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PHYSICOCHEMICAL EVALUATION AND STANDARDIZATION OF THE SIDDHA FORMULATION *SAMBIRANI POO KULIGAI* IN ACCORDANCE WITH AYUSH REGULATION

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ABSTRACT
Siddha formulations becomes popular cure as an ailment for treating several diseases throughout the globe, as a complementary and alternate medicine. Physicochemical evaluation of the preparation plays vital role in establishing the monograph of the formulation, as it becomes the documentary evidence to substantiate the standards of the preparation. It renders the useful information like genuinity, stability, selective characteristic feature and nature of the compound's present in the drug. Siddha also have standardized protocols for purification and detoxification of certain phytocomponents used in specific formulations. As this will greatly reduce the toxicity and also enhance the therapeutic efficacy of the formulation. Through systematic standardization a formulator can profile the preparation with respect to the following (i) Physiochemical parameters (ii) Category of phytocomponents (iii) Nature of individual chemical component (iv) Structural and functional group analysis (v) Correlation of mechanism with respect to the functional group present in bioactive phytocomponents (vi) Drug stability (vii) Pharmacokinetic profiling (viii) Receptors on which the drug acts and ix) Chances of possible interaction. The main aim of the present investigation is to systematically purify, standardize and to carry out the Invitro antioxidant evaluation of the novel siddha preparation *Sambirani Poo Kuligai* (SPK) as per AYUSH regulations. The organoleptic character of the drug SPK justifies the purity of the formulation. The results obtained from the physicochemical evaluation revels that the total ash value of SPK was 79.62 %, loss on drying value is 13.62% in which water soluble ash value is 16.75% and acid insoluble ash was found to be 0.225% respectively. Phytochemical analysis reveals the presence of phytocomponents such as alkaloids, carbohydrates, glycosides, phytosterol, triterpenes, proteins and amino acids. Biochemical investigation of the test drug SPK reveals the presence of the following radicals such as calcium, magnesium, sodium and phosphate. HPTLC analysis of the SPK reveals the presence of 11 phytocomponents. In conclusion the trial drug SPK confirms the regulatory requirement and also possess significant phytocomponents such as phytosterols which contributes to the beneficial effect on polycystic ovarian syndrome (PCOS).

KEYWORDS: Siddha, Standardization, Physicochemical, *Sambirani Poo Kuligai*, AYUSH regulations, HPTLC, PCOS.

1. INTRODUCTION
PCOS is characterized by small arrested antral follicle formation in the development process. In PCOS estrogen level decreases, however, the progesterone level increases and LH/FSH ratio becomes three times of the normal level. Where in the plant phytosterol which mimics the action of these functional hormones may render valuable therapeutic efficacy in management of such diseases.[1]

Phytosterols (plant sterols) are secondary plant metabolites which structural and biological counterparts of cholesterol. Plant sterols are responsible for permeability and fluidity of cell membranes. Isolated phytosterols have modulatory action that lead to

prevention of visceral fat obesity and improved hyperglycemia, hyperlipidemia and insulin resistance in metabolic syndrome.[2] In this context researchers tried to isolate various phytosterols and purify the active compounds from fraction of Aloe vera gel that ameliorated hyperglycaemia in diabetic C57BL/ KSLepr db (db/db) mice.[3], where the mice lack functional leptin receptor.[4] In addition these, β-sitosterol was shown to have glucose lowering effect in type 2 diabetes patients.[5] Recent study demonstrated that oral administration of anti-diabetic phytosterols isolated from Aloe vera, caused a reduction in pro-inflammatory cytokines such as monocyte chemotactic protein-1 (MCP-1) and serum adiponectin level, which are risk factors involved in cardiovascular disorders.[6]

Siddha system of medicine pioneering in emphasize the biological activity of the various phytocomponents with respect to the etiology and pathophysiology of various dread full disease emerging in humans and animals. It is evident that there are some medicinal plants used in siddha has potency of acting as an anesthetics, analgesics, anti-microbial, immune modulators, Hepato, neuro and nephron protectant. But the most pathetic scenario is most of these potential herbs are extinct and not be used currently. Siddha formulations offers tremendous advantage in clinical practice against metabolic and lifestyle disorders including neuro degenerative diseases. Often investigation on siddha preparations attempted on reverse pharmacology basis. Hence nearly 80% of the formulation already have proven track record clinically and now several investigation are being made on its preclinical aspect. Hence the main aim of the present investigation is to systematically purify, standardize and to carry out the phytochemical investigation of the novel siddha preparation *Sambirani Poo Kuligai*.

