*".

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Abstract

Aim and objective:
The aim of the study is to evaluate the Physico – Chemical and Phytochemical Analysis of *Siddha* Poly Herbal Formulation "*Siringipaerathi Chooranam" (SPC).*
Materials and methods:
Siddha system of medicine is the traditional system, which was found by Siddhars who believe the art of immortal corporeal human body. Standardization of a *Siddha* medicine is important to ensure its efficacy and safety but now-a-days it is a burden topic globally. "*Siringipaerathi Chooranam"* is one the Siddha drug chosen from *Sarabendra Vaidhiya Muraigal". Soolai, Moola, Kusta, Pitharoga Muraigal*, page no: 194-195 which is indicated for hepatoprotective activity. However an attempt was made to standarisize the *siddha* herbal preparation of "*Siringipaerathi Chooranam" through the scientific method of* Physico – Chemical, Phytochemical evaluation.
Results:
Through this method Organoleptic characters, pH, Ash (%),Acid Insoluble ash (%),Water soluble ash (%),Loss on drying (%),Solubility, Action on heat, Flame test, Ash test, Alkaloids, Carbohydrates, Glycosides, Saponins, Phenols, Phytosterols, Triterpenes, Flavanoids, Proteins and amino acids, Quinones, biochemical compound shows the presence or absence of this constituents. The organoleptic characters of a trail drug shows the nature of drug colour, texture, particle size and other morphology. The Phytochemical screening of the extract gives an idea nature of chemical substances in it.
Conclusion:
Thus the preliminary chemical analysis of a *Siddha* drug will give finger-prints to the clinical studies.

Keywords: Physico – Chemical, Phytochemical Analysis, *Siddha*, *Siringipaerathi Chooranam*, *SPC*

Introduction

Nature is a remedy for human ailments that have been diverged around the World. Siddha system is unique among the Indian system of medicine. It is believed to have been developed by the siddhar's the ancient supernatural spiritual saints of India. In Siddha system of medicine the drug sources are obtained from plant, mineral, metal and animals. Siddha system is unique among the Indian system of medicine. It is believed to have been developed by the siddhar's the ancient supernatural spiritual saints of India. In Siddha system of medicine the drug sources are obtained from plant, mineral, metal and animals.

Siddha system of medicine is the one of the oldest traditional medical systems, that derives the drugs from plants, animal products, minerals and metals. Siddhars were considered as superhuman who defines the age, laws of nature, to which all human being [1].

Herbal drugs have been used widely in many countries because of its availability, less cost effectiveness and safer than the synthetic drugs [2].

Siddha drugs have been practiced by ancient times without any standardization techniques. It required standardization for globally acceptance. Thus I have preferred to choose *"Siringipaerathi Chooranam"* was taken as a trial drug for Hepatoprotective activity from the Siddha literature *"Sarabendra Vaidhiya Muraigal". Soolai, Moola, Kusta, Pitharoga Muraigal*.

There is no such specific protocol for standardize the drug thus it was aimed to validate the qualitative and quantitative screening for *Siringipaerathi Chooranam* scientifically to prevent adulterations. Thus Organoleptic properties, Phyto-chemical screening, Biochemical and Physicochemical parameters, were carried out in this drug.

Materials and Methods

Drug selection:

In this research paper purified and prepared *"Siringipaerathi Chooranam"* was taken as a trial drug for Hepatoprotective activity from the Siddha literature *"Sarabendra Vaidhiya Muraigal". Soolai, Moola, Kusta, Pitharoga Muraigal*, page no: 194-195.

Table: 1. Ingredients:

Name of drugs	Botanical name	Quantity
Inji	*Zingiber officinalis*	560 gm (16 palam)
Milagu	*Piper nigrum*	50.4gm (12 varahan)
Thippili	*Piper longum*	33.6gm(8 varahan)
Thipili moolam	*Piper longum*	16.8gm(4 varahan)
Lavanga pathiri	*Cinnamomum tamala*	35gm(1 palam)
Elam	*Elettaria cardamomum*	42gm (10 varahan)
Kodiveli ver	*Plumbago zeylanica*	42gm (10 varahan)
Lavanga pattai	*Cinnamomum zeylanicum*	35gm (1 palam)
Moongil uppu	*Bambusa arundinaceae*	35gm (1 palam)
Sandhana thool	*Santalum album*	35gm (1 palam)
Vilamichu-ver	*Plectranthus vettiveroides*	35gm (1 palam)
Sathikkai	*Myristica fragrans*	35gm (1 palam)
Seeragam	*Cuminum cyminum*	35gm (1 palam)
Kirambu	*Syzygium aromaticum*	35gm (1 palam)
Sugar	*Saccharum officinarum*	Equal quantity
Nei	English Name : Ghee	30ml

