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Pharmacognostical and Phytochemical Evaluation of *Rheum emodi* Wall.

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Abstract
Rheum emodi (Polygonaceae) is a perennial stout herb; belongs to a large genus '*Rheum*. The most important constituents of the drug are anthraquinone derivatives such as Chrysophanol, Aloe-emodin, Emodin, Physcion, Rhein and its glycosides. The objective of the present investigation was the development of quality standards and phytochemical analysis of *Rheum emodi*. This included organoleptic properties, pH of aqueous solution, ash values, extractive values, successive extractive values, loss on drying, HPTLC finger printing profile and preliminary phytochemical screening. The findings of this study might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Key Words
Rheum emodi, extracts, phytochemical screening, HPTLC.

Introduction
The plant of *Rheum emodi* (Family: Polygonaceae) is a perennial stout herb; belong to a large genus '*Rheum*'. The Rhubarb distributed in the temperate and sub tropical regions of the world, chiefly in Asia. About ten are found in India[1-3]. The height of herb is about 1.5-3.0 meter and it has woody large roots, large leaves & branched leafy stem[1,4-6]. The main parts used as drug are roots and rhizomes. The rhizomes are collected in October – November[7]. Root of Indian Rhubarb is darker, inferior in aroma, coarser and untrimmed, is not decorticated[3]. Fresh rhizome is 6 to 12 inches long, and the freshly fractured surface is dull orange to yellowish brown. The most important constituents from rhubarb root are the anthraquinone derivatives such as Chrysophanol, Aloe-emodin, Emodin, Physcion, Rhein and its glycoside, Glucorhein etc. Tannins are also present in rhubarb which includes hydrolysable tannins, containing ester or glycosidic bonds composed of gallic acid, glucose and other monosaccharides and condensed tannins, derived primarily from the flavone derivatives catechin and leucocyanidin[8-10]. The root has a sharp bitter taste, alexiteric, purgative, emmenagogue, diuretic and useful in biliousness, lumbago, heating of brain, sore eyes, piles, chronic bronchitis, chronic fever, asthma, coryza, pains and bruises[6,10-13]. Keeping in view the importance the *Rheum emodi*, the present investigation was carried out to establish pharmacognostical and physicochemical standards which would help in authentication as well as in checking adulteration, if any; further the study will greatly help in quality assurance of finished products containing this herbal drug as component.

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Materials and Methods
Collection and Authentication of the Plant material
The dried rhizomes of *Rheum emodi* were purchased from the local market of Khari Baoli, Delhi, India. It was authenticated by Dr. H. B. Singh National Institute of Science Communication and Information Resources. The voucher specimen was also submitted in the Herbarium of NISCARE, New Delhi, India (NISCAIR/RHMD/06/766/83).

Macroscopical and microscopical study

Macroscopical and microscopical characters of the drugs were studied according to the WHO and pharmacopoeial guidelines[14,19].

Physico-chemical studies

Different physicochemical values such as extractive values (cold & hot extracts), ash values (total ash, acid-insoluble ash & water soluble ash), loss on drying, and pH of 1% and 10% solution of *Rheum emodi* were determined according to the standard methods[14].

Preliminary Phytochemical Analysis

The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents as per method described by Trease and Evans[15]. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins & amino acids, and lipids/fats.

Fluorescence Analysis

Chemical tests of powder drug with different reagents were performed to observe the colour reactions according to the reported method[16].

Determination of total phenolic content[17]

Preparation of standard curve

Standard Gallic acid (10 mg) was dissolved in 100 mL distilled water in a volumetric flask (100 µg/mL of stock solution). From the above stock solution 0.5 to 2.5 ml of aliquots were pipetted out into 25 mL volumetric flasks. Then 10 mL of distilled water and 1.5 mL of Folin-Ciocalteu reagent, diluted according to the label specification to each of the above volumetric flasks were added. After 5 min, 4 mL of 1M sodium carbonate was added and volume was made up to 25 mL with distilled water. After 30 min, absorbance at 765 nm was recorded and calibration curve of absorbance vs concentration was plotted.

Preparation of test sample

Plant material was dried at room temperature and grounded in a mortar. Powder (50 gm) was extracted with 500 mL of methanol by maceration (48 h). The solvent was removed under vacuum and the extract was freeze-dried. From the above prepared test solutions, 1 mL of solution was pipetted out into a 25 mL volumetric flask and then the same steps were followed as given above (standard curve preparation) for color development. The amounts of total phenolics using the standard curve of Gallic acid were determined. (McDonalds) Total phenol values are expressed in terms of gallic acid equivalent (mg/g).