2. MATERIALS AND METHODS

2.1. Source of raw drugs

The Required raw materials were procured from a well reputed indigenous drug shop from Parrys corner, Kanda Samy Temple, Chennai, Tamil Nadu, India. All raw drugs were authenticated by the Pharmacognosist, SCRI Chennai., Tamil Nadu, India. The test drug *Sambirani poo kuligai* was prepared as per Agasthiar Paripuranam 400.

2.2. Ingredients

The siddha formulation *Sambirani poo kuligai* Comprises of the following ingredients

Sambirani [Styrax benzoin]	- 250 g
Korosanai [Felbovinum purifactum]	- 6 g
Kirambu [Syzygium aromaticum]	- 20 g
Vetrilai [Piper betel]	- 50 ml

2.3. Purification of Raw Drug

***Styrax Benzoin*[7]:** The gums were purified by removing the sand, dust and odd particles.

***Fel bovinum purifactum*:** The unwanted debris substances were removed.

***Syzygium aromaticum*[8]:** The flower buds were removed and fried slightly.

***Piper betle*:** The stalk and the middle vein were removed.

2.4. Method of preparing *Sambirani poo kuligai*[9]

The purified *Styrax benzoin* was powdered well and was placed in a small pot. Then a paper was pasted on the inner surface of the big pot. The big pot was placed over the small pot and their mouths oppose each other. The gap between their mouths were covered by a seven layered muddy wet cloth and they allowed to dry. Then it was subjected to sublimation process for 12 hours (*4 samam*). After finishing sublimation process let the pot undisturbed to give away heat. Followed by this the seal were opened and the sublimed product was scrapped and collected.

2.4.1. Kuligai Process

Syzygium aromaticum and *Felbovinum* are powdered well and sieved through a white cloth. Finely powdered *Syzygium aromaticum* powder and *Felbovinum* powder are added along with the sublimate. Then all these substances are grounded well with *Piper betle* leaf juice for 48 minutes [2 *Nazhigai*]. The paste was made into pills in the size of seeds of *Abrus precatorius [Kundri size]* which was equivalent to 130 mg, dried in the shade and bottled up.

2.5. Organoleptic Investigation

The macroscopical evaluation of SPK was performed as per the methods of Khandelwal.[10] Organoleptic characters such as color and texture were studied.

2.6. Physico-chemical analysis[11]

2.6.1 Determination of pH

About 5 g of test sample will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the subjected to pH evaluation using pH meter.

2.6.2. Percentage Loss on Drying

10gm of test sample was accurately weighed in evaporating dish and was air dried at 105^{o}C for 5 hours and then weighed.

2.6.3. Determination of Total Ash

3 g of test sample was accurately weighed in silica dish and incinerated at the furnace a temperature 400ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

2.5.3. Determination of Acid Insoluble Ash

About 0.5gm of the ash obtained by total ash test boiled with 25 ml of dilute hydrochloric acid for 6mins. Then

the insoluble matter collected in crucible and was washed with hot water and ignited to constant weight. Percentage of acid insoluble ash calculated with reference to the weight of air-dried ash.

2.5.4. Tablet Disintegration test

Each SPK sample was placed in six tubes individually present in the basket of disintegration apparatus. The apparatus was handled using water as the immersion fluid maintained at 35-39 °C. The basket was lifted from the fluid and the state of the tablet was observed at the end of the 30 min. The six tablets disintegrated completely in about 45 minutes.

2.5.5. Weight variation test[12]

Weight variation was carried out to make certain that, each of the tablets contain the appropriate amount of drug. The test was carried out by weighing the 20 tablets separately using analytical balance, then manipulating the average weight, and comparing the individual tablet weights to the average.