Collection of the Plant materials:

All the raw materials were bought from the Ramasamy Mudhaliyar Store, Parry's corner, Chennai.

Identification and Authentication of the drug:

All the plant materials were identified and authenticated by the Botanists and Gunapadam experts in Government Siddha Medical College, Arumbakkam, and Chennai-106.

Purification of the drugs

All the drugs mentioned here were purified as per the Siddha literature [3].

Inji	-	Outer skin of ginger was peeled off.
Milagu	-	It was soaked in sour buttermilk for 3 hours and allowed to dry
Thippili	-	Soaked in lemon juice and allowed to dry.
Thippilimoolam	-	Remove the nodules and dried.
Lavangapathiri	-	Dried in sun light.
Elam	-	Roasted in the pan and outer skin was removed.
Kodiveli-ver	-	The root was cleaned with a white cloth.
Lavangapattai	-	Dried in sun light.
Sandhana kattai	-	The skin was peeled off to get purified and powdered
Vilamichu-ver	-	The root was cleaned with a white cloth.
Sathikkai	-	Cleaned and cut into small pieces and dried.
Seeragam	-	Clean the dust particles and allowed it to dry.
Kirambu	-	Flower buds were removed.

Preparation of the Drug:

Procedure:

In order to obtain the purified form of ginger, the upper skin of ginger was peeled off and then sliced into small pieces. The sliced pieces were dried in sunshade for two days. After complete drying 560 grams of dried ginger was taken and fried well in ghee and then powered.

50.4 grams of Purified Pepper, 33.6 grams of *Thippili*, 16.8 grams of *Thipplimoolam*, 42 grams of *Kodiveli-ver*, 35 grams of *Moongil uppu*, *Lavangapathiri*, *Sandhana thool*, *Vilamichu-ver*, *Lavanga Pattai*, *Adhikari*, *Seeragam*, *Kirambu* were taken and powered separately then mixed together with processed ginger powder.

Finally, the mixture was ground well which favors the homogenous preparation. Then the mixture powder was sieved through the thin clean white cloth. After that twice the weight of sugar was added to the mixture and again it was ground well.

Finally, the end product was obtained, which was kept in an air tight container and labeled as "***Siringipaerathi Chooranam" (SPC)***[4].

Purification of the Chooranam: steaming process (*Pittaviyal murai*)

The "*Siringipaerathi Chooranam"* was purified by *pittaviyal* method (steam cooking in milk) as per Siddha classical literature. A mud pot was taken and it was half filled by milk and mixed with equal quantity of pure water. The mouth of the pot was sealed by a cloth. This *chooranam* was placed over a clean dry cloth and tied firmly around the mouth of mud pot. The gap between mud pots was tied with a wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk reduced in the lower pot.

The same drug was later dried and powdered then sieved again. It was used for the further study [5].

Storage of the drug:

The prepared test drug was stored in a clean, air tight glass container.

Administration of the drug:

Form of the medicine : *Chooranam*
Route of Administration : Enteral Dose
: 2 gm twice a day depending on the severity
Adjuvant : honey

Indication:

Kamaalai, *Marbuvali, Kirani, Suram, Vaanthi, Peenisam.*

Standardization of the drug:

Standardization of the drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug. Thus standardization brings the efficacy and potency of the drug.

Organoleptic character:

The organoleptic characters of the sample drug were evaluated. 1gm of the test drug was taken and the color, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted.

Physicochemical analysis

Physicochemical studies of the trial drug have been done [6].

Determination of Ash Values:

Total Ash;

3g of the test drug was accurately weighed and incinerated in a crucible dish at a temperature not exceeding 450^{o}C until it was free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried powder was calculated.