Determination of total flavonoid content[18]

Preparation of standard curve

Aluminum chloride colorimetric method was used for flavonoids determination. Plant extract (0.5 mL of 1:10 mg/mL) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M sodium acetate and 2.8 mL of distilled water. It was kept at room temperature for 30 min and absorbance of the reaction mixture was measured at 415 nm with a double beam UV spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 10, 20, 40, 50 to 100 µg/mL in methanol. The results are reported in table.

Preparation of test sample

Plant powder (50 gm) was extracted with 500 mL of methanol by maceration (48 h). The solvent was removed under the vacuum and the extract was freeze-dried. From the above prepared test solutions, 1 mL of solution was pipetted out into a 25 mL volumetric flask and then the same steps were followed as given above (preparation standard curve) for the colour development.

Development of chromatographic fingerprint profile of different extracts by HPTLC

Preparation of extract

The plant material was coarsely powered and extracted in Soxhlet apparatus for 6-24 h using solvents; petroleum ether, chloroform, and methanol. The extracts were evaporated to dryness in a rota-vapour and the solvents were recovered. Gummy residues so obtained, were stored in deep freezer at -20 °C till further application. TLC and HPTLC samples were prepared by dissolving each extract in their respective solvent to get the concentration of 10 µg/mL. These solutions were further passed through syringe filter to remove any impurities and applied on TLC plate for finger printing analysis.

The extract was applied on TLC aluminum sheets silica gel 60 F 254 (Merck) 10 micro liter each with band length 6 mm using Linomat 5 sample applicator set at a speed of 100 nL/sec CAMAG, Switzerland. Different solvent systems were used for separation of constituents of different extracts. The chromatograms were developed in twin trough chamber for 20 min up to the distance of 80 mm and

the spots were visible without derivatization at 254 and 366 nm wavelengths.

Results and Discussion

Macroscopical characters

The rhizome of Rheum emodi was solid, compact somewhat cylindrical, barrel shaped, ranging from 3.5 to 5.5 cm in length and 2.5 to 3.5 cm in diameter. Outer surface was mostly irregularly longitudinally wrinkled, furrowed or ridged but few, of them showed transverse wrinkles and usually covered with brownish or yellowish brown cortex. Inner surface was yellow in colour. Fracture was hard and showed cambium line. The taste was bitter and the odour was aromatic and slightly pungent.

Microscopical characters

The transverse section of the root showed brown bark, which consist of 10-14 layers of cells. Cortical region was usually made up of a few layers of parenchymatous cells and cells were mostly oval to irregularly rounded and thin walled. Most of the cortical cells possessed starch grains, tannins, clusters of calcium oxalate and yellow brown content. The starch grains were abundant, speroidal or round in shape and found single or in 2 - 4 groups. A few cells of the region merged into the secondary phloem tissue, which formed a few layers of cells. Cambium was wavy and much compressed. Medullary rays were prominent and radially elongated and usually consisted of one to two layers of cells. The ray cells were radiating and often extended of layer of radial chain and the central cylinder of wood was formed in this way. The rest of wood was composed of trachied and xylem parenchyma. The vessels were mostly found to have scalariform and spiral thickenings.

Physico-chemical studies

Different physicochemical values such as extractive values (cold & hot extracts), ash values (total ash, acid-insoluble ash & water soluble ash), loss on drying, and pH of 1% and 10% solution of Rheum emodi were determined. The results are presented in Table 1.

Preliminary Phytochemical Analysis

The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents as per method described by Trease and Evans, 1985. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids, proteins & amino acids, and lipids/fats. Presence and absence of different phyto-constituents are presented in Table 2.

Fluorescence Analysis

Chemical tests of powder drug with different reagents were studied in day light, U.V. 254 nm and U.V. 366 nm, the results are presented in Table 3.

Total Phenolic content

Total phenolic content was measured by Folin Ciocalteu method in term of Gallic acid equivalent in mg/g of the extract. The amount of phenolic content was calculated with the help of graph (figure 1) was found to be 68.41 µg/mL (0.68% w/w). Results are presented in Table 4.

