International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 1157-1167 www.ijprajournal.com ISSN: 2249-7781

Characteristic determination of Rauvolfia tetraphylla leaf and its Drug forms

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Submitted: 01-04-2023 Accepted: 10-04-2023

ABSTRACT: The usage of plants in our traditional medicinal practise is well recognised worldwide. This practise comprising of different drug forms and among which the ash form, traditionally termed as 'Bhasma', is been studied in this paper. The ash forms of the leaves of R.tetraphylla are prepared and been studied through FTIR, UV, SEM-EDX spectroscopic techniques. A nanometer size transformation is observed when the raw leaf is made into drug form. The toxic nature of the raw leaf is also explored.

KEYWORDS: Spectroscopy, medicinal plants, Bhasma, R. tetraphylla, trace elements.

I. INTRODUCTION

India is the largest producer of medicinal herbs and is called the botanical garden of the world[1]. There are about 45,000 plant species in India with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman and Nicobar Islands. The officially documented plants with medicinal potential are around 3000 but traditional practitioners use more than 6000 plants and so validation of plants used in traditional practice is an open problem.

The knowledge of traditional medicinal plants and their healing properties have been transferred from generation to generation. The plants produce a great number of secondary metabolites (for e.g. alkaloids, terpenes and polyphenolic compounds), possess many therapeutic applications. Now a days plant derived chemicals are the most preferable ingredients in drug discovery. Plants, either singly or in poly herbal formulations, are being used traditionally worldwide to combat several ailments including microbial disease, snake bite, skin disease, diabetes, inflammation and cancer [1-12].

The human practise of utilizing the plants for medicinal purpose is totally dependent upon their availability in and around the region of utilization. Due to the adaptive nature of plants they will have different characteristics for different locality in the content of its chemical components. Hence it is hardly possible to earmark the uniqueness of a plant curing any particular ailment. So, location wise study of medicinal plants is mandatory for the benefit of location specific society.

In this context we have taken Rauvolfia tetraphylla, classified as a poisonous plant, for studying its medicinal value through spectroscopic techniques such as IR Spectroscopy, UV visible spectroscopy and EDAX. In our old traditional medicinal practice, there are various drug forms which are being used in treatments. The most crucial form among them is either raw intake or 'ash' form. We have prepared the drug forms such as fresh leaf powder, fried ash powder and furnace burned powder. In view of modern pharmaceutics, these drugs have been subjected to spectroscopic techniques and the difference in its content and response to various spectroscopic studies, between the raw leaf and drug forms are being studied.

II. DESCRIPTION OF THE PLANT

This plant is named in Sanskrit as 'Vanasarpagandha' or 'Sarparasini' and in Malayalam it is named as 'Pambumkolli'. In Tamil it is named as 'Pampukaalaachchedi' and in Hindi it is named as 'Barachandrika'. Due to the nature of its leaf and fruits it is called in English as 'Four leaf devil pepper'. As per the literature available, the plant contains alkaloids such as reserpine, rauvolscine, ajmalicine, ajmaline, canescine, pseudoyohimbine and yohimbine[13-25].

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Fig-1: Rauvolfia tetraphylla

III. MATERIALS AND METHODS

Rauvolfia tetraphylla leaves are collected from the garden of Tagore Government Arts and Science College, Pondicherry (11.961030; 79.814568).

The collected leaves are washed well in distilled water and shadow dried. The samples (dried leaves) are ground well into fine powder using motor and pestle and are pelletized using KBr palletiser. The pelletized samples are used for IR spectroscopy. The samples fried in a mud pot at suitable temperature is named as fried ash. For the burned ash powder of dried leaves it is burned in a muffle furnace (about 380^{o}C) for 30 minutes and thus obtained these three samples are analysed by IR spectroscopy, UV visible, and SEM- EDX and found out the information about functional groups, electronic transition, elemental composition and surface morphology. The stage wise process of the drug sample preparation are given as a schematic representation, in fig.(2-4) .