Water Soluble Ash;

The total ash was obtained as the above method for preparation of total ash. The ash was boiled with 25ml of water for 5mins. The insoluble ashes were collected using filter paper. It was then washed with hot water and transferred to the silica crucible. It was then ignited for 15minutes at temperature not exceeding 450^{o}C. For determination of weight of the water soluble ash the silica crucible and residue were weighed until constant weight was attained. The weight of the water soluble ash was determined by subtracting the weight of insoluble ash from the weight of total ash.

Acid insoluble Ash:

The total ash was obtained as the above method for preparation of total ash. The ash was boiled for 5minutes with 25ml 10% Hcl. The insoluble ashes were collected using filter paper and washed with hot water. It was then transferred to the silica crucible and ignited for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue were weighed until constant weight was attained.

Determination of Extractive Value:

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 resulting solution was shaken continuously for 6 hours. It was then allowed to stand and soak for 18 hours.

The solution was filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C. Then the content was cooled and weighed.

Water soluble Extractive value:

3g of test drug powder was weighed and macerated with chloroform and water, respectively, at 80^{o}C for 24 hrs. The resulting solution was shaken continuously for 6 hours and allowed to stand and soak for 24hrs then filtered. The solution from both chloroform and water respectively was filtered and evaporated of the filtrate in a flat bottomed shallow dish. It was dried at 105^{o}C then cooled and weighed.

Loss on Drying:

The powdered drug was taken and dried in the oven at 100- 105°C to constant weight. The result was noted.

Physical characterization:

Solubility: A little of the sample was shaken well with distilled water. **.** A little of the sample was shaken well with con Hcl and Con $H2SO_4$. Sparingly soluble character indicates the presence of Silicate.

pH value: Potentiometrically pH value was determined by a glass electrode and a suitable pH meter.

Action on heat: A small amount of the sample was taken in a dry test tube and heated gently. If there was a strong white fumes evolving it indicates the presence of Carbonate.

Flame test: A small amount of the sample was made into a paste with con.Hcl in a watch glass. It was then introduced into non-luminous part of the Bunsen flame. Appearance of bluish green flame indicates the presence of Copper.

Ash Test: A filter paper was soaked into a mixture of sample and cobalt nitrate solution. It was then introduced into the Bunsen flame and ignited. Appearance of yellow colour flame indicates the presence of Sodium.

Phytochemical analysis:

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the method illustrated [7].

Test for Alkaloids:

A small portion of solvent free extracts were stirred separately with few drops of dilute hydrochloric acid and filtered & tested carefully with various alkaloid reagents.

Mayer's reagent - Cream precipitate
Dragendroff's reagent - Orange brown precipitate
Hager's reagent - Yellow precipitate
Wagner's reagent - Reddish brown precipitate

Test for Carbohydrates and Reducing Sugars:

The minimum amount of extracts were dissolved in 5ml of distilled water & filtered. The filtrate was subjected to test for carbohydrates & glycosides.

a) Molisch's test:

The filtrate 1 ml was treated with 2-3 drops of 1% alcoholic alpha naphthol & 2ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate.

b) Benedict's test:

The filtrate 1 ml was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's test:

The filtrate 1 ml was treated with equal volume of Fehling's solution A and B and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Glycosides:

The extract was hydrolyzed with dil. HCl and subjected to test for glycosides.

a) Modified Borntrager's test:

To the hydrolysate extract, 1 ml of Ferric chloride solution was added and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammonial layer indicates the presence of Anthranol glycosides.

b) Legal's test:

The hydrolysate extract was treated with Sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of Cardiac glycosides.

Test for Saponins:

The extract 0.5 ml was shaken with 5 ml distilled water. The presence of saponins was indicated by formation of copious lather.

Test for Tannins:

Gelatin test:

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for Phenolic compounds:

To 0.5 ml of extract, 1 ml of alcoholic ferric chloride solution was added. Formation of bluish green or bluish black indicates the presence of Phenolic compounds.

Test for Phytosterols:

Ferric chloride – acetic acid test:

1 ml of extract was treated with 1 ml of chloroform and then, 2 ml of ferric chloride acetic acid reagent was added followed by 1 ml of conc. sulphuric acid. Appearance of reddish pink colour shows the presence of Phytosterols.

