UNIQUE JOURNAL OF AYURVEDIC AND HERBAL MEDICINES
Research Article

ELEMENTAL ANALYSIS OF SWARASA CHURNA BY SEM-ZAF METHOD

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Received 01-06-2013; Revised 19-06-2013; Accepted 03-07-2013

ABSTRACT

Eclipta alba (L) Hussk. (Fam. Asteraceae) is very much useful as, its whole plant juice extract(swarasa) dried in shade is called as Swarasa Churna in Ayurvedic system of medicine.It can be used in various compositions of medicines as Asava, Arissa and vati. It is useful as Vedanasthapaka(resist the pain), shotahara(anti-inflammatory), Vranashodhaka & ropaka(healing the wounds), Savarnikarana (Tvachya), Kesharanjana+Vardhaka(to grow hair withgood color),Deepana(appetizer),Rasayana(immunumodulater), Shulaprashamana(analgesic), Raktaprasadaka+ Vardhaka(improve the quality+ quantity of blood), Swedajanana(excretory action), Balya(tonic) and Kustahara(protectsskin). It is useful as external and internal purposes. Standardization and quality of the drug are the key factors in regulating the therapeutic efficacy of herbal drugs. The present study included Physico-chemical study, safety evaluation measures, such as heavy metal analysis, microbial load, aflotoxins contamination including Instrumental analysis of herbal based drugs provides data concerning the qualitative and quantitative aspects of bioactive molecules, which are responsible for therapeutic action and is widely accepted in the quality assessment of herbal drugs. However, such work related to traditional herbal medicines is deficit now a day. In the present study morphological, physico-chemical parameters and HPTLC finger print studies of *Eclipta alba* have been carried out and the results provide referential information for the quality control and standardization.
Concentrations of Trace elements in Swarasachurna were studied using SEM-ZAF (5-iterations) quantitative method. The aim of this study is to determine qualitatively and quantitatively trace elements in Swaraschurna and their medicinal roles in the human body. The whole plant was analyzed for their trace element contents. The plant samples were found to contain essential trace elements such as Carbon(C), Oxygen(O), Sodium(Na), Magnesium(Mg), Aluminium(Al), Silicon(Si), Phosphorous(P), Sulphur(S), Chlorine(Cl), Potassium(K) and Calcium(Ca) which are well known for their important roles in herbal drugs. Most of the medicinal plants were found to be rich in one or more of the essential elements under study. The elemental concentrations of different element in Swarasachurna and their biological effects are discussed.

Keywords: Swarasachurna, SEM-ZAF, trace elements, medicinal plants, herbal drugs.

INTRODUCTION

SEM-ZAF among the various analytical techniques used for the elemental analysis, is highly quantified for the identification and quantification of different elements in medicinal plants for various biological and environmental importance. Elemental research has definitely been part of this explosion of scientific knowledge. Impressive developments in this field of mineral elements have taken place in the chemical, biochemical and immunological areas of research. Deficiency of trace elements in human subjects can occur under the most practical dietary conditions and in many diseased statuses. In recent years, Scientist and nationalists have started believing in the therapeutic role of metals in human health. Trace elements play both curative and preventive role in combating diseases. There is a vast scope to exploit the preventive medicinal aspects of various trace elements. Medicinal plants play the most important role in traditional medicine. Mineral elements through usually form a small portion of total combination of most plant materials and of total body weight, it was nevertheless of treat physiological importance particularly in body metabolism.

A wide range of chemical compounds including coumestans, alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes and their glycosides have been isolated from this species. The plant is commonly used in hair oil all over India for healthy black and long hair. The fresh juice of leaves is used for increasing appetite, improving digestion and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma and popularly

used to enhance memory and learning[1]. The plant has a reputation as an anti ageing agent in Ayurveda. *Eclipta alba* is used as a general tonic for debility. Externally it is used for inflammation, minor cuts and burns and the fresh leaf-juice is considered very effective in stopping bleeding. Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections. *Eclipta alba* is a source of coumestan-type compounds used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis[2]. *Eclipta alba* is widely used in India as a cholagogne and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder[3]. Coumestan-type compounds, wedelolactone and dimethyl wedelolactone have been isolated as the main active principles of *Eclipta alba*, both constituents exhibiting antihepatotoxic activity[4,5].