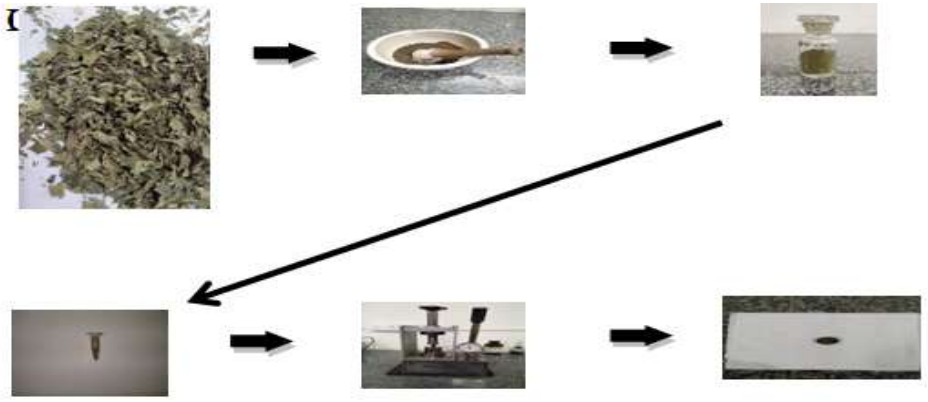

Fig-2: Stage wise process of preparation of R.tetraphylla raw leaf sample

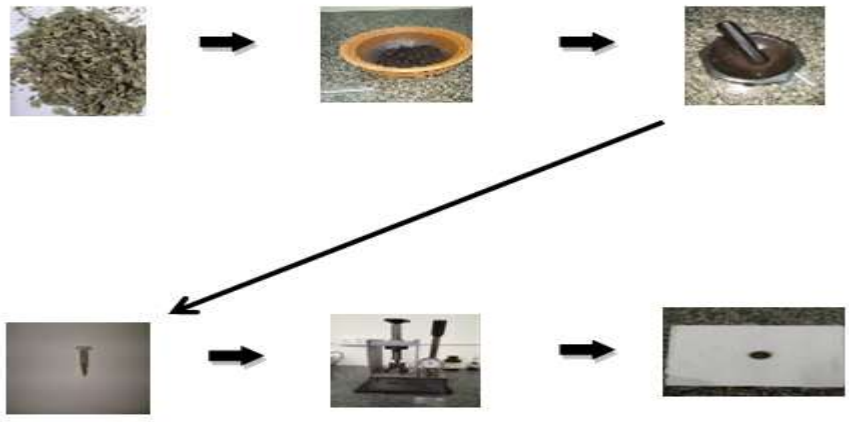

Fig-3: Stage wise process of preparation of R. tetraphylla fried ash form

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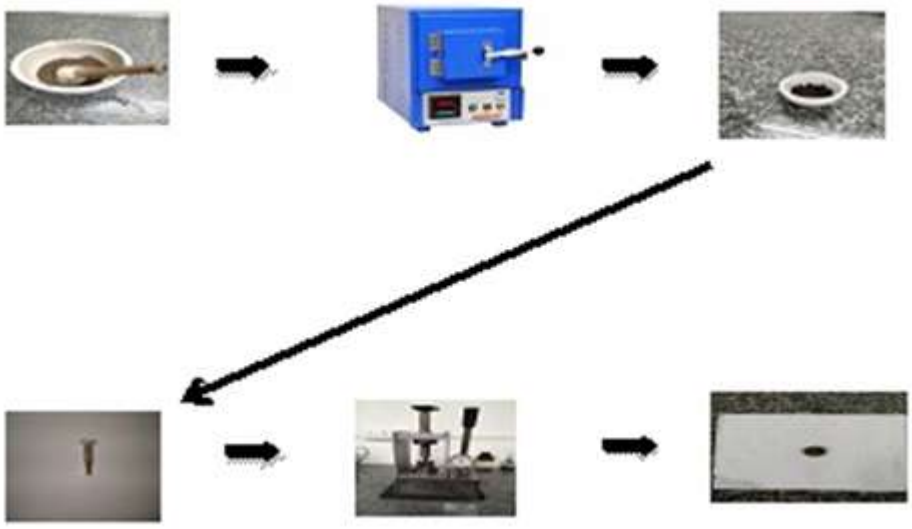

Fig-4: Stage wise process of preparation of R. tetraphylla burned ash form

IV. RESULTS AND DISCUSSION

(a) FTIR spectrum analysis of R. tetraphylla and its drug forms

According to the absorption peaks the compounds present in the samples are identified and are tabulated in table-1.