Test for Diterpenes:

Copper acetate test:

1 ml of extract was dissolved in water and treated with 3-4 drops of Copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Triterpenes:

Salkowski's test:

1 ml of extract was treated with 1 ml of chloroform followed by 1 ml of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour shows the presence of Triterpenes.

Test for Flavonoids:

a) Alkaline reagent test:

To 1 ml of extract, 1 ml of 10% sodium hydroxide solution was added. Formation of dark yellow colour indicates the presence of flavonoids.

b) Lead acetate test:

To 1 ml of extract, 3-4 drops of 10% lead acetate solution was added. Formation of yellow precipitate indicates the presence of flavonoids.

c) Ferric chloride test:

To 1 ml of extract, 3-4 drops of ferric chloride solution was added. Formation of dark green colour indicates the presence of flavonoids.

d) Shinoda test:

To 1 ml of extract, few mg of magnesium turnings was added followed by few drops of conc. hydrochloric acid and boiled for five minutes in a boiling water bath. Formation of red colour indicates the presence of flavonoids.

Test for Proteins and Free Amino Acids:

a) Xanthoproteic test:

To 1 ml of extract, 3-4 drops of conc. nitric acid was added. Formation of yellow precipitate indicates the presence of proteins.

b) Million's test:

To 0.5 ml of extract, 2.5 ml of Million's reagent was added. Formation of white precipitate and the precipitate warmed indicates the presence of proteins.

c) Biuret test:

To 0.5 ml of extract, 2.5 ml of diluted Biuret reagent was added. Appearance of purple colour or brick red precipitate showed the presence of proteins and free amino acids.

Test for Quinones:

Sodium hydroxide test:

To 0.5 ml of extract, 1 ml of 10% sodium hydroxide was added. Appearance of blue or green or red colour shows the presence of quinones.

Bio-chemical analysis:

Preliminary Basic and Acidic radical studies[8] :

Preparation of extract:

10g of sample was taken in a 250 ml of clean beaker and 50 ml of distilled water was added to it. Then it was boiled well for about 10 mins. Then it was allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Test for Basic radicals:

1. Test for Potassium:

To a pinch of the *SPC* 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium:

To 2 ml of *SPC* extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate**.**

3. Test for Magnesium:

To 2ml of *SPC* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

To 2ml of *SPC* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium:

Hydrochloric acid was added with a pinch of the *SPC*, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. **Test for Iron (Ferrous):**

The *SPC* extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc:

To 2 ml of the *SPC* extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium:

To the 2m1 of the *SPC* extract sodium hydroxide was added in drops and changes are noted for white precipitate formation.

9. Test for Lead:

To 2 ml of *SPC* extract 2ml of potassium iodide solution was added and noted for yellow colored precipitate**.**

10. Test for Copper:

a. A pinch of *SPC* was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue color appearance.

b. To 2 ml of *SPC* extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury:

To 2m1 of the *SPC* extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

12. Test for Arsenic:

To 2 ml of the *SPC* extract 2ml of sodium hydroxide solution was added and brown or red precipitate formation was noted.

Test for acid radicals:

1. Test for Sulphate:

To 2 ml of the *SPC* extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride:

The *SPC* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. Test for Phosphate:

The *SPC* extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. Test for Carbonate:

The *SPC* extract was treated with conc.Hcl and observed forth appearance of effervescence.

5. Test for Fluoride & Oxalate:

To 2ml of *SPC* extract 2ml of dil.acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate:

To 1 gm of the *SPC*, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for yellowish red color.

HPTLC finger print studies:

HPTLC finger printing was carried out as per the reference[9].

