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Identification of Bioactive Phytoconstituents in Rheum Emodi via Gas Chromatography-Mass Spectrometry

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ABSTRACT

Rheum emodi Wall. (Polygonaceae), an important Himalayan medicinal herb, is traditionally valued for treating liver disorders, gastrointestinal problems, infections, inflammation, and other ailments. The present study investigated the phytochemical composition of *R. emodi* collected from the Siran Valley, Pakistan, with emphasis on comparing the rhizome (conventionally used part) with fruits, leaves, and callus. Methanolic extracts (2 g/250 mL) of each plant part were prepared using Soxhlet extraction and subjected to preliminary qualitative phytochemical screening. The chemical profiling was further performed through Gas Chromatography–Mass Spectrometry (GC–MS) using an Rxi®-5Sil MS capillary column, and compounds were tentatively identified by matching mass spectra with NIST and Wiley libraries. Phytochemical screening showed the widest metabolite diversity in rhizomes, including anthraquinones, while leaves and fruits showed strong presence of phenolics/tannins and flavonoids. GC–MS analysis revealed distinct organ-wise fingerprints; the rhizome was dominated by the anthraquinone chrysophanol (43.97% peak area) along with physcion, whereas leaves were enriched with fatty acid methyl esters and antioxidant-associated constituents such as squalene and vitamin E. Callus exhibited a comparatively reduced profile, indicating altered metabolite accumulation under in vitro conditions. These findings provide baseline chemical evidence supporting traditional use and highlight potential bioactive value in non-rhizome tissues.

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Keywords: *Rheum emodi*; GC–MS; anthraquinones; phytochemical screening; Siran Valley

1. Introduction

Rheum emodi Wall. (Polygonaceae), commonly known as Indian rhubarb, is a high-value medicinal herb of the Himalayan belt. In Pakistan, it occurs in suitable high-altitude niches of the north, including the Siran Valley region, where the temperate climate and mountainous ecology provide favorable conditions for its growth. The species is generally reported from Himalayan temperate to sub-alpine zones, commonly around ~2800–3000 m elevation.[1,2] Owing to its long-standing traditional use, R. emodi is employed for the management of a wide range of ailments, including jaundice, headache, migraine, paralysis, sciatica, asthma, diarrhea, cancer, and liver-related disorders.[3]

The rhizome is considered the most pharmacologically important part and is a rich source of anthraquinones such as emodin, emodin glycoside, chrysophanol, chrysophanol glycoside, physcion, aloe-emodin, and rhein.[4] These anthraquinones are not only utilized in various industries (e.g., textiles, paints, cosmetics, foods, and imaging applications), but are also associated with diverse biological activities, including antiarthritic, anti-inflammatory, antifungal, antiviral, antibacterial, antidiabetic, laxative, and neuroprotective effects.[5] Importantly, several anthraquinones from R. emodi have been linked with anticancer potential; for example, emodin and aloe-emodin have been reported to inhibit proliferation in human gastric cancer cell lines,[6] rhein has been associated with apoptosis induction in human colon cancer cells,[7] while physcion and chrysophanol have been implicated in anticancer responses in cervical and liver cancer models, respectively.[8,9] In addition to these, other phytoconstituents present in R. emodi are known to contribute to antioxidant activity, supporting its therapeutic relevance.[10]

With increasing concern over the adverse effects and limitations of some synthetic drugs, plant-derived therapeutics are gaining stronger attention, and the growing demand for herbal medicines underscores the need for systematic screening of medicinal flora for bioactive molecules. In this context, a review of available literature suggests that chemical investigations of R. emodi have largely remained

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focused on the rhizome, whereas the chemical composition of other plant parts is comparatively underreported. Therefore, the present study emphasizes the chemical characterization of R. emodi collected from the Siran Valley, Pakistan, aiming to isolate and identify important phytoconstituents not only from the conventionally used rhizome but also from other plant parts, thereby generating a more complete phytochemical profile to support future pharmacological exploration.