Table-1: FTIR analysis of raw leaf, fried ash and burned ash of R. tetraphylla

Sl. No.	Absorption wave Number(cm^{-1})			Group	Compound Class
	Raw leaf	Fried leaf	Burned leaf		
1.	-	3614.91	-	O-H	Alcohol
2.	-	3477.99	3414.35	O-H	Alcohol
3.	-	3402.78	-	O-H	Alcohol
4.	-	3402.78	-	O-H	Alcohol
5.	3343.96	3324.68	-	N-H	Secondary amine
6.	-	3208	-	O-H	Alcohol
7.	2923.56	3078.8	-	C-H	Alkane
8.	-	2766.39	-	O-H	Alcohol
9.	-	2221.59	-	CΞC	Alkyne
10.	1642.09	-	1614.13	C=C	Alkene
11.	-	1547	-	N-O stretching	Nitro compound
12.	1321.96	-	1381.75	O-H Bending	Phenol
13.	1244.83	-	-	C-O	Alkyl aryl ether
14.	1084.76	-	-	C-O	Primary alcohol
15.	-	-	1105.98	C-O	Secondary alcohol
16.	-	-	748.245	C=C Bending	Alkene
17.	618.074	681.713	-	C-Br	Halo compound
18.	589.147	604.294	594.932	C-I Stretching	Halo compound
19.	566.005	-	-	C-Cl	Halo compound

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20.	541.899	-	-	C-Br	Halo compound
21.	504.294	504.294	552.506	C-I stretching	Halo compound

The FTIR spectrum obtained for raw leaf, fried leaf ash and burned leaf ash are given in FIG (5-7) respectively.

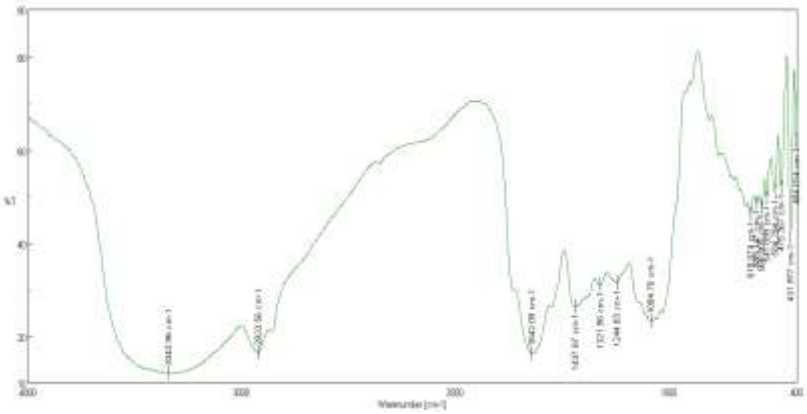

Fig-5 : FTIR spectrum of raw leaf of R. tetraphylla.

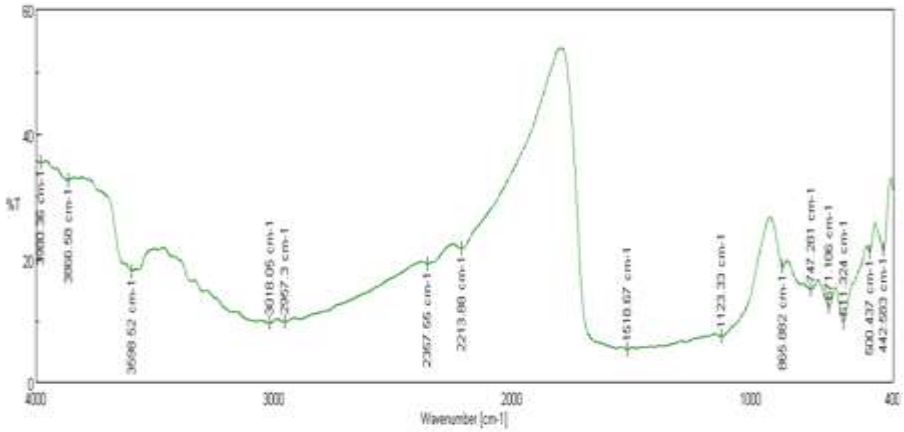

Fig-6: FTIR spectrum of fried leaf ash of R. tetraphylla

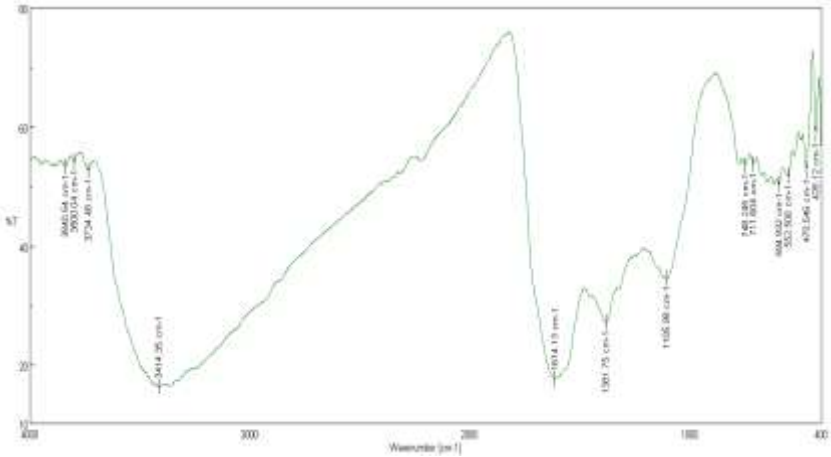

Fig-7: FTIR spectrum of burned leaf ash of R. tetraphylla

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According to the compounds present in the samples, the medicinal values of the compounds are given below:

Alcohol is used in the pharmaceutical industry in a variety of manufacturing processes. It has bactericidal activity and is often used as a topical disinfectant, especially in alcohol gel for hands. It is also widely used as a solvent and preservative in pharmaceutical preparations[26].

Alcohols, in various forms, are used within medicine as an antiseptic, disinfectant, and antidote. Alcohols applied to the skin are used to disinfect skin before a needle stick and before surgery[27].

Alkane is an anionic surfactant used to treat varicose veins of the lower extremitie, and to maintain alcohol abstinence in patients with alcohol dependence. It is also used in cosmetics and pharmaceuticals as a fat emulsifier, wetting agent, and detergent.

Alkenes are the raw materials for the manufacture of chemicals like alcohols, aldehydes etc. A number of neurotropic agents contain a conjugated alkene group incorporated in an iminostilbene or dibenzosuberene ring system [28]. Alkenes are suitable functional groups to carry out biorthogonal ligations and they have good compatibility with water and high selectivity. It was demonstrated that highly strained alkenes (electron-rich dienophile), such as transcyclooctene and norbornene, can react rapidly with tetrazines. This approach was successfully employed to functionalize thioredoxin and to label the cell surface of living cells [29]. Propylene glycol which is an alkene is used as a solvent in many pharmaceuticals, including oral, injectable and topical formulations,which are insoluble in water[30]. These are also used as general anesthesia. Ethane is a plant hormone which controls growth, seed germination and fruit development. Therefore, ethane is used for artificial ripening of fruits, flower maturation, etc.

(b) UV visible analysis of R. tertraphylla and its drug forms

UV visible spectroscopy is based on the absorption of light by the sample. The result of UV visible analysis of R. tetraphylla shows that the relation between absorption and wavelength. The peaks represent the active range of UV visible spectrum. Using these wavelengths we can identify the electronic transition of active compounds present in the leaf. For raw leaf of R. tertaphylla the electronic transition is n to π^* since the peaks obtained are at 221nm and 271nm (Fig.8).

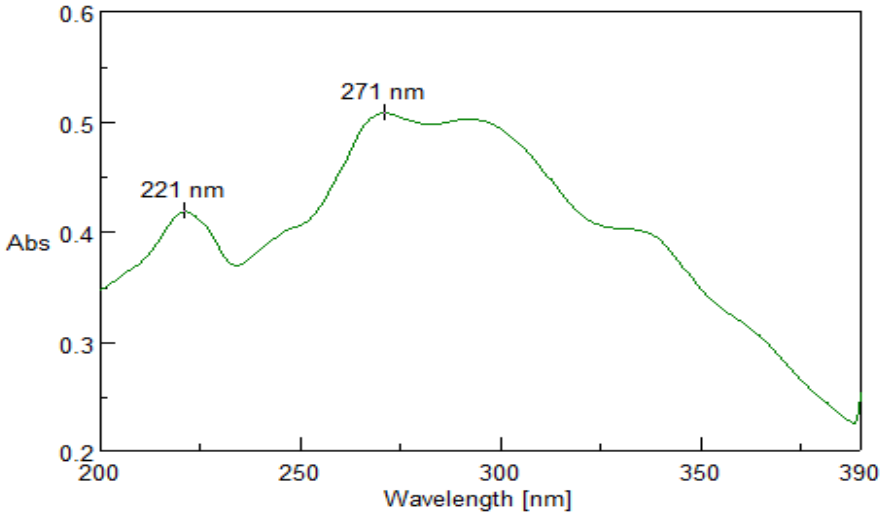

Fig-8: UV spectrum of R. tetraphylla raw leaf.