Preparation of spray reagent-vanillin-sulphuric acid reagent:

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add 5ml of cooled concentrated sulphuric acid then Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions:

Instrument : CAMAG (Switzerland).
Sample Applicator : CamagLinomat - IV applicator with N_2 gas flow
Photo documentation System : Digi store - 2 documentation system with Win Cat& video scan software.
Scanner: Camag HPTLC scanner - 3 (030618), Win Cats - IV.
Development Chamber : Camag HPTLC 10X10, 10 X 20 twin trough linear Development chamber.
Quantity applied : 5, 10 µl for extracts and 5 µl for standards
Stationary phase : Aluminium plate pre-coated with silica gel 60(E. Merck)
Plate thickness : 0.2 mm.
Mobile Phase : For Chloroform extract - Toluene: Ethyl acetate (9:1) and ethanol extract - Toluene: Ethyl acetate (1:1).
Scanning wavelength : 254 nm
Laboratory condition : 26 ± 5°C and 53 % relative humidity

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally the plates were derivatized using vanillin-sulphuric acid reagent heated at 105° till colour spots appeared.

Results and Discussion

Table: 2. Organoleptic characters of SPC

Colour	Brown
Odour	Pleasant
Taste	Bitter
Texture	Fine powder
Particle size	Completely pass through sieve no 92

Table: 3.Physicochemical Analyses

S.No	Parameter	Result
1.	pH	6.4
2.	Ash (%)	13.23
3.	Acid Insoluble ash (%)	0.79
4.	Water soluble ash (%)	5.79
5.	Loss on drying (%)	9.26
6.	Solubility	Positive
7.	Action on heat	Negative
8.	Flame test	Negative
9.	Ash test	Negative

Interpretation

The physicochemical analysis of the drug result reveals the pH, Moisture, Solubility, Water soluble ash, Ash and Acid insoluble ash.

1. pH:

pH is a measure of hydrogen ion concentration; it is the measure of the acidic or alkaline nature. 7.0 is a neutral, above 7.0 is an alkaline and below are acidic. The pH of the drug *SiringipaerathiChooranam* is 6.4 which are weak acidic in nature.Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are better absorbed in stomach. [10]

2. Ash:

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug. Total ash value will determine the amount of minerals and earthy materials present in the drug. The total ash value of *SPC* is 13.23% which determines the absence of inorganic content.

3. Acid insoluble ash:

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. Acid insoluable ash is 0.79 for *SPC*.

4. Water soluble ash:

Water-soluble ash is the part of the total ash content, which is soluble in water. It is 5.79 for *SPC.*

5. Moisture (Loss on drying):

The moisture present in the drug was established in loss on drying.The moisture content of the drug reveals the stability and its 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 of *SPC* is 9.26% [11].

Phytochemical analysis:

Table:4. Phytochemicals screening test:

Phytochemicals	Test	Result
1. Alkaloids	Mayer's test	Present
2. Carbohydrates	Molisch's test	Present
3. Glycosides	Modified Borntrager's test	Absent
4. Saponins	Froth test	Absent
5. Phenols	Alcoholic Ferric chloride test	Present
6. Phytosterols	Ferric chloride acetic acid test	Absent
7. Triterpenes	Salkowski's test	Present
8. Flavanoids	Alkaline reagent test	Present
9. Proteins and amino acids	Xanthoproteic test	Present
10. Quinones	Sodium hydroxide test	Present

Interpretation:

Phytochemicals are natural bioactive compounds, found in plants and fibres, which act as a defense system against diseases and more accurately protect against diseases. The phytochemical analysis reveals that the presence of Alkaloids, glycosides, phenol, Triterpenes, Flavanoids and Quinones [12].

1. Alkaloids

Alkaloids possess antispasmodic, analgesic, bactericidal effects. Alkaloids are the active principles producing many essential effects in protecting the body [13].

2. Carbohydrates

Carbohydrates play important role in storage of glucose. Carbohydrates play important role in homeostasis of glucose and fatty acids in liver [14].

3. Phenols

They possess rich Anti-Oxidant property and protect body from oxidative stress. Phenol groups are the essential part of many anti-oxidant compounds. It is an Anaesthetic or pain reliever [15].

5. Phytosterols

Phytosterols are plant sterols, Phytosterols have anti-inflammatory effect, Phytosterols reduce oxidative stress .Various bioactivities of Phenolic compounds are responsible for their chemo preventive properties .Phytosterols have an anti-oxidant property [16].

6. Triterpenes

Suppress the inflammatory response. The Triterpenes are the best immunomodulator and have anti-oxidant property. Anti microbial activity. Anti bacterial agent [17].