Swarasachurna contains the source of chemicals of Bringaraja having immense medicinal and pharmaceutical importance and it is widely used as hepatoprotective, spasmogenic, hypotensive, antileprotic, healing of fractures, promoter for blackening and growth of hair, antiviral and avicidal, antibacterial, analgesics, antioxidant, antimyotoxic, antihemorrhagic, anticancer and antihepatotoxic etc[6,7]. It is also used for the treatment of sleepada, granthi, vrana, shirroga, Yakritpleehavridhi, Arshas, Kusta, Raktavikara, Charmaroga, Swarsakasa, Sleela-pitta and Mutraroga[8,9].

The surface area, PHZPC and density of the Bringaraja Swarasa churna sample were analyzed. The ICP-OES analysis revealed that the main components present in it. In the present study work carried out to evaluate the trace element content in Swaras churna through SEM-ZAF.

MATERIALS AND METHODS

Plant collection

Fresh plants parts were collected from Local market of Hyderabad in Andhra Pradesh State of India. After confirmation of its botanical identity with the help of botanist, the whole plant was crushed and the juice is extracted and shade dried and stored in air tight bottles which is further carried for analysis.

Morphological Studies: Routine procedures were followed for external morphology of the drug.

Roots: Well developed, a number of secondary branches arise from main root, upto about 7mm in diameter, cylindrical, grayish.Stem: Herbaceous, branched, occasionally rooting at nodes, cylindrical or flat, rough due to oppressed white hairs, node distinct, greenish, occasionally brownish.

Leaf: Opposite, sessile to sub sessile, 2.2-8.5cm long, 1.2-2.3 cm wide, usually oblong, lanceolate sub entire, sub acute or acute, strigose with oppressed hairs on both surfaces.

Flower: Solitary or together on unequal axillary peduncles, involuceral bracts about 8, ovate, obtuse or acute, herbaceous, strigose with oppressed hairs, ray flowers ligulate, ligule small, spreading, scarcely as long as bracts not toothed, white, disc flowers.

Physico-chemical Parameters: Physico-chemical parameters such as ash and extractive values were determined according to the methods described in Unani Pharmacopoeia of India, 2008[1] and GBC-908 AA model Atomic Absorption Spectrophotometer (AAS) was used to determine the concentration of heavy metals. Microbial load and aflatoxins contamination were analyzed as per the methods described in WHO guidelines (Anonymous, 1998).

Apparatus

HPTLC system composed of an Automatic TLC applicator, Basic Marathon Auto sampler, Densitometer CD 60 of DESAGA Sarstedt Gruppe system and UV Vis Cabinet for recognition of spots. The chromatographic and the integrated data were recorded using computer based software DESAGA ProQuant 1.6 Version.

Preparation of Extract of the drug sample for HPTLC:

The whole plant was crushed into mortar and pestle to produce juice which is extracted and shade dried. Five grams Swarasa churna was macerated in 100 ml of methanol in a Stoppard conical flask and was kept for 2 hours shaking gently in regular intervals. Later the contents were filtered through Whattmann No. 41 filter paper and the filtrate was evaporated to get 20 ml of solution. The solution thus obtained was used as sample for the determination of components.

Development of HPTLC technique:

Development and determination of the solvent system:

Sample Applied: Sample drug solution about 10µl.

Solvent system: Toluene: Ethyl acetate: Methanol (7: 2: 1)

Migration distance: 95mm

Scanning wavelength: 366nm

The sample was spotted with the help of Automatic TLC applicator system of the DESAGA Sarstedt Gruppe on Precoated Aluminum Sheets of Silica Gel 60 F_{254} (Merck). After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated above is selected in its proportional ratio and developed in the Twin-through glass TLC chamber to the maximum height of the plate so that the components are separated on the polar phase of silica gel and mobile phase of solvent system.