Total Flavonoid content

The results of total flavonoids content of *Rheum emodi*, determined by aluminum chloride colorimetric method, are showed in Table 5. The standard calibration curve is shown in the form of Figure 2. The total flavonoid content was found to be 28.41 µg/mL (0.28 % w/w).

Chromatographic fingerprint profile of different extracts of Rheum emodi by HPTLC

HPTLC analysis was also performed of methanolic, extracts of *Rheum emodi* for the development of characteristic fingerprint profile, which may be used as markers for quality evaluation and standardization of the drug. The chromatograms obtained after development in different solvent systems followed by scanning at 254 (Table 6) and 366 nm (Table 7) in absorbance mode depicted presence of number of substances in the extracts. Finger 3 to 8 show the 3D view of different extract of *Rheum emodi* with different solvent systems at 254 nm and from figure 9 to 14 at 366 nm.

Discussion

This study on the pharmacognostical and phytochemical analysis of *Rheum emodi*, revealed a set of parameters which may enable to those who handle this plant to maintain its quality control. Adulteration and substitution have become a major problem due to the absence of standards relating to genuineness of drug. Skill hand and cost factors for pharmaceuticals purposes, the quality of medicine must be as high as that of other medicinal preparations. Quality refers to intrinsic value of the drug, the amount of medicinal principles or active constituents present.

The pharmacognostical parameters including HPTLC are helpful for the future identification and authentification of this plant in the herbal industry. The physical parameters, such as loss on drying, ash values and extractive values will be helpful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in future. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. The plant may be considered as biosynthetic laboratory for a variety of compounds (secondary metabolites) like alkaloids, glycosides, flavonoids, volatile oils, and saponins that exert physiological effects. The curative properties of medicinal plants are due to the presence of various secondary metabolites. Thus the preliminary screening tests may be useful in the detection of bioactive principles. HPTLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. Phytochemical study was also useful to isolate the pharmacologically active principles present in the drug. More phytochemical research work is required for isolation, purification and characterization of biologically compounds.

Conclusions

Standardization of herbal drugs is a topic of great concern. They are subject to variability as derived from heterogeneous sources. This variability can have both merits and demerits. The main demerits are that the activity of the material may vary and that inferior material may be produced. *Rheum emodi* is an important plant and has been found to have various biological properties. So efforts have been made to provide scientific data to standardize the plant material for further studies. Microscopic, macroscopic data and other physical values including HPTLC will help to identify the correct species of the plant. The research out comings of the standardization parameters can also be used for evaluating the quality and purity of the drug and its formulation.

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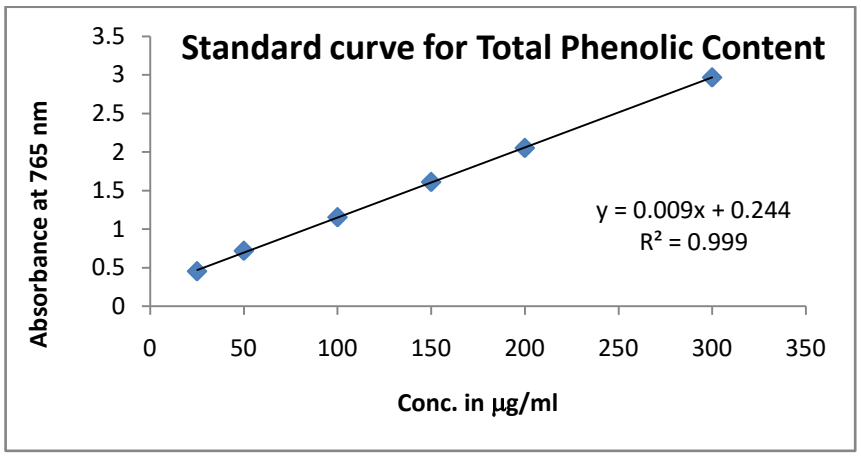

Figure 1: Standard calibration curve for determination of total phenolic content.