7. Flavonoids

It is the most important group of polyphenol compounds in plants. Flavonoids are a group of plants metabolites which provide health benefits through cell signaling pathways and antioxidant effects. Flavonoids can exert their Anti-Oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation. Flavanoids are immunomodulator [18].

8. Protein and amino acids

Proteins and amino acids helps in liver regeneration and energy production. Boosts glutathione production to protect the liver. Increases satiety to promote weight loss and reduce fat accumulation in the liver. Protein is an amalgamation of amino acids .It is an important component of every cell in the body. Body uses protein to build and repair tissues [19].

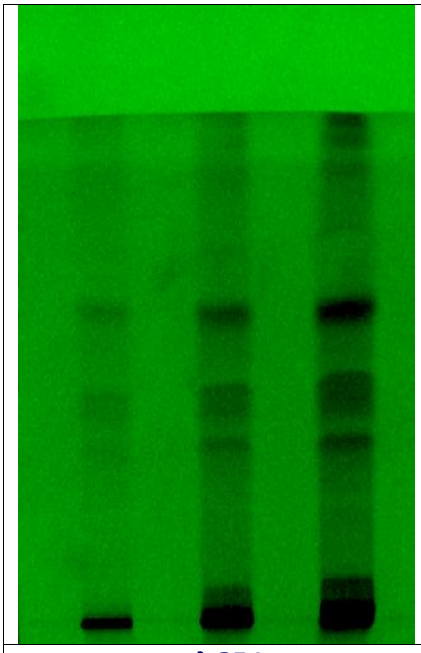
A synergistic effect of all these flavonoids, alkaloids, carbohydrates, phenols, Phytosterols**,** Triterpenes increases the potency of the drug against hepatic damage.

HPTLC analysis of chloroform extract:

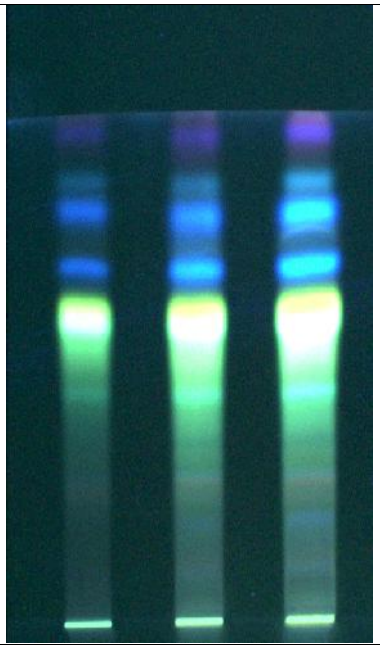
HPTLC analysis:

Chloroform extract was applied in TLC aluminum sheet silica gel 60(E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate (9:1). After development, the plate is allowed to dry in air and examined under UV - 254nm, 366 nm and Visible light (Vanillin - Sulphuric acid).

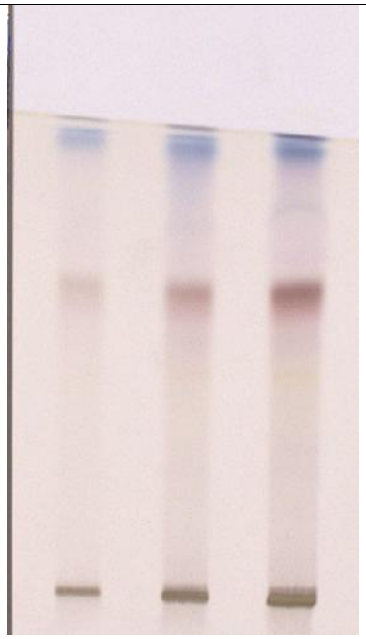
Fig:1. HPTLC Chloroform extracts Photos:



λ 254 nm



λ 366 nm



White light

Table: 5.Rf Values for the chloroform extract

Colour	R_f value(s)	Colour	R_f value(s)	Colour	R_f value(s)
Green	0.08	Green	0.12	Red	0.60
Green	0.35	Pale green	0.46	Violet blue	0.90
Green	0.47	Fluorescent yellow	0.59	Magenta blue	0.95
Green	0.62	Blue	0.70	Magenta blue	0.98
		Blue	0.82		
		Magenta blue	0.88		
		Violet	0.98		

HPTLC finger print analysis for chloroform extract

The finger print chromatogram was recorded at 366 nm. It showed 8 peaks of which peaks at Rf. and were the major peaks and others were moderately smaller peaks.