2. Material and method

2.1 Plant Collection

Fresh *Rheum emodi* plant samples (rhizome and aerial parts) were collected from the Siran Valley, Khyber Pakhtunkhwa, Pakistan, during the active growing season. The specimens were harvested from naturally growing populations, cleaned to remove soil and debris, and shade-dried. A representative sample was submitted for botanical authentication, and a voucher specimen was deposited for future reference.

2.2 Preparation of Crude Extract

Plant materials (rhizomes, fruits, leaves, and callus) were air-dried at room temperature and ground separately into fine powder. For each sample, 2 g of powder was extracted with 250 mL methanol using a Soxhlet apparatus. The resulting methanolic extract was concentrated under reduced pressure using a rotary evaporator at 70°C to obtain a solid residue. The dried crude extract was collected and stored at 4°C until further analysis. Prior to GC–MS, each extract was re-dissolved in methanol.

2.3 Phytochemical Analysis

Preliminary phytochemical screening of *Rheum emodi* methanolic extracts (rhizome, leaves, fruits, and callus) was performed to assess the presence of major secondary metabolite groups using standard qualitative tests. The extracts were screened for alkaloids, flavonoids, phenolics/tannins, saponins, terpenoids, steroids, glycosides, and anthraquinone derivatives. The outcomes were recorded as present (+) or absent (−) and used to support the GC–MS-based chemical profiling.

2.3.1 Preparation of test solution

Each crude methanolic extract was dissolved in methanol (or distilled water where required) to prepare a working solution of approximately **10 mg/mL**. All tests were

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performed in triplicate and results were recorded as **(+), (++), (+++)** or **(−)** based on intensity of reaction.

2.3.2 Qualitative phytochemical screening

The extracts were subjected to the following standard tests:

1) Alkaloids (Dragendorff's/Mayer's test): A small volume of extract was acidified with dilute HCl and filtered. The filtrate was treated with Dragendorff's reagent (orange precipitate) and/or Mayer's reagent (cream precipitate). Formation of precipitate indicated alkaloids.

2) Flavonoids (Alkaline reagent test / Shinoda test): For the alkaline reagent test, NaOH solution was added to the extract, producing an intense yellow color that disappeared on addition of dilute acid, indicating flavonoids. For Shinoda test, magnesium turnings and concentrated HCl were added; a pink/red coloration confirmed flavonoids.

3) Phenolics and Tannins (Ferric chloride test): A few drops of 1% $FeCl_3$ were added to the extract. A blue-green/black coloration indicated phenolic compounds and tannins.

4) Saponins (Froth test): The extract was vigorously shaken with distilled water. Persistent froth formation (for ~10–15 min) suggested the presence of saponins.

5) Terpenoids (Salkowski test): The extract was mixed with chloroform and concentrated H_2SO_4 was carefully added along the side of the tube. A reddish-brown ring at the interface indicated terpenoids.

6) Steroids (Liebermann–Burchard test): The extract was treated with acetic anhydride followed by concentrated H_2SO_4. A blue-green coloration indicated steroids.

7) Glycosides (Keller–Killiani test for cardiac glycosides): The extract was treated with glacial acetic acid containing $FeCl_3$ and then carefully layered with concentrated H_2SO_4. A brown ring at the interface indicated deoxy-sugar characteristic of cardiac glycosides.

8) Anthraquinones (Bornträger's test): The extract was shaken with benzene/chloroform and the organic layer was separated and treated with dilute ammonia. A pink/red coloration in the ammoniacal layer confirmed free

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anthraquinones. For combined anthraquinones, the extract was first hydrolyzed with dilute acid before performing the test.

