Section A-Research paper
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Analytical profiling of *Rheum palmatum* using Chromatographic Technique

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ABSTRACT

The current study focuses on analytical approaches, which involve isolating active components from the *Rheum palmatum* plant family *Polygonaceae*. This study emphasises that *Rheum palmatum* plant was subjected to fractionalization by the column chromatography. During the optimization of the chromatography conditions, various assays were conducted with a gradient of Hexane: Ethyl acetate as the mobile phase.The eluted fractions were run in TLC mobile phase with the solvent Hexane: ethyl acetate '7:3'. The fraction showed Rf value equal to standard Aloin in TLC were combined and crystallized. The characterisation technique like UV-visible Spectroscopy and FTIR (Fourier Transform Infrared spectroscopy)are used to portrait that, the probable compound may be the substitute of Aloin.

Keywords: *Rheum palmatum*, *Polygonaceae*, isolation

INTRODUCTION

The use of *Rheum palmatum* root in ancient Chinese medicine included treating stomach disorders, acting as a "cathartic" (a substance that relieves severe constipation), and treating "fevers and edoema" (swelling brought on by fluid retention in body tissues) with a poultice (a preparation of fresh, moistened, or crushed dried herbs applied externally) **(Miraj 2016; Grabley et al., 1991; Dawson et al., 1992).** The leaves are poisonous. The leaves contain a lot of oxalic acid. Oxalic acid, while completely harmless in moderation, can lock up several minerals in the body (particularly calcium), which might result in nutritional deficiencies. Oxalic acid content in the plant will decrease during cooking. People who have a history of rheumatism, arthritis, gout, kidney stones, or hyperacidity should exercise extra caution when consuming this plant because it can make their condition worse (**Cheng et al., 2019; Shang et al., 2019; Wang et al., 2010; Arokiyaraj et al., 2017).** Medicinal plants contain

inalienable dynamic fixings to remedy infection or soothe torment. It has been closely observed how traditional medicines and medicinal plants are used as therapeutic agents in the majority of underdeveloped countries to support excellent wellbeing.Consumers all over the world are becoming moreconscious of the nutrition value, health benefits andsafety of their food and its ingredients. In addition,there is a preference for natural functional foodingredients that are believed to be safer, healthier andless subject to hazards than their artificial counterparts **(Jha et al., 2010).** Theworld wellbeing organization assessed that 80% of the populace of creating countrieslies on conventional medications, generally home grown plant drugs for their essential wellbeing care (**Sharma et al., 2014**). India maybe the biggest maker of restorative herbs and is called Botanical Plant ofthe World. Restorative herbs have been in utilize for thousands of a long time, in one frame oranother, beneath the inborn frameworks of pharmaceutical like Ayurveda, Sidha and Unani (**Mehrola et al., 1990).**The term "traditional medicine" alludes to ways of securing and reestablishing wellbeing thatexisted some time recently the entry of modern medicine.These health philosophies are indigenous to each nation, as the name implies, and have been passed down through the generations. A conventional framework requires to meet wants of the nearby communities formany centuries (**Mehrola et al., 1990**).The helpful property of the therapeutic plants is the result of the active constituents; these pharmacologically dynamic constituents were synthesized and put away totally different plant parts. Analysts are attempting to investigate this treasure of bio dynamic particles to change over the characteristic chemicals in a shape valuable for cutting edge frameworks of pharmaceutical (**Tachjian et al., 2010**). Crude extract mixtures of plant are better than pure isolated chemicals. Several biologically dynamic compounds in a plant work together to deliver more prominent impact then single chemical on its claim. The blend of chemicals found in herbs can be more potent than the single filtered fixing so cherished of drugs companies. Chemical partnerships explain why entire herbs can work superior than single decontaminated fixings **(Mehrola et al., 1990**). A moment approach is essentially to gather each promptly accessible plant, get ready extricate and test each extract for one (or) more types of pharmacological activity. This random collection, broad screening method is a reason able approach that eventually should produce valuable drugs, but it is unexpected as the accessibility of satisfactory discoveries and appropriate predict able bioassay frameworks (**Handa et al., 1992**). Many herbs, such as goldenseal and garlic, have characteristic anti-microbial and antiviral propertiesas well. Licorice relieves the throat, ginger avoids blood clots and reduces the chance of heart disease and her balte as help reduce tension. There are herbs that can address respiratory,

gastrointestinal, neurological and sexual issues as well. In many cases natural herbal remedies can even help in managing the side effects of aggressive treatments like chemotherapy. In rundown, there's much to pick up from the utilize of natural remedies. They offer a secure and common elective to routine pharmaceutical and are oftentimes more viable (**Handa et al., 1992).**

Materials and methods

Collection and authentication of plant material

The medicinal plant *Rheum palmatum* extracts was collected from Bhopal; M.P. Authentication of parts of medicinal plant *Rheum palmatum* extract was performed by a plant taxonomist in order to confirm its identity and purity.