The percentage of weight variation was calculated by using the following formula.

$$\% \text{ of weight variation} = \frac{\text{Individual weight} - \text{Average weight}}{\text{Average weight.}} \times 100$$

2.6. Preliminary phytochemical Evaluation[13]

5g of SPK sample was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water and the extract was subjected to class of preliminary phytochemical screening of the following components.

Test for Alkaloid- Mayer's reagent

To the test drug about 2ml of Mayer's reagent was added and was observed for the presence of alkaloids. Appearance of dull white precipitate indicates the presence of alkaloids.

Test for flavonoid

To 0.1ml of 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 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 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 Triterepnoids

To the test solution 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml) at the side of the test tube. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Test for Steroids - Salkowski test

To the test solution 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.

Test for Carbohydrates - Benedict's test

To 0.5 ml of test drug 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.

Test – Phenol- Lead acetate test

The test sample is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Test for tannins

About 0.5ml of test sample is boiled in 20 mL of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. The 0.1% FeCl3 is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of tannins

Test for Saponins

The test drugs were shaken with water vigorously for 10 mins, copious lather formation indicates the presence of saponins.

Test for Proteins (Biuret Test)

Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple colour indicates the presence of proteins and free amino acids.

Test of Coumarins

1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for Quinones

The test samples were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of Quinones.

2.7. Biochemical analysis of Basic and acidic radicals

Biochemical analysis of the trial drug SPK was subjected for qualitative analyses of cations and anions as per the standard procedure described.[14,15]

2.8. Thin layer chromatography (TLC)

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 liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Toulene: Ethyl Acetate: Acetic Acid (1.5:1:0.5) 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.[16]

2.9. High Performance Thin layer chromatography (HPTLC)

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.[17]

2.9.1. 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.

2.9.2. 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.

3. RESULTS

3.1. Physico-chemical Evaluation and standardization of SPK

The organoleptic characters of the drug *Sambirani Poo Kuligai* showed that the colour of the *Kuligai* is brown in color since prepared from dry herbs, and on sight they are hard, solid in consistency, spherical in shape. The results were tabulated in Table 01.

Table. No.1. Organoleptic characters of *Sambirani Poo Kuligai.*

Sl.no	Parameters	Results
1	Colour	Brown
2	Odour	Characteristic odour
3	State of matter	Solid
4	Consistency	Hard
5	Shape	Spherical

3.2. Physico-chemical Evaluation and standardization of SPK

The results obtained from the physicochemical evaluation revels that the total ash value of SPK was found to 79.62 %. Similarly loss on drying value at 105^{o}C was fond to be 13.62% in which water soluble ash value is 16.75% and acid insoluble ash was found to be 0.225% respectively. The average weight of 20 SPK tablets was found to be 0.132 g and the disintegration time was found to be 25 mins. pH plays a vital role in drug disintegration and absorption further the pH of the formulation SPK was found to be 5.2. The results were tabulated in Table 02.

Table 2: Physicochemical Evaluation of *SPK.*

S.No	Parameter	Mean
1.	Loss on drying at 105^{0}C	13.62 (%)
2.	Total Ash	79.62 (%)
3.	Water soluble ash	16.75 (%)
4.	Acid insoluble ash	0.225 (%)
5.	Disintegration Test	25 min
6.	Average weight of 20 tablets	0.132 g
7.	pH	5.2

3.3. Result Analysis of phytochemical analysis of *SPK*

Phytochemicals are significantly the most important components majorly responsible for the biological activity of the formulation. The results obtained from the phytochemical analysis reveals the presence of phytocomponents such as alkaloids, carbohydrates, glycosides, phytosterol, triterpenes, proteins and amino acids. The results were tabulated in Table 03.