After developing, the TLC plate was dried completely and detected with the suitable detection system like UV Cabinet system for detection of spots at 366nm. Further it is scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 366nm appearing a maximum number of components. A corresponding densitogram was obtained in which peaks appeared for the spots corresponding to R_f values of each component as shown in the table 2a - 2b for bringaraja and Swarna churna.

RESULTS AND DISCUSSION

using SEM and ZAF method showed in table 1. The SEM EDX spectra of the crude extract of the Swarasa churna showed in figure 1. Carbon (C), Oxygen (O), Sodium (Na), Magnesium (Mg), Aluminium (Al), Silicon (Si), Phosphorous (P), Sulphur (S), Chlorine (Cl), Potassium (K) and Calcium(Ca) are used as the standards. In all these elements, Carbon and oxygen presented as high concentration while Si, Cl, K and Ca presented as moderate amount. But Mg, Na, Al, S are presented only in trace quantities. The trace elements play a vital role in the medical value of plants as curative and

preventive agents in combating disease, nutritive and catalytic disorders. The concentration of mineral and trace elements in plants is so meager that their importance was ignored for a long time. There is a vast scope to explore the preventive medicinal aspects of various trace elements. Health treatment is based on medicinal plants recommended as nutritional supplements for the treatment of everyday problems. There is resurgence of interest in herbal medicine for the treatment of main ailments. The main advantage of herbal medicines is that naturally occurring products without any side effects. Trace elements play a major role in health and diseases. In the building up and restoration phenomenon, it was observed that during the last 20 years remarkable progress has occurred in this area of health and science [10].

The elemental concentrations were determined to verify the biological role of trace elements in antiparasitic medicinal plants. The variation in elemental concentration is mainly attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants are cultivated. Other factors responsible for variation in elemental content are preferential absorbability of the plant, use of fertilizers, irrigation water and climatologically conditions [11].

The elements Fe, K, Mg, Na, Ca, Co, Mn, Zn and Cu have been classified as essential elements, Ni, Cr are possibly essential while Cd, Pb and Li are non essential elements for the human body. Among the various elements detected in different medicinal plants used in the treatment of different diseases, Calcium and potassium are found in major concentrations in these plants. It is known that potassium is necessary for muscle contraction (especially cardiac fiber), for the synthesis of some proteins and as an enzymic cofactor. Since the minerals are essential part of nucleoproteins metalloproteins, chromoproteins, lipoproteins, etc., the determination of minerals is important in the case of a disease [12]. We hope that our results will provide a starting point for discovering new compounds with better activity than agents currently available. Calcium is needed in the development of bone and teeth and it regulate heart rhythm, helps in normal blood clothing, maintain proper nerve and muscle functions and lower blood pressure [13]. Kanneez et al.,[14] stated that magnesium (Mg) in plant lowers the cholesterol level. Magnesium (Mg) plays an important role in regulating muscular activity of heart rhythm and also Magnesium is important cofactor of convert blood glucose into energy [15].

The element potassium is an extremely important element in human body. Potassium is essential for the transport of nutrients inside the cell. Without potassium, nutrients could not able enter into the cell that leads cell death. Silicon is also another important element to prevent the hardening of veins and arteries. Chloride works with sodium and potassium carry an electrical charge when dissolved body fluids and to regulate the pH in the body. Chloride is also important for digest the food properly and absorb many elements that what we need to survive. Toxic elements such as Cd, Hg and Sb were not detected in sample. From pharmacological and toxicological points of view the concentrations of these elements present in the sample analyzed are found to be very low to cause any kind of effect.

Based on the conclusion, the data obtained in the present work will be helpful in the synthesis of new ayurvedic drugs which can be used for the control of various diseases. The results of the present research work will be helpful to ayurvedic clinicians and scientists who would like to pursue further research in the areas of Ayurvedic and alternative medicines. Trace elements present in Swarasa churna has a lot of biological activities to prevent organs from diseases. In future, Swarasa churna could be used as good pharmaceutical and therapeutic agents.