= 366 nm

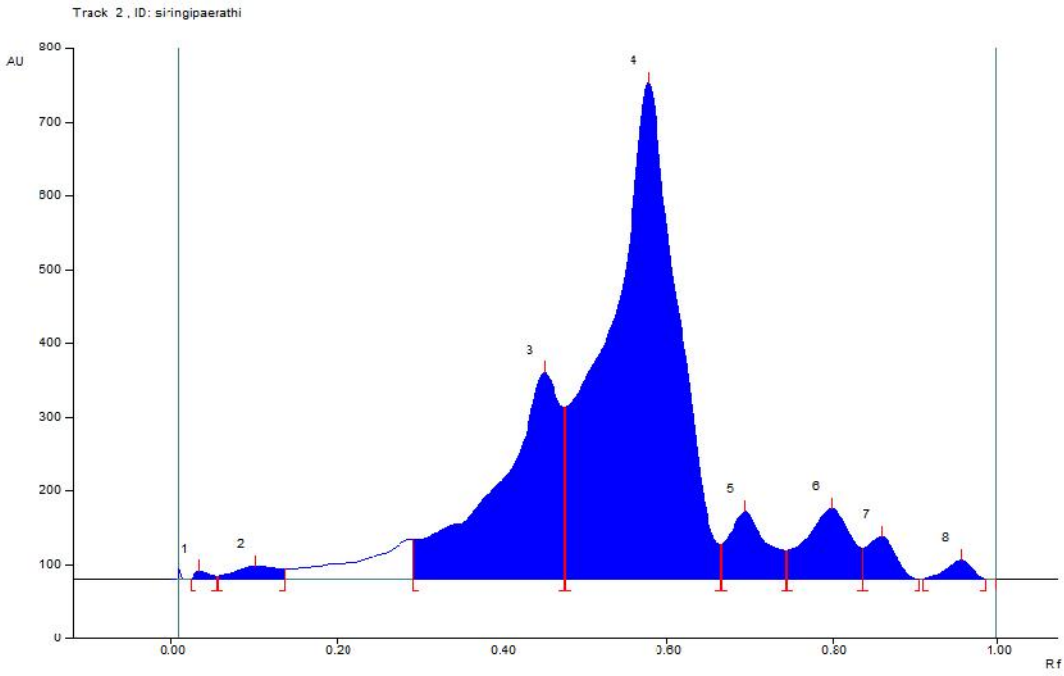


Fig:2. HPTLC finger print for chloroform extract

Table: 6.Chloroform`` extracts - Rf values in HPTLC finger print

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.4 AU	0.03 Rf	10.4 AU	0.84 %	0.06 Rf	3.9 AU	143.0 AU	0.20 %
2	0.06 Rf	4.0 AU	0.10 Rf	16.8 AU	1.35 %	0.14 Rf	13.4 AU	677.5 AU	0.96 %
3	0.29 Rf	52.4 AU	0.45 Rf	279.5 AU	22.47 %	0.48 Rf	31.7 AU	16844.8 AU	23.90 %
4	0.48 Rf	232.2 AU	0.58 Rf	671.0 AU	53.95 %	0.67 Rf	46.7 AU	43492.0 AU	61.71 %
5	0.67 Rf	47.1 AU	0.70 Rf	90.2 AU	7.25 %	0.75 Rf	38.2 AU	3275.3 AU	4.65 %
6	0.75 Rf	38.5 AU	0.80 Rf	94.7 AU	7.61 %	0.84 Rf	41.4 AU	3963.5 AU	5.62 %
7	0.84 Rf	41.5 AU	0.86 Rf	56.8 AU	4.57 %	0.91 Rf	0.0 AU	1507.4 AU	2.14 %
8	0.91 Rf	0.1 AU	0.96 Rf	24.4 AU	1.96 %	0.99 Rf	0.0 AU	573.5 AU	0.81 %

Interpretation:

The quantitative analysis of compounds present in the *SPC* has been performed by HPTLC. The method may be applied to identify the *SPC* from other manufacturing process. It provides the identification of constituents, determination of impurities and quantitative determination of active substance present in the *SPC*. They provide the identification of constituents, determination of impurities and quantitative determination of active substance present in the *SPC*.[20]The Rf value of the *SPC* supports the better standardization of the drug. The present study revealed that *SPC* showed best results in Toluene: Ethyl acetate (9:1). Solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366 nm and visible light range, best results were shown at visible light range. Thus the plate showed different colour phyto constituents of chloroform extract of *SPC*. The bands revealed presence of six green, two blue, and one fluorescent yellow, bands showing the presence of alkaloids, glycosides, phenols, Triterpenes, flavonoids and quinones. The results from HPTLC finger print scanned for chloroform extract of *SPC*. There are thirteen polyvalent phyto constituents and corresponding ascending order of Rf values start from 0.02 to 0.91 in which highest concentrations of the phyto constituents was found to be 53.95% and 22.47 % with its corresponding Rf value were found to be 0.02 and 0.91 respectively.

Table: 7.Results of basic radicals studies

S.No	Parameter	Observation	Result
1	Test for Potassium	Formation of yellow colour precipitate	Positive
2	Test for Calcium	Formation of white colour precipitate	Negative
3	Test For Magnesium	Formation of white colour precipitate	Positive
4	Test For Ammonium	Appearance of brown colour	Negative
5	Test For Sodium	Appearance of intense yellow colour	Negative
6	Test for Iron (Ferrous)	Appearance of blood red colour	Positive
7	Test For Zinc	Formation of white colour precipitate	Positive
8	Test For Aluminium	Characteristic changes	Negative
9	Test For Lead	Formation of yellow colour precipitate	Negative
10	Test for Copper	Formation of blue colour precipitate	Negative
11	Test For Mercury	Formation of yellow colour precipitate	Negative
12	Test for Arsenic	Formation of brownish red precipitate	Negative

Table: 8. Results of acid radical studies

S.No	Parameter	Observation	Result
1	Test for Sulphate	Formation of white precipitate	Positive
2	Test for Chloride	Formation of white precipitate	Negative
3	Test for Phosphate	Formation of yellow precipitate	Negative
4	Test for Carbonate	Formation of effervescence	Negative
5	Test for fluoride &oxalate	Formation of cloudy appearance	Negative
6	Test For Nitrate	Characteristic changes	Negative

Interpretation of Basic and acid radicals:

The sample contains Potassium, Magnesium, Iron, Phosphorus, and Zinc. These trace quantities of minerals along may play an important role in the functioning of various enzymes in biological systems and have immunomodulatory functions and thus influence the susceptibility to the course and the outcome of a variety of viral infections.

1. Potassium:

Potassium is important for maintaining the integrity of cell membranes and functions as a vital electrolyte. Potassium is absorbed through the small intestine; severe lack of potassium can disrupt liver function. If potassium level falls below 30% to 40% are prone to liver disease [21].

2. Magnesium:

It enhances immune system. Depletion of Magnesium level leads to Cirrhosis, Fatty liver syndrome, Thus magnesium is essential for liver to prevent Liver diseases.[22]

3. Iron:

Needed for energy metabolism. It is crucial for oxygen transport, energy production, and cellular growth and proliferation.

4. Zinc

The liver plays a central role in zinc homeostasis. Zinc is a trace mineral that is essential to the normal functioning of the immune system. Zinc is essential for many metabolic and enzymatic functions. Liver as a powerful antioxidant. Deficiency of zinc leads to malabsorption syndrome , Cirrhosis of liver.[23]

The basic radical test shows the presence of **Potassium, Magnesium, Iron and Zinc** absence of heavy metals such as lead, arsenic and mercury.

5. Sulphate:

The acidic radicals test shows the presence of sulphate.

Conclusion

The present study shows the preliminary screening of physico-chemical, phyto-chemical, acid and basic radicals screening and HPTLC analysis shows the presence of Alkaloid, Carbohydrates, Triterpenes, Flavanoids, Proteins, Amino acids, Phenols, Quinones, Potassium, Magnesium, Iron, Zinc and Sulphate will provide footprints for further clinical studies.

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