Extraction of plant by Soxhlet extraction method

Coarsely powered parts of *Rheum palmatum* was then extracted by successive extraction using different organic solvents, defatted with petroleum ether and successively extracted with methanol for 36 hrs using soxhlet apparatus **(Alara et al., 2019)**.

Phytochemical screening of the extract

A variety of phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were qualitatively analysed in the extract of *Rheum palmatum* (**Kokateet al.,2006**).

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study was of analytical grade.

Isolation

Thin Layer Chromatography

Thin Layer Chromatography of *Rheum palmatum* extract was carried out on TLC plates of silica gel 60 F_{254} pre coated with layer thickness of 0.2 mm using different solvent systems. Spots on TLC plates were visualised with spraying reagent: sulphuric acid solution, then in UV light. R_f values were calculated **(Kumar et al., 2018).**

Column chromatography

Methanol extract was subjected to silica gel column chromatography for isolation of bioactive components from *Rheum palmatum* extract. Gradient elusion technique was followed for column chromatography. The column was eluted with Hexane: ethyl acetate '7:3'and number of elutes were collected **(Srivastava et al., 2021)**.

Characterization-

UV-visible Spectroscopy

The isolated fraction of sample was diluted to 1:10 with the same solvent. The extract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1800) and the characteristic peaks were detected and recorded **(Perkampus et al., 2013)**.

FT-IR

To establish the presence of the functional groups, FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer **(Luciene et al., 2008).**

RESULT

Plant Collection

Table 1 Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Rheum palmatum*	Roots	346 gm

The total weight of the *Rheum palmatum* root powder used was 346 gm.

Percentage yield

Table 2 Percentage yield of *Rheum palmatum* root extract

S. No.	Solvent	Color of extract	Theoretical weight (gm)	Yield in gms	% Yield
1.	Pet. Ether	Transparent	346	No. yield	-
2.	Ethyl acetate	Yellow	322.16	1.97	0.611
3.	Methanol	Brown	301.56	5.87	1.94

After performing extraction of *Rheum palmatum* root powder, the percentage yield of extracts in different solvents like Ethyl acetate, and methanol were found to be 0.611% (1.97gm), and 1.94% (5.87gm) respectively.

Qualitative Phytochemical Analysis of *Rheum palmatum* root extracts

Table 3 Qualitative phytochemical analysis of *Rheum palmatum* root extracts

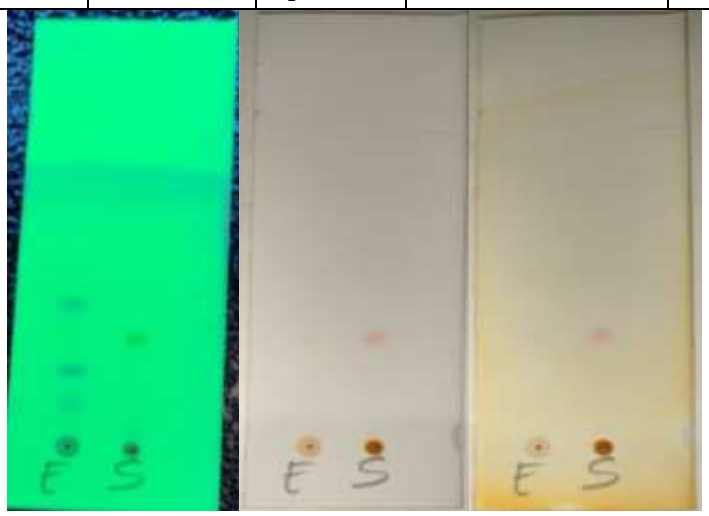
S. No.	Experiment	Result	
		Ethyl acetate	Ethanol
Test for Carbohydrates			
1.	Molisch's Test	-	-
2.	Fehling's Test	-	-
3.	Benedict's Test	-	-
4.	Bareford's Test	-	-
Test for Alkaloids			
1.	Mayer's Test	+	+
2.	Hager's Test	+	+
3.	Wagner's Test	+	+
4.	Dragendroff's Test	+	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	+	+
Test for Flavonoids			
1.	Lead Acetate Test	+	+
2.	Alkaline Reagent Test	+	+
3.	Shinoda Test	+	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	+	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+
4.	Dilute Iodine Solution Test	+	+
Test for Saponins			
1.	Froth Test	+	-
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	-	+