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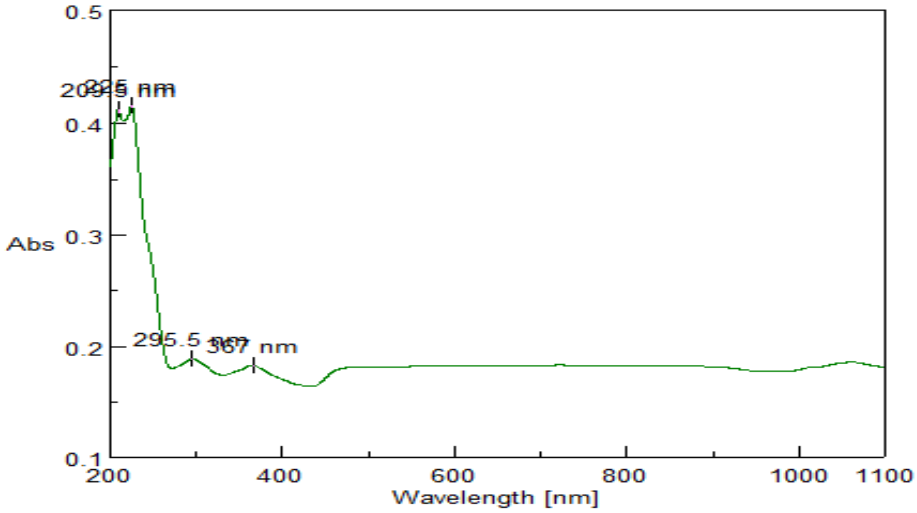

Fig-9: UV spectrum of R. tetraphylla leaf in a fried ash form.

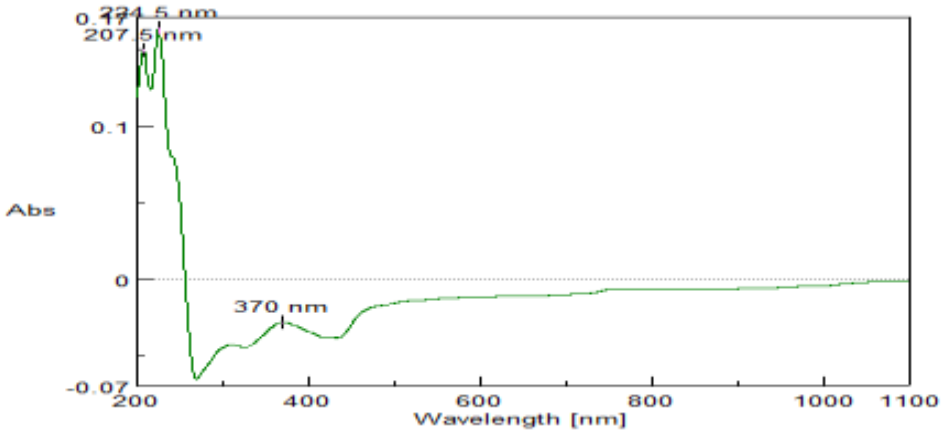

Fig-10: UV absorption spectrum of R. tetraphylla leaf in a burned ash form using muffle furnace at 380°c

For the fried leaf of R. tetraphylla, the electronic transition is n to π^*since the peaks are obtained in the spectrum at 209nm to 367nm. This is given in (Fig.9).

The burned leaf of R. tetraphylla shows the same transition of n to π^* and the region of absorption is from 207nm to 370nm.(Fig.10).

(c) SEM-EDX Analysis of Drug Forms of R.Tetraphylla

When the leaf is transformed into ash which is been followed by the modern pharmaceutical industry, traditionally termed as 'Bhasma', by frying the leaf at steady heat and burned in the muffle furnace show tremendous changes of its size from micrometre to nanometre.

A crystalline structure of the leaf sample (fig.11) is been transformed to a plate like crystals when fried (fig.12) but the burned sample shows a rigid huge crystalloid structure (fig.13).

The SEM analysis explain a transition of micrometre to nanometre size when the leaf is transferred to Bhasma form.

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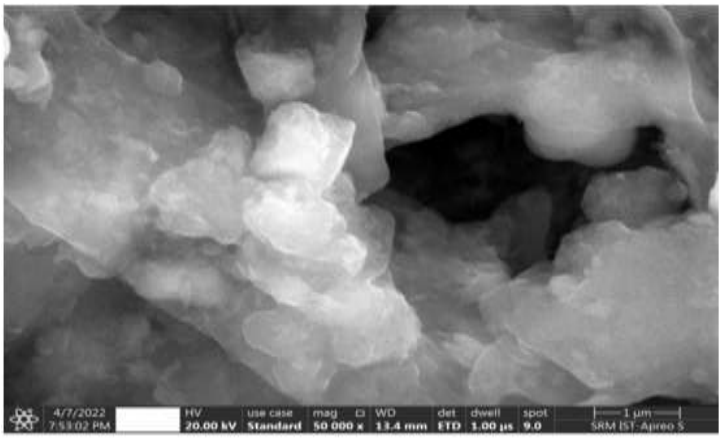