Table No: 3. Results of phytochemical analysis of *SPK.*

Phytochemicals	Observation
1. Alkaloids	+
2. Carbohydrates	+
3. Reducing sugars	-
4.Glycosides	+
5. Cardiac glycosides	-
6. Saponins	-
7. Tannins	-
8. Phenols	-
9. Phytosterols	+
`10. Diterpenes	-
11. Triterpenes	+
12. Flavanoids	-
13. Proteins and amino acid	+
14. Quinones	-

+ Presence and - Absence

3.4. Result Analysis of acid and basic radical analysis of *SPK*

Biochemical investigation of the test drug SPK reveals the presence of the following radicals such as calcium, magnesium, sodium and phosphate. The results were tabulated in Table 04.

Table No: 4. Results of acid and basic radical analysis of *SPK.*

Parameter	Result
Test for Potassium	-ve
Test for Calcium	+ve
Test For Magnesium	+ve
Test For Ammonium	-ve
Test For Sodium	+ve
Test for Iron (Ferrous)	-ve
Test For Zinc	-ve
Test For Aluminium	-ve
Test For Lead	-ve
Test for Copper	-ve
Test For Mercury	-ve
Test for Arsenic	-ve
Test for Sulphate	-ve
Test for Chloride	-ve
Test for Phosphate	+ve
Test for Carbonate	-ve
Test for fluoride &oxalate	-ve
Test For Nitrate	-ve

3.6. TLC Result Analysis of *SPK*

TLC chromatogram of the sample SPK indicates the presence of colored florescent (pink, green, blue) compounds under 254 and 366 nm which may belongs to category of alkaloids, glycosides, terpenoids etc. The chromatogram represented in figure 1.

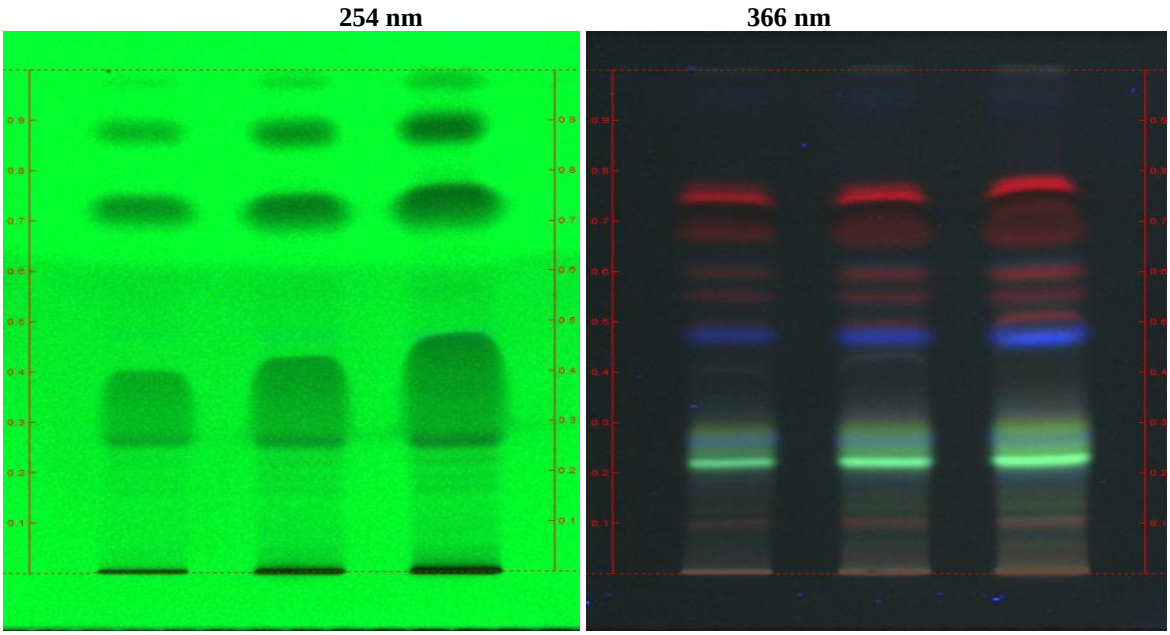

Figure 1: TLC finger printing of Sample SPK

3.7. HPTLC Result Analysis of *SPK*

HPTLC finger printing analysis of the sample SPK reveals the presence of eleven prominent peaks corresponds to presence of eleven versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.07 to 0.73. Further the peak 3 occupies the major percentage of area of 43.16 % which denotes the abundant existence of such compound.