3.	Million's Test	-	+
Test for Glycosides			
1.	Legal's Test	-	+
2.	Keller Killani Test	-	+
3.	Borntrager's Test	-	+

Qualitative phytochemical screening of methnaolic extract of *Rheum palmatum* showed the presence of phytoconstituent such as terpenoids, flavonoids, alkaloids, glycosides, steroids and phenols.

Table 4 TLC of *Rheum palmatum* extract

S. No.	Extract	Number of spots		R_f value	Solvent system
1.	Methanolic extract	4	Spot 1	3.9/4.2 = 0.92	Petroleum ether: Ethyl acetate: methanol (5:4:1)
			Spot 2	3.0/4.2 = 0.71	
			Spot 3	2.1/4.2 = 0.50	
			Spot 4	1.3/4.2 = 0.30	
		4	Spot 1	3.0/3.9 = 0.76	Hexane: Ethyl acetate: one drop of acetic acid (7:3)
			Spot 2	2.4/3.9 = 0.61	
			Spot 3	1.6/3.9 = 0.41	
			Spot 4	0.8/3.9 = 0.20	
		4	Spot 1	2.6/4.0 = 0.65	Toluene: Ethyl acetate (9:1)
			Spot 2	2.0/4.0 = 0.50	
			Spot 3	1.3/4.0 = 0.32	
			Spot 4	0.5/4.0 = 0.12	



a) **b)** **c)**

Figure 1: TLC of *Rheum palmatum* methanolic extract, in a) UV light, b) alcoholic KOH solution, and c) Iodine in Hexane: Ethyl acetate: one drop of acetic acid (7:3)solvent system.

Table 5: Fractions isolated in different Solvents using Column Chromatography

S. No.	Solvent	Elutes	Colour	Fractions
1	Hexane (100%)	E1	Colourless	F1
2	Hexane (100%)	E2	Colourless	
3	Hexane (100%)	E3	Colourless	
4	Hexane (100%)	E4	Colourless	
5	Hexane: Ethyl acetate (90:10)	E5	Colourless	F2
6	Hexane: Ethyl acetate (90:10)	E6	Colourless	
7	Hexane: Ethyl acetate (90:10)	E7	Colourless	
8	Hexane: Ethyl acetate (90:10)	E8	Colourless	
9	Hexane: Ethyl acetate (90:10)	E9	Colourless	
10	Hexane: Ethyl acetate (90:10)	E10	Colourless	
11	Hexane: Ethyl acetate (90:10)	E11	Colourless	
12	Hexane: Ethyl acetate (90:10)	E12	Colourless	
13	Hexane: Ethyl acetate (80:20)	E13	Colourless	F3
14	Hexane: Ethyl acetate (80:20)	E14	Colourless	
15	Hexane: Ethyl acetate (80:20)	E15	Colourless	
16	Hexane: Ethyl acetate (80:20)	E16	Colourless	
17	Hexane: Ethyl acetate (80:20)	E17	Colourless	
18	Hexane: Ethyl acetate (80:20)	E18	Colourless	
19	Hexane: Ethyl acetate (80:20)	E19	Colourless	
20	Hexane: Ethyl acetate (70:30)	E20	Light Yellow	F4
21	Hexane: Ethyl acetate (70:30)	E21	Light Yellow	
22	Hexane: Ethyl acetate (70:30)	E22	Light Yellow	
23	Hexane: Ethyl acetate (70:30)	E23	Light Yellow	
24	Hexane: Ethyl acetate (70:30)	E24	Light Yellow	
25	Hexane: Ethyl acetate (70:30)	E25	Light Yellow	
26	Hexane: Ethyl acetate (70:30)	E26	Light Yellow	
27	Hexane: Ethyl acetate (60:40)	E27	Colourless	F5
28	Hexane: Ethyl acetate (60:40)	E28	Colourless	
29	Hexane: Ethyl acetate (60:40)	E29	Colourless	