Fig-11: SEM-image of raw leaf form of R.tetraphylla

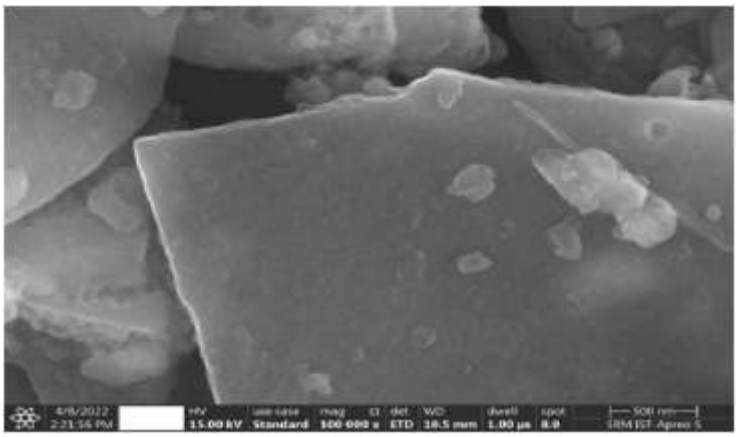

Fig-12: SEM-image of fried leaf form of R.tetraphylla

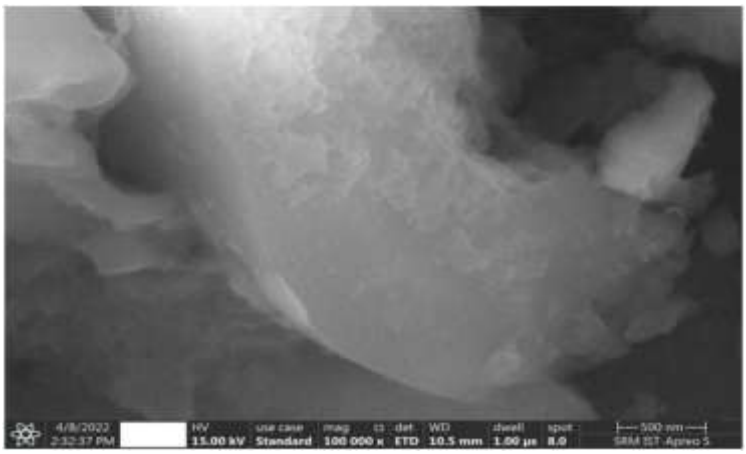

Fig-13: SEM-image of burned leaf form of R.tetraphylla

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TABLE-2. Elemental analysis of R. tetraphylla leaf and its drug forms

At. No	Element	Net count			Weight %			Atom %		
		Raw leaf	Leaf fried ash	Leaf burned ash	Raw leaf	Leaf fried ash	Leaf burned ash	Raw leaf	Leaf fried ash	Leaf burned ash
6	C	12589	4330	10744	30.40	27.63	35.76	47.94	44.88	53.71
7	N		343	610	-	4.36	5.86	-	6.08	7.55
8	O	6181	330	1110	17.02	1.75	4.60	20.15	2.13	5.19
11	S	435	-	-	0.22	-	-	0.18	-	-
12	Mg	3164	-	1299	1.13	-	0.78	0.88	-	0.58
13	Al	117732	80524	78936	35.02	62.70	44.41	24.58	45.33	29.70
15	P	2891	351	-	1.04	0.43	-	0.64	0.27	-
16	S	8575	-	-	2.79	-	-	1.65	-	-
17	Cl	4001	167	1163	1.50	0.23	1.04	0.80	0.13	0.53
19	K	-	776	2362	-	1.22	2.49	-	0.61	1.15
20	Ca	9713	468	2094	4.16	0.82	2.48	1.96	0.40	1.12
26	Fe	439	-	-	0.43	-	-	0.15	-	-
29	Cu	199	-	-	0.33	-	-	0.10	-	-
42	Mo	-	433	2059	-	0.85	2.58	-	0.17	0.49
49	In	8631	-	-	5.95	-	-	0.98	-	-

From table-2, the hierarchy of elemental abundance in the raw plant leaf is Al, C, Ca, In, S, O, Cl, Mg, P, Fe, Na, and Cu.