Followed by this peak 4 and 11 occupies the percentage area of 19.71 and 13.86%. The results were tabulated in Table 5 and chromatogram represented at figure 2.

Table No 5: Results of Peak Table of HPTLC analysis of *SPK.*

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.1 AU	0.10 Rf	36.1 AU	2.84 %	0.12 Rf	6.4 AU	581.1 AU	1.75 %
2	0.14 Rf	10.7 AU	0.17 Rf	29.0 AU	2.29 %	0.18 Rf	23.3 AU	612.7 AU	1.84 %
3	0.18 Rf	23.4 AU	0.22 Rf	547.4 AU	43.16 %	0.24 Rf	18.3 AU	11664.1 AU	35.10 %
4	0.24 Rf	218.6 AU	0.26 Rf	250.0 AU	19.71 %	0.33 Rf	32.8 AU	10511.6 AU	31.63 %
5	0.33 Rf	35.2 AU	0.34 Rf	47.3 AU	3.73 %	0.38 Rf	2.9 AU	530.9 AU	1.60 %
6	0.38 Rf	2.8 AU	0.41 Rf	14.8 AU	1.17 %	0.42 Rf	7.8 AU	267.6 AU	0.81 %
7	0.44 Rf	7.1 AU	0.48 Rf	50.0 AU	3.94 %	0.51 Rf	17.3 AU	1577.3 AU	4.75 %
8	0.53 Rf	10.2 AU	0.56 Rf	33.2 AU	2.62 %	0.58 Rf	15.7 AU	876.3 AU	2.64 %
9	0.58 Rf	15.8 AU	0.60 Rf	46.1 AU	3.63 %	0.65 Rf	11.6 AU	1543.4 AU	4.64 %
10	0.65 Rf	11.6 AU	0.68 Rf	38.7 AU	3.05 %	0.73 Rf	0.2 AU	1299.0 AU	3.91 %
11	0.73 Rf	0.2 AU	0.75 Rf	175.8 AU	13.86 %	0.80 Rf	10.6 AU	3770.4 AU	11.34 %