30	Hexane: Ethyl acetate (60:40)	E30	Colourless	
31	Hexane: Ethyl acetate (50:50)	E31	Light Yellow	F6
32	Hexane: Ethyl acetate (50:50)	E32	Light Yellow	
33	Hexane: Ethyl acetate (50:50)	E33	Light Yellow	
3	Hexane: Ethyl acetate (50:50)	E34	Light Yellow	
23	Hexane: Ethyl acetate (40:60)	E35	Light Yellow	F7
24	Hexane: Ethyl acetate (40:60)	E36	Light Yellow	
25	Hexane: Ethyl acetate (40:60)	E37	Light Yellow	
26	Hexane: Ethyl acetate (40:60)	E38	Light Yellow	
29	Hexane: Ethyl acetate (30:70)	E39	Dark Yellow	F8
30	Hexane: Ethyl acetate (30:70)	E40	Dark Yellow	
31	Hexane: Ethyl acetate (30:70)	E41	Dark Yellow	
32	Hexane: Ethyl acetate (30:70)	E42	Dark Yellow	
33	Hexane: Ethyl acetate (20:80)	E43	Dark Yellow	F9
34	Hexane: Ethyl acetate (20:80)	E44	Dark Yellow	
35	Hexane: Ethyl acetate (20:80)	E45	Dark Yellow	
36	Hexane: Ethyl acetate (20:80)	E46	Dark Yellow	

Table 6: Total yield of fractions isolated from column chromatography

S. No.	**Fractions**	**Yield (mg)**
1	F1	No yield
2	F2	No yield
3	F3	25
4	F4	63
5	F5	15
6	F6	33
7	F7	71
8	F8	110
9	F9	160

Table 7: TLC of finalised fraction (F4)

S. No.	**Fractions**	**spots**	**R_f value**	**Solvent system**
1	Fraction 4	1	0.61	Hexane: Ethyl acetate: one drop of acetic acid (7:3)

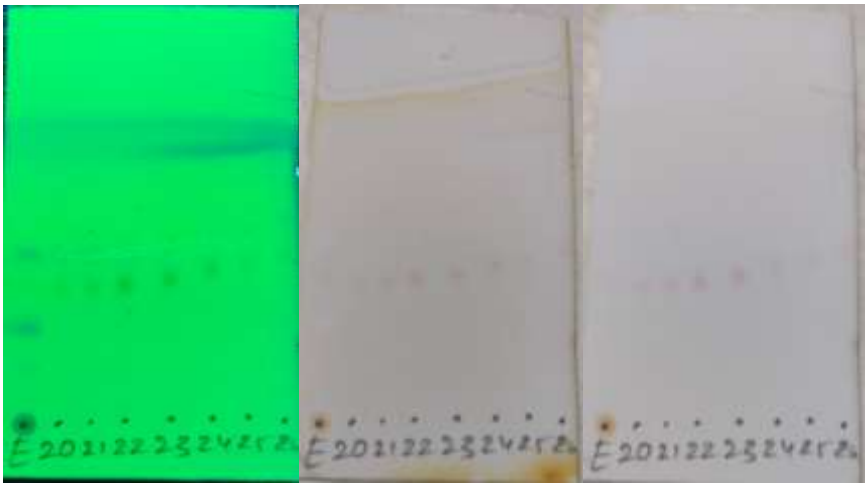
TLC of *Rheum palmatum* methanolic extract was performed on different solvent systems (solvent system was selected on the basis of literature survey). TLC performed in Hexane: Ethyl acetate: one drop of acetic acid (7:3) showed maximum number of spots that were clearly visible. The fractions/elutes obtained from silica gel column chromatography of

Rheum palmatum methanolic extract were tested for the detection of various phytocompounds using TLC. The phytocompounds showing the same R_f values were pooled into a single fraction. TLC of the isolated components was performed and the R_f value was compared with the standard anthraquinone (Aloin). F4 fraction was found to show the same R_f value.

Table 8: FT-IR Interpretation

S. No.	Peak (cm^{-1})	Reference Range (cm^{-1})	Functional group present	Name of functional group
1	3448.51	3500–3200	O-H stretching	alcohols
2	2925.77	3000–2850	C-H stretching	alkanes
4	1718.13	1740–1720	C=O stretch	carbonyls
5	1458.84	1500–1400	C–C stretch	aromatics
6	1100.09	1320–1000	C–O stretch	alcohols

Figure 2: TLC of isolated fraction(F4) in a) UV light, b) Iodine, and c) visible light in Petroleum ether: ethyl acetate: methanol (4:4:2) solvent system.



a) **b)** **c)**

Figure: 3 UV spectra of isolated fraction (F7)

The results show the UV spectra obtained from the isolated fraction (F4) of *Rheum palmatum* extract. The peak was observed is 431.0 nm.