Physico-Chemical Characters

The Physico-Chemical Parameters data expressed here as mean values of three readings. The total ash found to be 30.67-31.32 %; whereas Alcohol soluble matter in terms of % w/w was found to be 4.55-4.78 and water soluble matter as 11.21-11.67. Loss of weight on drying at 105^0C found to be 4.82-4.83 %. P^H of the 1% solution measured as 6.14 and that of 10% solution as 5.95 & other results are depicted in table 1.

Multi-wavelength Scan

When multi-wavelength scan was carried out from range of 250nm to 400nm in the steps of 4nm from which it shows that at UV 366nm wavelength more number of peaks are obtained and also found better intense peaks.

HPTLC ANALYSIS

It is evident from the table-2a and 2b that there are thirteen and ten spots with Rf values respectively at 0.11 (Blue), 0.21 (Blue), 0.33 (Blue), 0.37 (Blue), 0.51 (Blue), 0.57 (Pink), 0.64 (Brown), 0.72 (Red), 0.80 (Blue), 0.84 (Red), 0.89 (Red), 0.95 (yellowish orange) & 0.98 (Violet) as shown in figure 2a and 2b for Bringaraja and Swarna churna respectively indicating the occurrence of at least thirteen different components in the extract of the drug. And the densitogram of Bringaraja and Swarna churna showing peaks of the corresponding spots obtained on the chromatogram is shown in figure 3a and 3b respectively. Thus developed chromatogram will be specific with selected solvent system and constant Rf values, and serve the better tool for quality control purpose and standardization of the drug.

For safety evaluation studies of the drug, estimation of heavy metals such as cadmium, and arsenic were carried out and found to be absent except the presence of lead & mercury which was within the permissible limits as given in table 3. Similarly, Aflatoxins were analyzed and found to be absent as given in the table 4. Microbial load were analyzed and found to be within the permissible limits as given in the table 5, inferring the drug to be safe and non toxic.

Chemical compounds, some of which are having therapeutic activities, are species specific and vary from species to species. These compounds can be visualized by developing chromatograms. Characteristic TLC/HPTLC finger printing of a particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species, Thus the present study will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization and quality

CONCLUSION

In addition to organoleptic parameters, chromatographic finger printing of herbal medicines will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of Swarasa churna i.e. shade dried juice extract of whole plant providing standard fingerprinting profile and can be used as a reference for the identification and quality control of the drug.

ACKNOWLEDGEMENT

We, the authors are thankful to our Principal, Dr. B.R.K.R. Govt. Ayurvedic College for providing encouragement and constant support.

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Table 1: The Physico-chemical parameter of the drug

Parameters	Results
Total ash (% w/w)	30.67-31.32
Acid insol. ash (% w/w)	15.38-15.59
Alcohol sol. Matter (%w/w)	4.55-4.58
Water sol. matter (% w/w)	11.21-11.67
PH of 1% Aqueous Solution PH of 10% Aqueous Solution	6.14 5.95
Loss of weight on drying at 1050C	4.82-4.83

Table 2a: Peak list of area and R_f Values of the component for Bringaraja

Peak no.	Name	Migration distance(mm)	Area	Area percentage (%)	Height (mm)	R_f Value
1	Component 1	19.0	750.10	12.6	199.48	0.11
2	Component 2	27.1	33.19	0.6	16.02	0.21
3	Component 3	36.7	267.87	4.5	98.78	0.33
4	Component 4	39.9	20.69	0.3	9.68	0.37
5	Component 5	51.3	31.77	0.5	10.47	0.51

6	Component 6	56.3	12.58	0.2	5.96	0.57
7	Component 7	62.0	160.03	2.7	54.18	0.64
8	Component 8	68.6	68.06	1.1	24.33	0.72
9	Component 9	75.0	769.40	12.9	199.99	0.80
10	Component 10	78.2	431.14	7.3	185.11	0.84
11	Component 11	81.9	1288.78	21.7	343.99	0.89
12	Component 12	86.9	1363.51	22.9	413.36	0.95
13	Component 13	89.1	748.26	12.6	303.35	0.98