It is clear from the analysis that the essential elements such as Mg and Fe are very less in ppm except Ca. The abundance of Al is 10 times to the presence of next abundant element C.

In fried and burned forms Na, P, S, Fe, Cu and I_n are not present. Al dominates in all the forms of fried and burned leaf ash forms. The carbon content is 7 times less than Al.

Considering the net count of raw, fried and burned leaf of tetraphylla the aluminium content is more in all the forms compared to other elements, which indicates its toxic nature. Even though it is toxic the lower dose of it can be used for medicinal purposes.

The spectrum obtained for the samples (raw leaf, fried leaf ash and burned leaf ash) are given in Fig. 14-16.

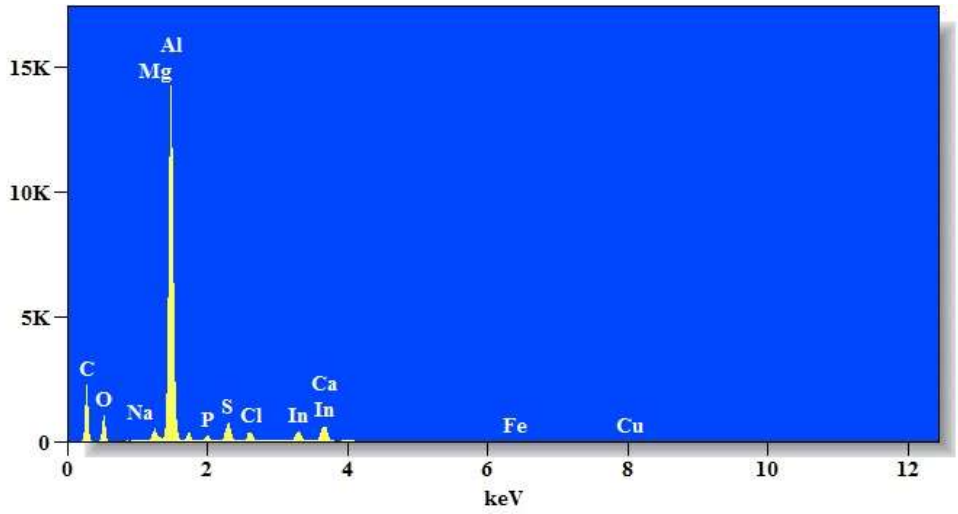

Fig-14: EDX spectrum of R. tetraphylla raw leaf

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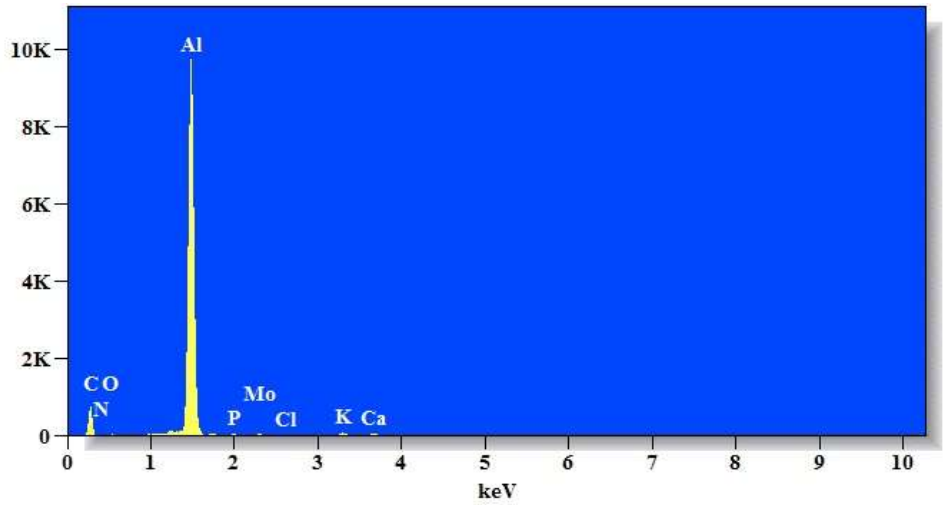