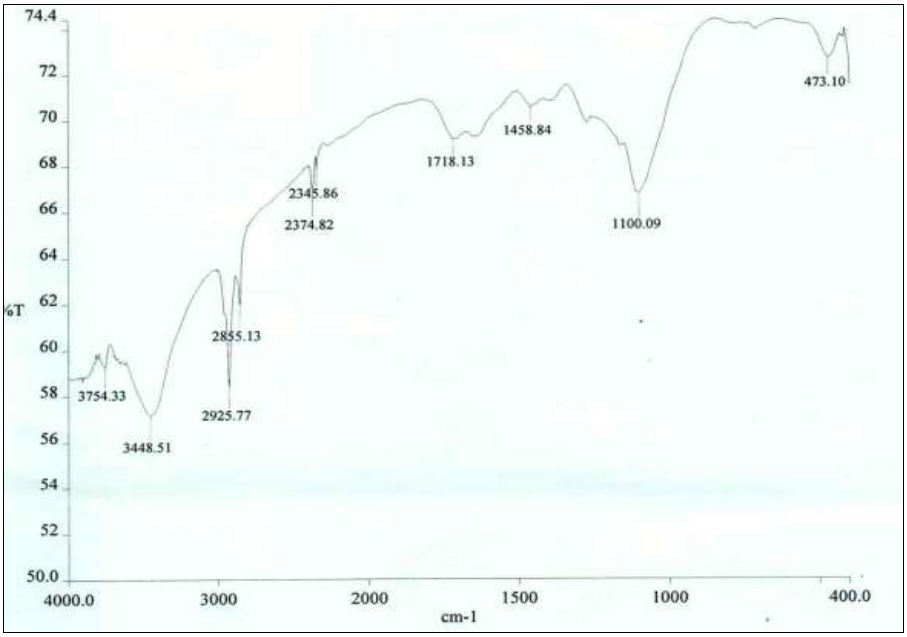


Figure 3: FT-IR spectra of isolated fraction (F4)

The IR spectrum of isolated fraction (F4)was interpreted and the functional groups were identified. It confirms that the main functional group alcohol, carboxylic acid and aromatic ring are present in the *Rheum palmatum* extract.

DISCUSSION

Qualitative phytochemical screening of methnaolic extract of *Rheum palmatum* showed the presence of phytoconstituent such as terpenoids, flavonoids, alkaloids, glycosides, steroids and phenols.TLC analysis of the methanolic extract from *Rheum palmatum* involved the use of different solvent systems, which were selected based on a comprehensive review of existing literature.

The most effective separation was achieved using a Hexane:Ethyl acetate (7:3) solvent with the addition of one drop of acetic acid, resulting in the highest number of well-defined spots.The fractions obtained through silica gel column chromatography of the methanolic extract of *Rheum palmatum* were subjected to TLC to detect various phytochemical compounds. Subsequently, a separate TLC analysis was performed on the isolated components, and their Rf values were compared to a standard anthraquinone compound known as Aloin. It was observed that the F4 fraction exhibited the same Rf value as Aloin.

The results include the UV spectra obtained from the isolated F4 fraction of the *Rheum palmatum* extract, revealing a peak at 431.0 nm. In the IR spectrum of the isolated F4 fraction, a thorough analysis was conducted to identify the various functional groups. Notably, a significant peak corresponding to alcohol (–OH) groups was observed at 3448.51 cm-1, falling within the typical range of 3500–3200 cm-1. The stretching peak for alcohol (C-O) bonds was detected at 1100.09 cm-1, aligning with the expected range of 1320–1000 cm-1. Furthermore, a carbonyl (C=O) stretching peak was identified at 1718.13 cm-1, which falls within the standard range of 1740–1720 cm-1. The presence of alkane (C-H) stretching peaks at 3066.60 cm-1 further confirms the existence of key functional groups, including alcohol, carboxylic acid, and an aromatic ring, within the *Rheum palmatum* extract.

CONCLUSION

The characterisationHexane: ethyl acetate technique like UV-visible Spectroscopy and FTIR (Fourier Transform Infrared spectroscopy) are used to portrait that, the probable compound may be the substitute of Aloin. These results not only advance our understanding of the chemical composition of *Rheum palmatum* but also provide opportunities for future research and potential applications, including the exploration of a possible Aloin substitute across various contexts.

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