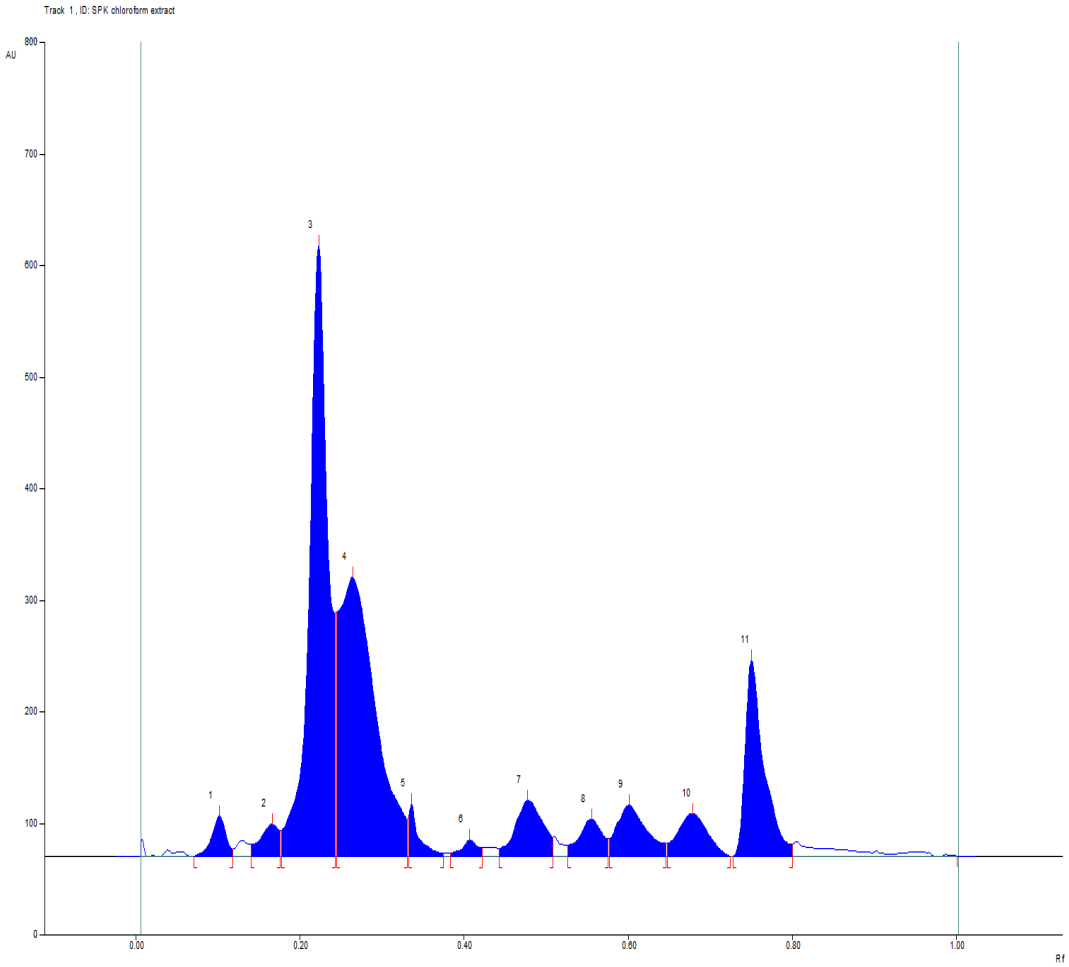

Figure 2: HPTLC finger printing of Sample SPK.

4. DISCUSSION

Standardization is one such regulatory process which ensures the quality, safety and genuinity of the prepared formulation. Further the standards of the prepared formulations have been rightly identified by some parameters which include ash value, extract value, moisture content, total foreign materials, pesticide residue etc. The most of drug contain definite chemical

constituents to which their pharmacological and Biological activity depended. The results obtained from the physicochemical evaluation revels that the total ash value of SPK was found to 79.62 %. Similarly loss on drying value at 105^{o}C was fond to be 13.62% in which water soluble ash value is 16.75% and acid insoluble ash was found to be 0.225% respectively. The average weight of 20 SPK tablets was found to be 0.132 g and the disintegration time was found to be 25 mins. pH plays a vital role in drug disintegration and absorption further the pH of the formulation SPK was found to be 5.2. Biochemical investigation of the test drug SPK reveals the presence of radicals such as calcium, magnesium, sodium and phosphate, These radicals make contribute to basic elemental need of the body to counteracts and stabilize the physiology by specific enzyme and hormone synthesis.

Qualitative chemical test used to identify drug quality and purity. The identification, isolation and purification of active chemical constituents is depends chemical methods of evaluation. Preliminary phytochemical investigation is also a part of chemical evaluation. Some Qualitative chemical test for chemical evaluation crude drug are Saponification value and acid value etc [18-20]. The results obtained from the phytochemical analysis reveals the presence of phytocomponents such as alkaloids, carbohydrates, glycosides, phytosterol, triterpenes, proteins and amino acids.

The presence of sterols, phenols, alkaloids, polyphenols, flavonoids and lignin have attributed to various efficacies.[21] Recently, several researchers have tried to check in the efficacy of phyto-components from AVG in the content of its hypoglycemic and anti-inflammatory potential.[22]

Phytochemical evaluation of the formulation SPK reveals the presence of phytosterol which may be responsible for its action against PCOS. TLC and HPTLC chromatogram of the sample SPK indicates the presence of eleven colored florescent (pink, green, blue) compounds which may belongs to category of alkaloids, glycosides, terpenoids etc. These phytocompounds also has promised track records on its mechanism against PCOS.

5. CONCLUSION

From the result obtained from the present investigation it was concluded that the formulation SPK possess significant biologically active phyto therapeutics and may act therapeutically in treating several stress and metabolic disorder's. Further present investigation had generated an evidence based data with respect to purity, standards and phytochemical nature of the formulation SPK

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