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Extraction and Phtytochemical screening of rhizome extracts of *Rheum emodi*: A Himalayan rhubarb

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Abstract
The aim of present study was to investigate the moisture content, ash value and presence of phytochemicals in the rhizome extracts of *Rheum emodi.* The rhizome powder was analysed for moisture content and ash value detection. The extracts of rhizome were screened for the presence of phytochemicals using standard procedure. The moisture content of the powdered rhizome was 0.50 and total ash value was 6.9%. The phytochemical screening of extract reveals the presence of Carbohydrates, Fats, Phytosterols, Saponins, Flavonoids, Phenols and glycosides, while amino acids, proteins and alkaloids were absent. The presence of these phytochemicals in *Rheum emodi* may serve as a potential source of useful drugs in future.

Keywords: moisture content, ash value, phytochemical screening

1. Introduction
Rheum emodi Wall. ex Meissn, is a leafy perennial herb [1], 1.5-3.0 m in height [2] belongs to the family Polygonaceae. It grows in the alpine zone on rocky soils, moraines and cervices [3] this stout herb is distributed in the temperate and subtropical region from Kashmir to Sikkim in India at altitudes ranging from 2800 m to 3800 m **[4]**. The flowers are small, greenish-white, and borne in large compound leafy inflorescences. Fruit ovoid-oblong, 13 mm long, purple, base cordate, apex notched, wings narrower than the disk [5]. Root of Indian Rhubarb is darker, inferior in aroma, coarser and untrimmed, is not decorticated. Fresh rhizome is 6 to 12 inches long, and the freshly fractured surface is dull orange to yellowish brown [6]. This herb has been included in Indian Pharmacopeia, since it is an important medicinal plant and explored traditionally in many system of medicine like Ayurveda and Unani systems of medicine [7]. It is used to treat kidney stones and other liver associated diseases like gout and jaundice [8]. The Roots are regarded as panacea in local home remedies and is used in stomach problems, cuts, wound, and muscular swelling, tonsillitis and mumps [9]. Some persons chew the root [10]. Roots powder are used for cleaning teeth and sprinkled over ulcers for quick healing [11]. Leaves and leaf-stalk, flowers are cooked as vegetable [12]. It is however stated that cook stalks act as a powerful purgative [11]. Besides the medicinal uses, it is also used for coloration of textile and wooden material [13]. In this study, the phytochemical screening of extracts of *Rheum emodi* rhizome was carried out with a view to provide scientific basis on the claim by traditional healers of its uses in traditional medicine in the context of the continued search for active therapeutic agents from plants.

Taxonomic classification of Herb

Kingdom	*Plantae*
Division	Magnoliophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Polygonaceae
Genus	Rheum
Species	*emodi*
Botanical name	*Rheum emodi*
Vernacular names	
English	Himalayan Rhubarb [14]
Sanskrit	Pita [15]
Kashmiri	Pumbehakh [16]

Fig 1: *Rheum emodi*

2. Materials and Methods
2.1 Collection and Identification of plant.
The rhizome of *Rheum emodi* was collected from Pabbar valley of Himachal Pradesh in the month of September-October 2017. The plant was identified by ---------- -------- Head of the PG Department of Botany, ---------- College Bhopal (MP). The voucher specimen no. …….. are kept in the herbarium of Bhoj Mahavidyalaya Bhopal (M.P.) future reference.

2.2 Processing of plant material
The collected rhizomes were washed thoroughly under running tap water and then were rinsed in distilled water, they were allowed to dry for some time. Then these rhizomes were shade dried for about 5 to 7 weeks and coarse powder was made with the help of a grinder.