Fig-15: EDX spectrum of R. tetraphylla fried leaf ash

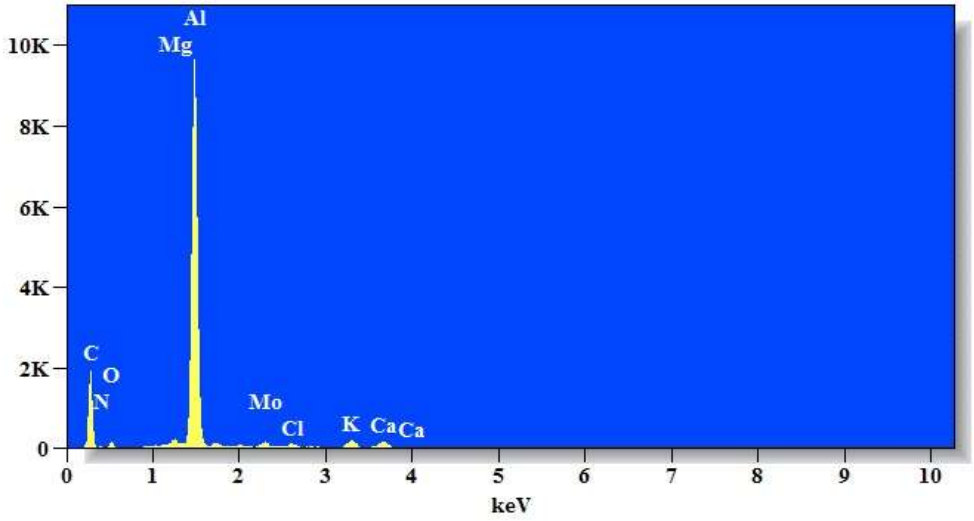

Fig-16: EDX spectrum of R. tetraphylla leaf in a burned ash form.

The medicinal usage of elements present dominantly in the studied plant is discussed below.

Magnesium is important for normal bone structure in the body. It is responsible for proper function of nerves and muscles. In stomach, it helps to neutralize stomach acid. Low magnesium levels in the body leads to diseases such as osteoporosis, high blood pressure, clogged arteries, hereditary heart diseases, diabetes and stroke.

High level of **aluminium** in the body causes neurotoxicity, alzheimer's disease, and breast cancer. Today, we use aluminium compounds to make vaccines more efficient.

Phosphorous is a mineral that uses to build bones and teeth and to make proteins for growing and repairing cells. It offers numerous health benefits like muscles contract, filtering and removing waste from the kidneys, nerve conduction throughout the body, making DNA and RNA. Receiving too much of phosphorus also causes diseases.

Sulphur is used to treat many type of skin disorders. Oral supplement of sulphur cures shortness of breath, allergies, swelling in the back of the throat, high cholesterol, clogged arteries and upper respiratory tract infections.

Potassium plays an important role in the transmission of nerve signals, muscle contrast, fluid balance and various chemical reactions. Potassium is used for treating high blood pressure and preventing stroke.

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Chlorine is used to treat diabetes, high blood pressure, high cholesterol, asthma, seizures, cancer and depression.

Taking **calcium** helps the bones to rebuild and stay strong. It also helps the heart, nerves, and blood clotting systems. It is used for high blood pressure, cancer, and stroke. The deficiency of calcium resulting weak nails, slower hair growth, and breaking of bones.

Iron is commonly used for preventing and treating different types of anemia, heart failure, fatigue, ADHD. Iron helps cells to carry oxygen from the lungs to cells.

Copper helps to make red blood cells, keep nerve cells healthy, support immune system, protect cells, making of bones and tissues. It is used for treating anemia, irregular heartbeat, thyroid problems, etc.

Molybdenum plays an important role in body functions. It works in the body to break down proteins and other substances.

V. SUMMARY

The leaf of the plant R. tetraphylla is subjected to FTIR, UV and SEM-EDX analysis after making it into two different form of drug namely, fried leaf ash and burned leaf ash and is compared with the raw leaf.

From the FTIR analysis it is found that Alcohol content in the raw leaf is completely absent and it is quite high in the fried leaf ash compared to burned leaf form. This indicates the studied plant is a drug friendly and which can be easily absorbed by the body. In the raw leaf the halo compounds are quite high compared to other drug forms.

As for as UV absorption spectrum is concerned the electronic transition due to the absorption of UV by the samples is n to π^* transition but the reactivity is quite high in the dry forms than the raw leaf.

The SEM analysis explain a transition of micrometre to nanometre size when the leaf is transferred to Bhasma form which reveals the exemplaric value of our traditional medicinal practise.

Form the EDX data, it is found that in the leaf of Rauvolfia tetraphylla, the presence of Al and Mg is high. The excessive content of Aluminium shows the poisonous character of the plant. For the pharmaceutical purposes, the amount of plant extract used in medicine is in the range of µg to mg. While making this plant leaf into drug much importance should be given to the interaction of other elements in the biological system.

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