Table 2b: Peak list of area and R_f Values of the component for Swarasa Churna

Peak no.	Name	Migration distance (mm)	Area	Area percentage (%)	Height (mm)	R_f Value
1	Component 1	10.1	2778.57	20.9	758.28	0.05
2	Component 2	14.9	1144.54	8.6	230.10	0.10
3	Component 3	21.9	678.21	5.1	119.39	0.18
4	Component 4	38.9	646.72	4.9	244.03	0.36
5	Component 5	43.5	8.77	0.1	8.32	0.41
6	Component 6	52.3	23.56	0.2	9.50	0.50
7	Component 7	60.3	201.88	1.5	48.37	0.59
8	Component 8	74.5	7631.26	57.5	1001.22	0.74
9	Component 9	90.7	23.58	0.2	10.17	0.91
10	Component 10	95.2	139.62	1.1	51.50	0.96

Table 3: Heavy Metal Analysis

Sl.No	Parameter analyzed	Results	Permissible limits as per WHO
1	Arsenic	Nil	Not more than 3.0 ppm
2	Cadmium	Nil	Not more than 0.3 ppm
3	Lead	0.020	Not more than 10.0 ppm
4	Mercury	0.205	Not more than 1.0 ppm

Table 4: Aflatoxin Contamination

Sl.No	Parameter analyzed	Results	Permissible limits as per WHO
1	B1	Nil	Not more than 0.50 ppm
2	B2	Nil	Not more than 0.10 ppm
3	G1	Nil	Not more than 0.50 ppm
4	G2	Nil	Not more than 0.10 ppm

Table 5: Microbial Contamination

Sl. No	Parameter analyzed	Results	Permissible limits as per WHO
1	Total *Bacterial* Load	11×10^4	Not more than 10^5/g
2	Total *Fungal* count	5 x 10	Not more than 10^3/g
3	E.Coli	Nil	Nil
4	*Salmonella Spp*	Nil	Nil

Figure 1: Macroscopic feature of Dried Swarasa Churna.