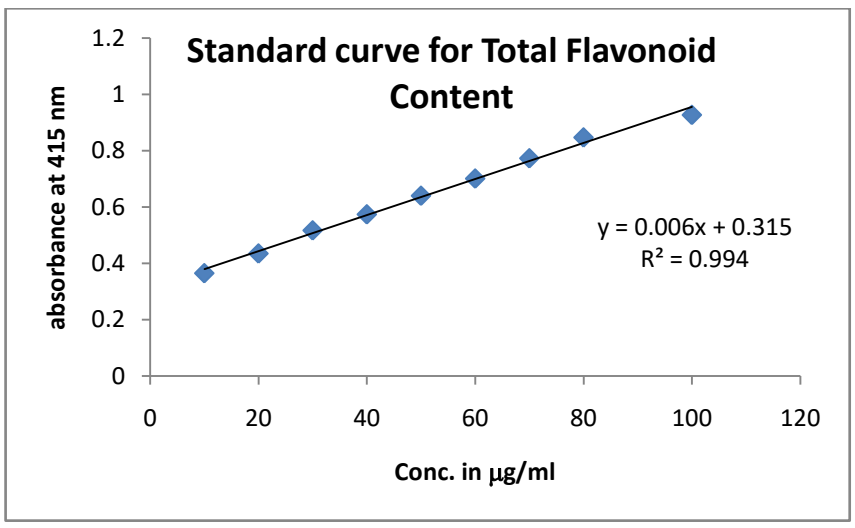

Figure 2: Standard calibration curve for determination of total Flavonoid content.

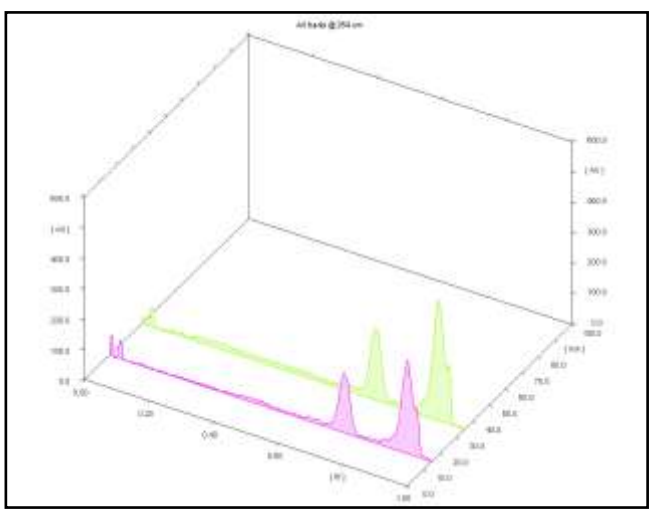

Fig. 3: 3D view of Petroleum ether extract of *R. emodi* at 254 nm

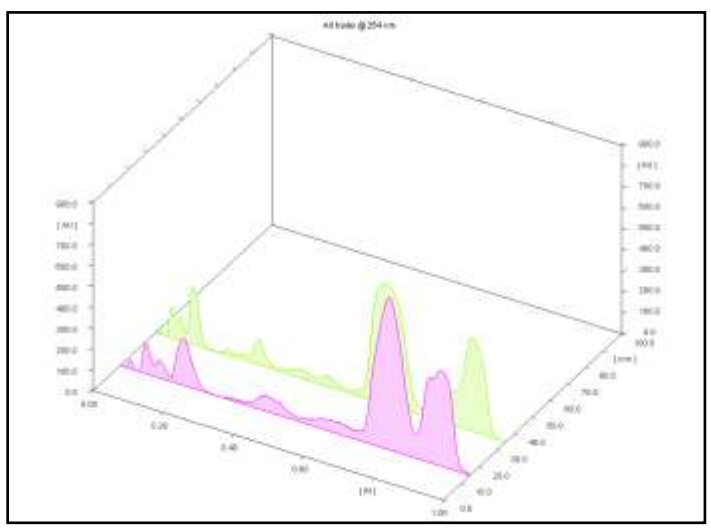

Fig. 4: 3D view of Chloroform extract of *R. emodi* at 254 nm.

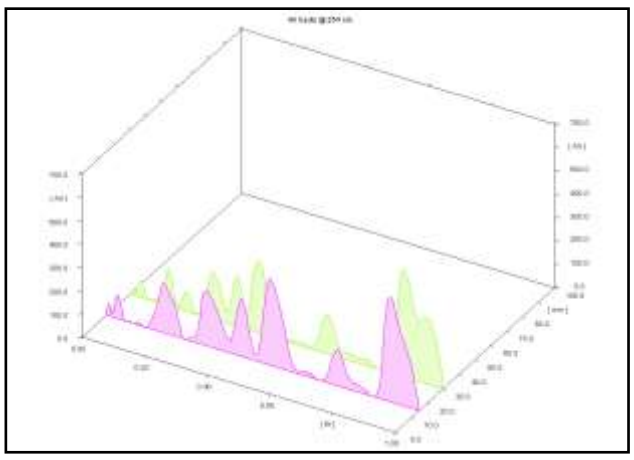

Fig. 5: 3D view of Ethyl acetate extract of *R. emodi* at 254 nm.