2.3 Weight of plant material after drying and percentage loss.
The weight and percentage loss of the dried rhizome was calculated by the following formula and shown in the (Table No. 1)

$$\% \text{ loss on drying} = \frac{\text{loss in weight of sample}}{\text{Weight of sample}} \text{ X } 100$$

2.4 Organolyptic properties of plant material
The rhizomes coarse powder was used to determined the various organolyptic properties *viz* colour, odour, taste and texture shown in (Table No.2)

2.5 Determination of moisture content or Loss on drying (LOD)
2 gm of rhizome powder was taken in a Petri dish. The powder was distributed evenly. Then Petri dish was kept open, in an oven and the sample was dried at a temperature between 100 to 105^0C for 2 h until a constant weight was recorded. Then it was cooled in a dessicator at room temperature and weighed. (Table No. 3) % Loss on drying was calculated by using the following formula.

$$\% \text{ loss on drying} = \frac{\text{loss in weight of sample}}{\text{Weight of sample}} \text{ X } 100$$

2.6 Determination of Total ash value
10 gm rhizome powder was placed in a previously ignited crucible of silica. The material was spreaded in an even layer and ignited by gradually increasing the heat to 500-600 ^{o}C until it became white, which indicates the absence of carbon. Cooled in a dessicator and weighted. The percentage of ash content was calculated by the following formula (Table No. 4)

$$\% \text{ Ash content} = \frac{\text{weight of ash}}{\text{Weight of powder sample}} \text{ X } 100$$

2.7 Extraction of plant material
The coarse powder of rhizome was extracted sucessively in Soxhlet apparatus using solvents viz petroleum ether, chloroform, ethyl acetate and 90% ethanol. The extraction was done for 48 hours in each solvent. The crude extracts thus obtained were then filtered through Whatmann filter paper No. 1. The filtered extracts were concentrated under reduced pressure in a rotary vaccum evaporator. The amount of crude extracts obtained thus weighed and yield was calculated (Table-5)

$$\% \text{ Yield} = \frac{\text{weight of extract}}{\text{Weight of powder}} \text{X } 100$$

2.8 Solublity and organoleptic evaluation of extracts
The organolyptic and solubility properties of extracts were analysed which are shown in (Table No.6) and (Table No.7) respectively.

2.10 Phytochemical screening of extracts
Phytochemical screening of the extracts was carried out according to the standard procedures [17, 18]. The Petroleum ether, chloroform, ethyl acetate and 90% ethanol extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. Anthraquinones, Terpinoids, Steroids, Flavonoids, Carbohydrates, glycosides, Saponins and phenols. (Table 8)

2.9.1 Test for alkaloids
Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of the yellow coloured precipitate. 2.9.2 Test for carbohydrates Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Molish test Treat the test solution with few drops of alcoholic alpha-napthol. Add 0.2ml of Conc. Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction.

2.9.3 Test for Glycosides
Extracts were hydrolysed with dilute HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthraquinone glycosides.

Legal's Test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

2.9.4 Test for saponins
Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

2.9.5 Test for phytosterols
Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicates the presence of triterpenes.

2.9.6 Test for phenols
Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.9.7 Test for flavonoids
Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.

Shinoda test: Crude extract was mixed with few fragments of magnesium ribbons and conc. hydrochloric acid was added drop wise. Pink scarlet color appears after few minutes, indicated the presence of flavanoids.

2.9.8 Test for Fats
Saponification Test To the petroleum ether extracts, few drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein were added and heated on a water bath for 1-2 hours. Formation of soap and/or partial neutralization of alkali indicated the presence of fixed oils and fats.

2.9.9 Test for Amino acid and Proteins
Xanthoproteic Test: The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins

Ninhydrin test: To the 3ml of crude sample, 3 drops 5% ninhydrin was mixed and heated for 10min in boiling water bath. Purple or bluish color indicated presence of amino acids

3. Results and discussion
The weight loss after drying the rhizome is 58.33%. Moisture content of the powdered rhizome is 0.50, Total ash value 6.9%. The yield of extracts varies from petroleum ether (0.052%) to 90% ethanol (3.2%) shown in (Table No.5). This variation in the yield of extracts may be due to the solvation of phyto-constituents in different polarities of the solvents. The solubilities of extracts are shown in (Table No.7). The results of phytochemical screening reveled the presence of Carbohydrates, Fats, Phytosterols, Saponins, Flavonoids, Phenols and glycosides (Table No.8) Flavonoids were detected in ethyl acetate and 90% ethanol only. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties [19]. They exist widely in the plant kingdom and displayed positive correlation between increased consumption of flavonoids and reduced risk of cardiovascular and cancer diseases [20]. Correspondingly, these extracts also show positive results for phenolic compounds. The phenolic compounds are aromatic secondary metabolites that impart colour, flavour and associated with health benefits such as reduced risk of heart and cardiovascular diseases [21, 22]. Phenolic compounds account for most of the antioxidant activities in plants [23]. Terpenoids were detected in chloroform extract. terpenoids such as triterpenes, sesquiterpenes and diterpenes have been referred to as antibiotics, insecticidal, anthelmintic and antiseptic in pharmaceutical industry [24, 25]. Ethyl acetate and 90% ethanol extract also showed the positive result for saponins. These are glycosides of steroids, steroid alkaloids found in plants, especially in the plant skins where they form a waxy protective coating. Saponins are helpful in lowering cholesterol, as antioxidant and anti-inflammatory agents [26].