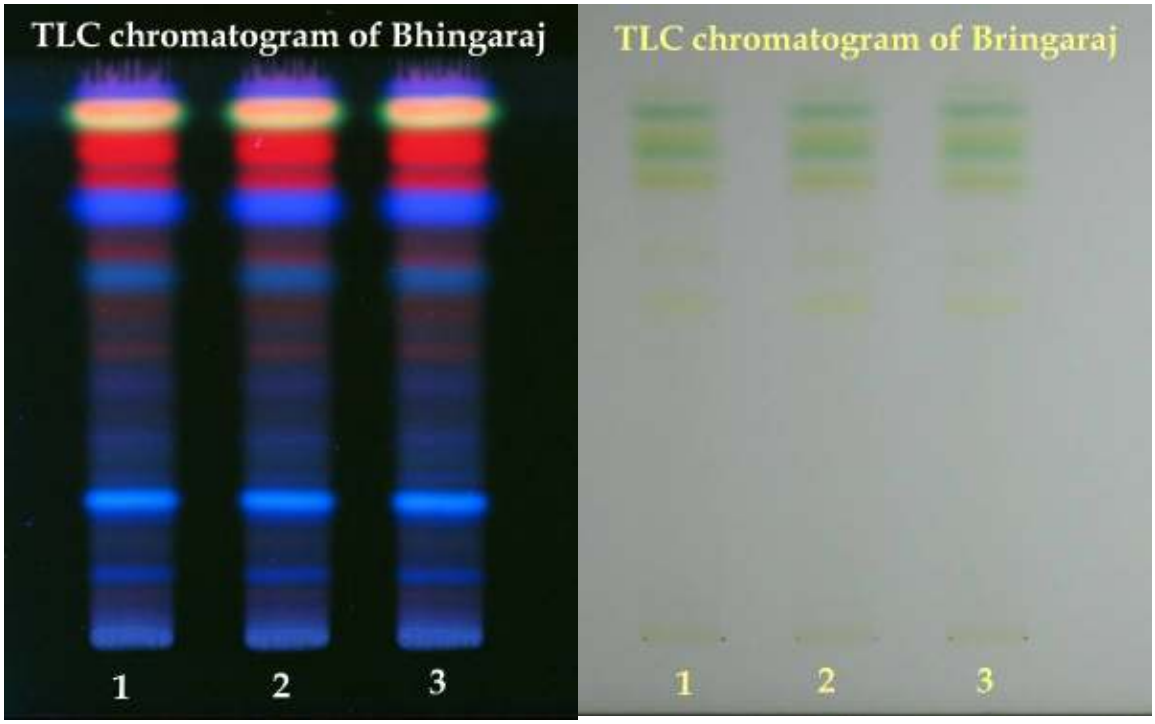

Figure 2: Finger print TLC Plate of Swarasa Churna at UV 366nm and visible region

UV 366nm **Visible region**

Swarasa Churna

Solvent system: Toluene: Ethyl Acetate: methanol= 7: 2: 1

Detection System: Under UV at 366 nm

Spots: Thirteen

Rf values: 0.11 (Blue) , 0.21 (Blue), 0.33 (Blue), 0.37 (Blue), 0.51 (Blue), 0.57 (Pink), 0.64 (Brown), 0.72 (Red), 0.80 (Blue), 0.84 (Red), 0.89 (Red), 0.95 (yellowish orange), 0.98 (Violet).

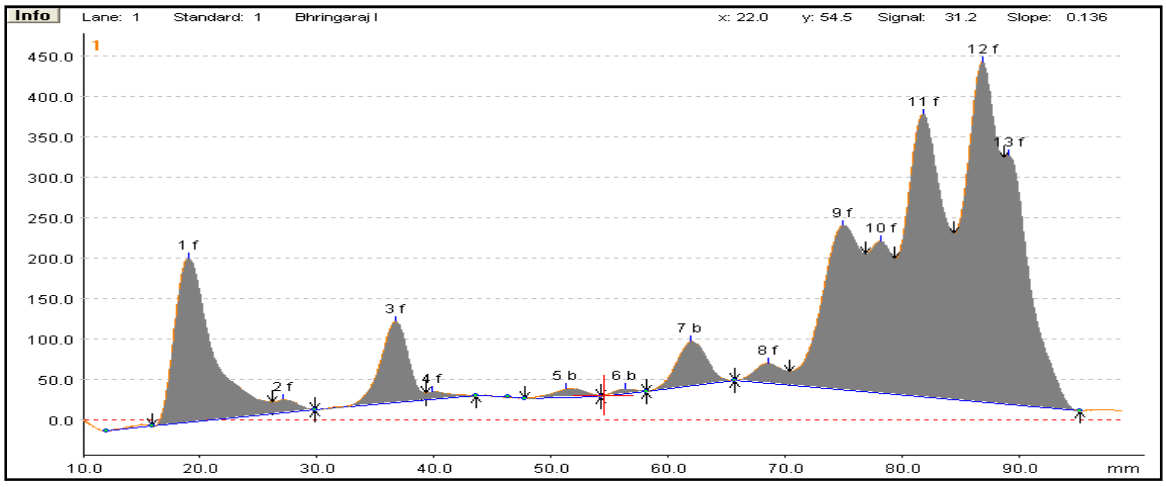

Figure 3a: Methanolic Extract, Finger print densitogram of Bringaraja showing peaks of the corresponding spots obtained on the chromatogram.