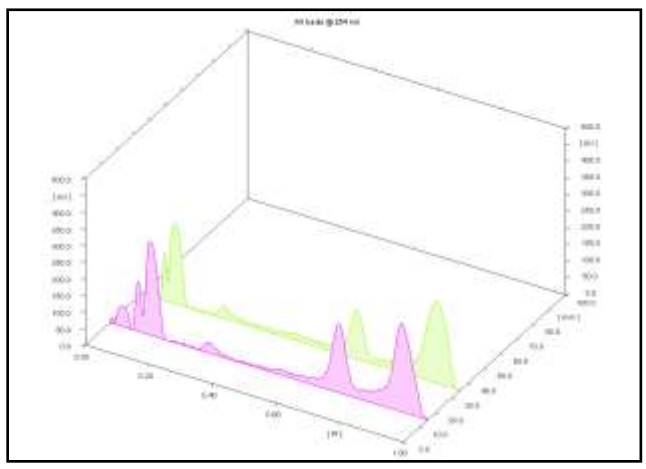

Fig. 6: 3D view of Methanol extract of R. emodi at 254 nm.

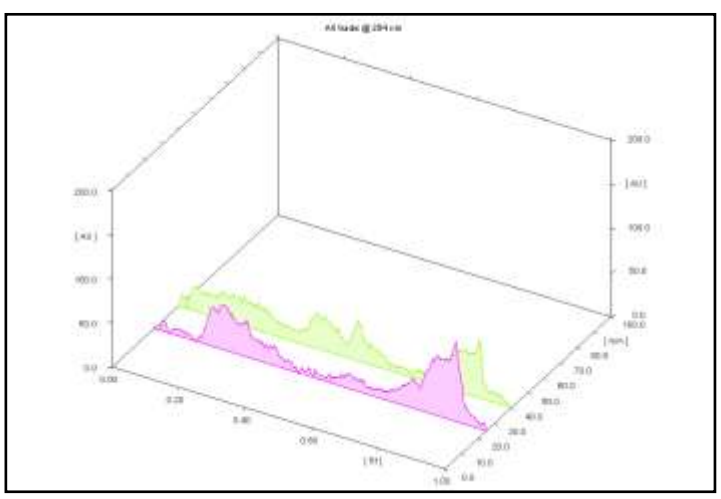

Fig. 7: 3D view of water extract of *R. emodi* at 254 nm

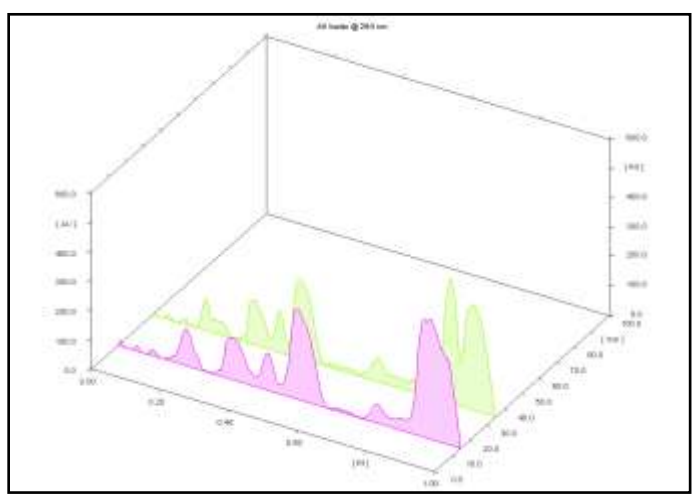

Fig. 8: 3D view of Methanol direct extract of *R.*

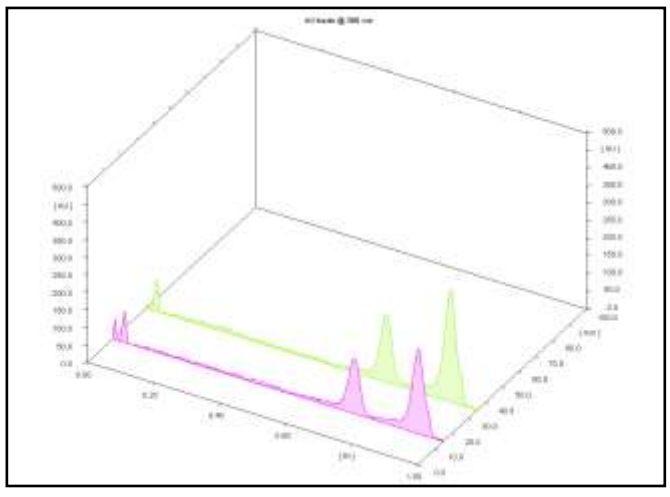