4. Conclusion
The phytochemical analysis result revealed that extracts gave a positive test for a particular class of secondary metabolites whereas other extract gave negative test. The results obtained exposed the presence of medicinally important phytochemicals constituents in the rhizomes extract of *Rhium emodi*. Presence of these phytochemicals give physiological as well as medicinal properties to the plant. As a result, extracts from the plant studied might be seen as a good source for useful drugs. More research should be carried out to purify, isolate and characterize the active constituents which may be responsible for the medicinal value of *Rhium emodi.*

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Table 1: Weight of plant material after drying and percentage loss.

Description	Weight in gms.	% loss
Weight of plant material in wet, fresh condition	2400	58.33
Weight of plant material after drying at room temperature	1000	
Loss in weight on drying	2400-1000=1400	

Table 2: Showing organoleptic evaluation of *Rheum emodi* rhizome powder

Parameters	***Rheum emodi* rhizome**
Colour	Brown
Odour	Aromatic
Taste	Bitter
Texture	Coarse

Table 3: Showing moisture content of rhizome powder of *Rheum emodi*

Weight of powered material	Loss of weight	Moisture content
2 gm.	0.0101 gm.	0.50

Table 4: Showing the total ash content of plant material

Name of plant	Weight of powered material	Weight of (ash)	% Ash content
Rheum emodi	10 gm.	0.69 gm.	6.9

Table 5: Showing % Yield of solvent extracts of plant material.

Weight of powdered material (gm)	Extract	Weight of extract (gm)	% yield
1000	Petroleum ether	0.52	0.052
	Chloroform	1.11	0.11
	Ethylacetate	1.54	0.15
	90% Ethanol	32	3.2

Table 6: Showing organoleptic evaluation of extracts.

	Colour	Odour	Consistency
Petroleum ether	Light brown	Aromatic	Somewhat sticky
Chloroform	Light brown	Astringent	Non sticky
Ethylacetate	Yellow	Astringent	Non sticky
90% Ethanol	Yellow brown	Astringent	Little bit sticky

Table 7: Solublity of extracts in solvents

S. No.	Solvent	Petroleum ether	Chloroform	Ethylacetate	90% Ethanol
1	Hexane	Freely Soluble	Slightly soluble	insoluble	insoluble
2	Chloroform	Slightly soluble	Freely soluble	soluble	Slightly soluble
3	DMSO	Soluble	Freely soluble	Freely soluble	Soluble
4	Acetone	Partially soluble	Slightly souble	Partially soluble	soluble
5	Ethanol	insoluble	Partially soluble	soluble	soluble
6	Methanol	insoluble	Partially soluble	soluble	soluble
7	Water	insoluble	Slightly soluble	Moderately soluble	soluble

Table 8: Phytochemical screening of *rheum emodi* rhizome extracts.

Phytoconstituents	Test	Extracts			
		Pet-ether	Chloroform	Ethyl acetate	90% Ethanol
Alkaloids	Dragendroff	_	_	_	-
	Hager	_	_	_	
Carbohydrates	Fehling	+	+	_	-
	Molish	+	+	_	-
Glycosides	Modified Borntrager	_	+	_	-
	Legal	_	+	+	-
Saponins	Froth	_	_	+	+
Phytosterols	Salkowski		+	-	-
Phenols	Ferric Chloride	_	_	+	+
Flavonoids	Alkaline Reagent	_	_	+	+
	Shinoda	-	-	+	+
Fats	Saponification	+	-	-	-
Amino acid and Proteins	Xanthoproteic	_	_	_	-
	Ninhydrin	_	_	_	-

Key: + = present - = absent

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