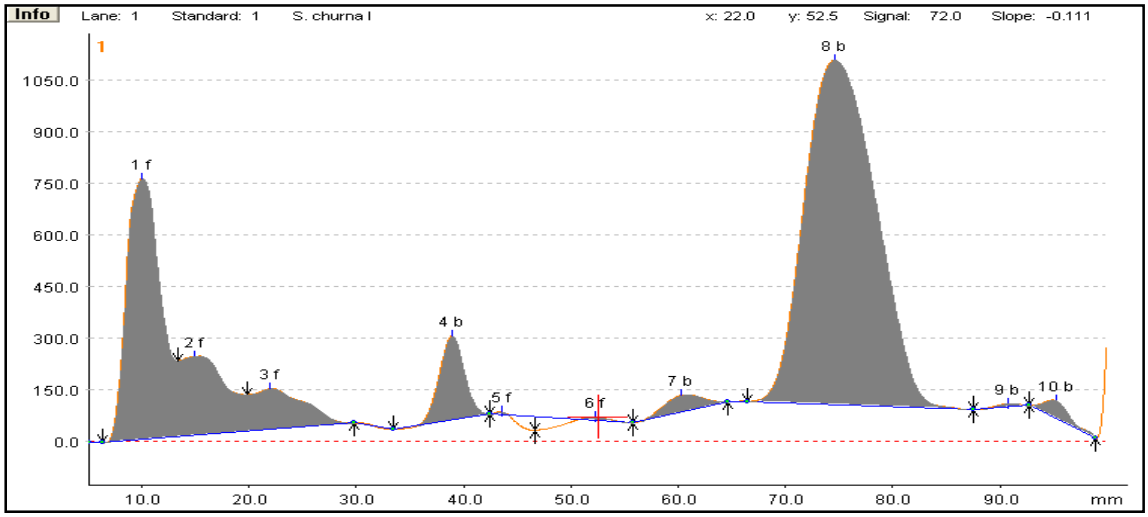

Figure 3b: Methanolic Extract, Finger print densitogram of Dried Swarasa Churna showing peaks of the corresponding spots obtained on the chromatogram.

Table 6: Elemental composition of Swarasa churna

S.No.	Element	Element %	Atomic %
1.	C	34.92	45.69
2.	O	46.32	45.50
3.	Na	0.32	0.22
4.	Mg	0.53	0.34
5.	Al	1.02	0.60
6.	Si	4.38	2.45
7.	P	0.18*	0.09*
8.	S	0.83	0.41
9.	Cl	2.51	1.11
10.	K	6.18	2.48
11.	Ca	2.82	1.10
12.	**Total**	**100.00**	**100.00**

* = <2 sigma

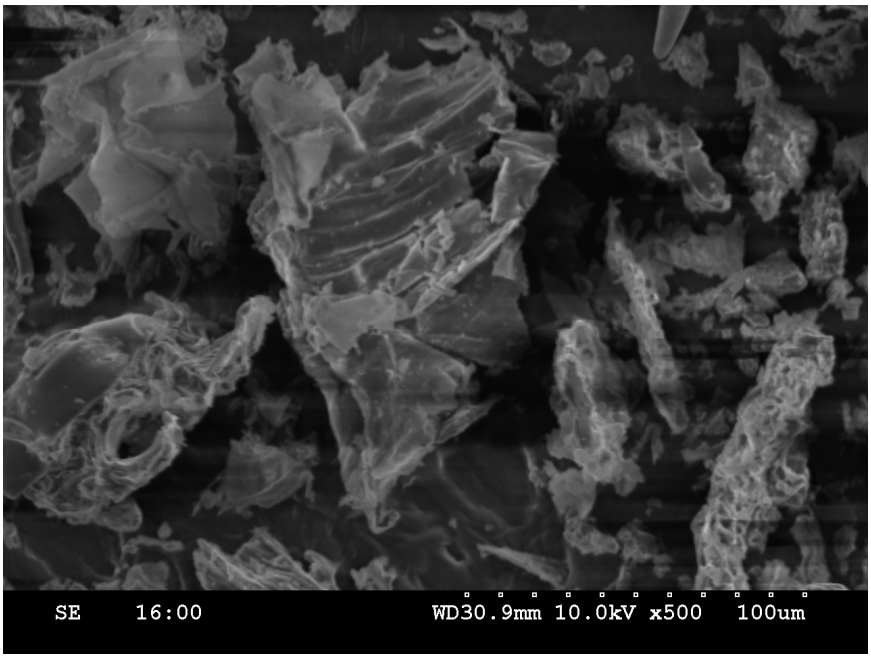

Figure 4: shows EDX Spectra of Swarasa churna

Source of support: Nil, Conflict of interest: None Declared