Fig. 9: 3D view of Petroleum ether extract of *R. emodi* at 366 nm.

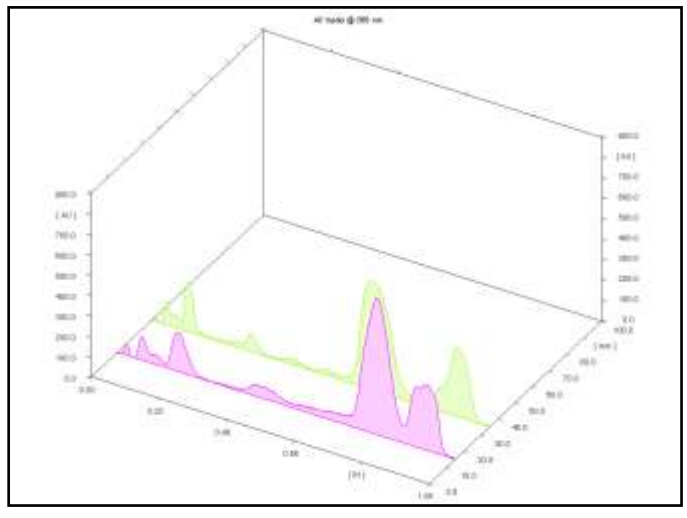

Fig. 10: 3D view of Chloroform extract of *R. emodi* at 366 nm.

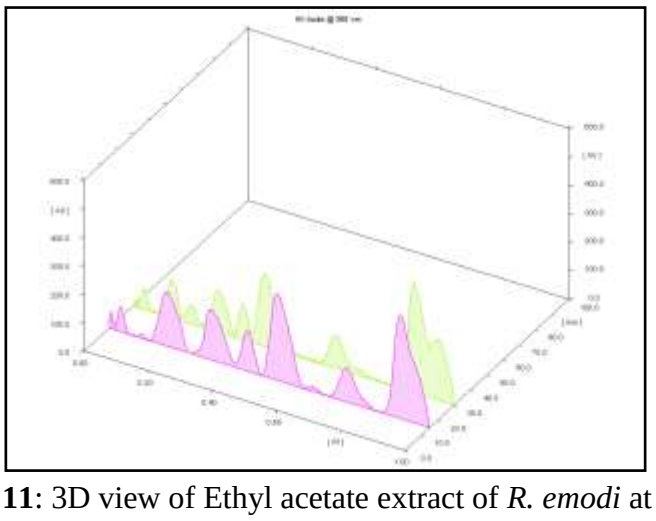

Fig. 11: 3D view of Ethyl acetate extract of *R. emodi* at 366 nm.

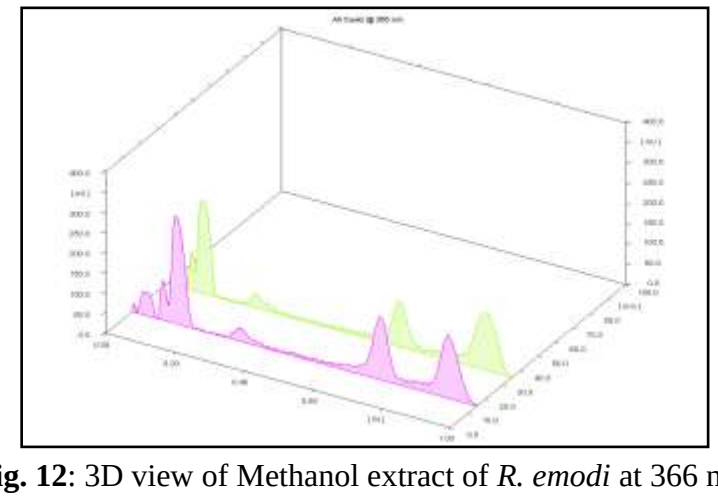

Fig. 12: 3D view of Methanol extract of *R. emodi* at 366 nm.

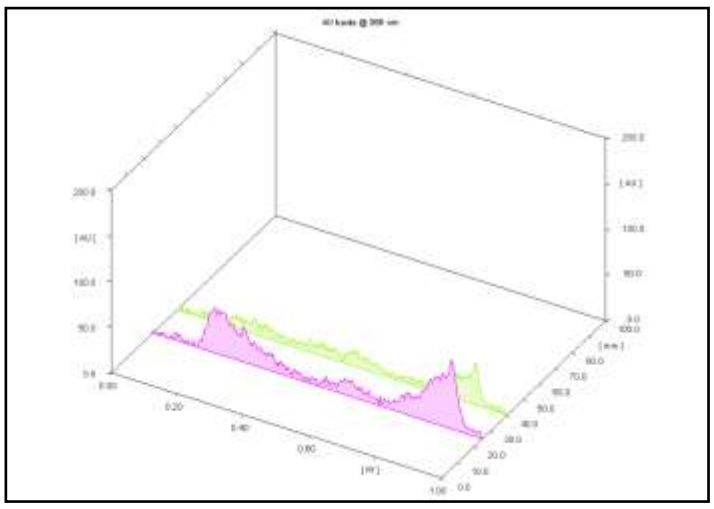

Fig. 13: 3D view of water extract of *R. emodi* at 366 nm.

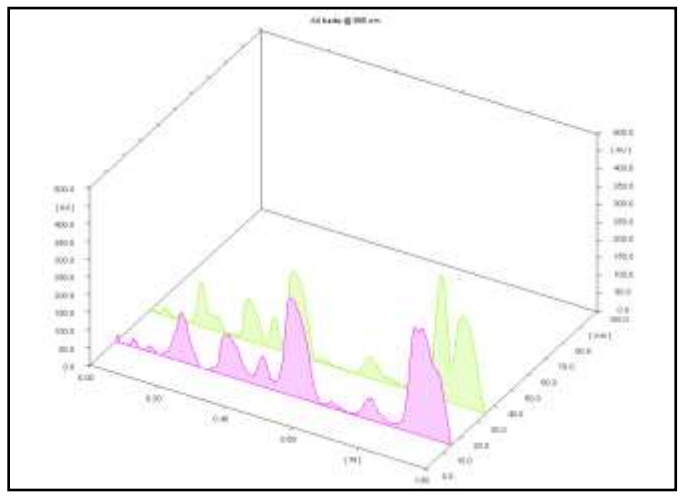

Fig. 14: 3D view of Methanol direct extract of *R. emodi* at 366 nm.

Table 1: Results of Physico-chemical analysis.

S. No.	Parameter	Value
1.	**Extractive Values (Cold extract)**	**% Extractable matter**
(i)	Petroleum ether extract	5.8
(ii)	Chloroform extract	13.8
(iii)	Methanol cold extract	12.4
(iv)	Aqueous extract	11.5
2.	**Extractive Values (Hot extract)**	**% Extractable matter**
(i)	Petroleum ether extract	6.9
(ii)	Chloroform extract	14.5
(iii)	Methanol extract	23.6
(iv)	Aqueous extract	16.1
3.	**Successive extraction**	**% Extractable matter**
(i)	Petroleum ether extract	6.9
(ii)	Chloroform extract	12.6
(iii)	Methanol extract	17.5
(iv)	Aqueous extract	12.2
4.	**Ash value**	**% Total ash**
(i)	Total ash	4
(ii)	Acid insoluble ash	0.4
(iii)	Water soluble ash	2.6
5.	**Loss on drying in crude drug (%)**	5.8
6.	**pH of the drug 1%**	4.96
7.	**pH of the drug 10%**	5.16

Table 2: Results of Phytochemical analysis.

Constituent	Extract			
	Petroleum ether	**Chloroform**	**Methanol**	**Aqueous**
Alkaloids	Absent	Absent	Absent	Absent
Carbohydrates	Absent	Absent	Present	Present
Phenolic compounds	Absent	Present	Present	Present
Flavonoids	Absent	Present	Present	Present
Proteins and amino-acids	Absent	Absent	Absent	Present
Lipids / Fats	Present	Absent	Absent	Absent

Table 3: Results of fluorescence analysis of crude drug powder.

S. No.	Treatment	Day light	UV light (254 nm)	UV light (366 nm)
1.	Powder as such	Brown	Dark brown	Brown
2.	Distilled water	Brown	Yellowish brown	Dark yellow
3.	1N NaOH	Reddish brown	Brown	Reddish brown
5.	HNO_3	Light yellowish brown	Yellowish brown	Greenish Brown
6.	H_2SO_4	Blackish brown	Yellow brown	Greenish brown
7.	Iodine	Blackish brown	Greenish yellow	Light greenish yellow
8.	Conc. HCl	Brown	Yellowish green	Light green
9.	Ferric chloride	Yellowish brown	Dark yellowish brown	Dark yellow
10.	Ammonia	Blackish green	Light greenish black	Dark green
11.	Glacial acetic acid	Light brown	Brown	Light yellowish brown
12.	Picric acid	Brown	Light yellow	Greenish yellow

Table 4: Phenolic content of *Rheum emodi.*

S. No	Concentration of the standard solution (µg/mL)	Absorbance (765 nm)
1.	25	0.4523
2.	50	0.7176
3.	100	1.1533
4.	150	1.6103
5.	200	2.0516
6.	300	2.9666
7.	Sample	0.867

Table 5: Total flavonoid content of *Rheum emodi.*

S. No.	Concentration of the Standard solution (µg/mL)	Absorbance (415 nm)
1.	10	0.365
2.	20	0.435
3.	30	0.517
4.	40	0.574
5.	50	0.640
6.	60	0.701
7.	70	0.773
8.	80	0.847
9.	100	0.927
10.	Sample	0.498

Table 6: Fingerprint of different extracts at 254 nm of *Rheum emodi.*

S. No	Extract	Solvent system	No. of peaks observed (Rf values)
1	Petroleum ether	Toluene: ethyl acetate (6: 4)	5 (0.03, 0.13, 0.18, 0.73, 0.92)
2	Chloroform	Chloroform: methanol (9: 1)	11 (0.02, 0.05, 0.07, 0.11, 0.21, 0.25, 0.29, 0.41, 0.49, 0.66, 0.91)
3	Ethyl acetate	Chloroform: methanol (5: 1)	10 (0.03, 0.08, 0.12, 0.18, 0.26, 0.34, 0.41, 0.63, 0.76, 0.87)
4	Methanol	Chloroform: methanol (9: 1)	8 (0.04, 0.07, 0.1, 0.25, 0.4, 0.44, 0.68, 0.93)
5	Water	Toluene: ethyl acetate: methanol (7: 2: 1)	9 (0.03, 0.18, 0.21, 0.28, 0.36, 0.59, 0.78, 0.86, 0.91)
6	Methanol direct	Chloroform: methanol (5: 1)	11 (0.05, 0.09, 0.15, 0.2, 0.29, 0.37, 0.43, 0.58, 0.66, 0.77, 0.87)

Table 7: Fingerprint of different extracts at 366 nm of *Rheum emodi.*

S. No	Extract	Solvent system	No. of peaks observed (Rf values)
1	Petroleum ether	Toluene: ethyl acetate (6: 4)	3 (0.03, 0.73, 0.92)
2	Chloroform	Chloroform: methanol (9: 1)	9 (0.01, 0.05, 0.07, 0.11, 0.21, 0.26, 0.3, 0.42, 0.49)
3	Ethyl acetate	Chloroform: methanol (5: 1)	10 (0.03, 0.09, 0.12, 0.18, 0.27, 0.34, 0.41, 0.63, 0.71, 0.87)
4	Methanol	Chloroform: methanol (9: 1)	8 (0.02, 0.04, 0.07, 0.1, 0.26, 0.29, 0.67, 0.93)
5	Water	Toluene: ethyl acetate: methanol (7: 2: 1)	7 (0.19, 0.21, 0.28, 0.52, 0.58, 0.86, 0.91)
6	Methanol direct	Chloroform: methanol (5: 1)	10 (0.05, 0.09, 0.15, 0.2, 0.29, 0.37, 0.43, 0.52, 0.66, 0.87)

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