2.4 GC–MS Analysis

GC–MS analysis of the methanolic extracts was carried out using a Shimadzu GC–MS system (QP2010 Plus, SHIMADZU, USA) fitted with an Rxi®-5Sil MS capillary column (5% phenyl, 95% dimethylpolysiloxane; 30 m × 0.25 mm i.d. × 0.25 µm film thickness). Helium (He) was used as the carrier gas at a flow rate of 1.21 mL/min. The oven temperature was initially set at 80°C and then increased to 250°C at a rate of 5°C/min. An injection volume of 1 µL was used. Mass spectra were recorded in the range of m/z 40–700. Compounds were identified by comparing their mass fragmentation patterns with those available in the National Institute of Standards and Technology (NIST) library and the Wiley Registry of Mass Spectral Data (Wiley, New York). The relative abundance of each detected constituent was calculated by peak area normalization and expressed as percentage peak area.

3. Results and Discussion

3.1 Phytochemical Analysis methanolic extracts of *Rheum emodi* (rhizomes, fruits, leaves, and callus)

Preliminary qualitative phytochemical screening of methanolic extracts of *Rheum emodi* (rhizomes, fruits, leaves, and callus) indicated the presence of multiple secondary metabolite groups with variation among plant parts. Overall, rhizomes showed the richest phytochemical profile, particularly for anthraquinones, phenolics/tannins, and terpenoid-type constituents. Leaves were notably positive for phenolics/tannins and flavonoids, reflecting their protective metabolic role. Fruits exhibited a moderate profile, whereas callus showed fewer detectable phytochemical groups, suggesting comparatively lower accumulation of certain specialized metabolites under in vitro conditions.

Table 1: ***Qualitative phytochemical profile (+ present, − absent)***

Phytochemical group	Rhizomes	Fruits	Leaves	Callus
Alkaloids	+	−	−	−
Flavonoids	+	+	+	−

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Phenolics / Tannins	+	+	+	+
Saponins	–	+	–	–
Terpenoids	+	+	+	–
Steroids	+	–	+	–
Glycosides	+	+	+	+
Anthraquinones (Bornträger's)	+	–	+	–

The qualitative phytochemical screening of *Rheum emodi* revealed clear variation in metabolite distribution among rhizomes, fruits, leaves, and callus, reflecting both organ-specific biosynthesis and differences in ecological function. Rhizomes showed the broadest phytochemical spectrum, testing positive for alkaloids, flavonoids, phenolics/tannins, terpenoids, steroids, glycosides, and anthraquinones[11]. This enriched profile supports the traditional preference for rhizomes as the principal medicinal part of *R. emodi*, and particularly aligns with the well-documented presence of anthraquinones that contribute to multiple pharmacological properties such as antimicrobial, anti-inflammatory, laxative, and anticancer effects. Leaves were also positive for flavonoids, phenolics/tannins, terpenoids, steroids, glycosides, and anthraquinones, suggesting a strong antioxidant and protective chemical makeup[12]. The dominance of phenolics/tannins and flavonoids in leaves is consistent with their role in combating oxidative stress and environmental exposure (UV, herbivory), and these constituents may contribute to anti-inflammatory and free-radical scavenging potential. Fruits exhibited a moderate phytochemical profile, being positive for flavonoids, phenolics/tannins, saponins, terpenoids, and glycosides, but negative for alkaloids, steroids, and anthraquinones. The presence of saponins in fruits may be linked to defense and antimicrobial protection, as well as possible bioactivities such as membrane-modulating and cholesterol-lowering effects. Interestingly, callus showed positivity mainly for phenolics/tannins and glycosides, while most other groups were absent. This reduction likely reflects in vitro growth conditions, where differentiation-related pathways and stress cues required for specialized metabolite accumulation are limited. Overall, the findings justify rhizome-focused use while also highlighting leaves and fruits as potential supplementary sources of bioactive constituents[13].

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3.2 GC–MS Analysis

Table 2: ***GC–MS Analysis of methanolic extracts of Rheum emodi (rhizomes, fruits, leaves, and callus)***

S.No.	Compounds	Formula	Molecular weight (g/mol)	Rhizome (Area %)	Fruit (Area %)	Leaves (Area %)	Callus (Area %)
1	Guanosine	$C_{10}H_{13}N_5O_5$	283.244	3.16	-	-	7.96
2	Beta-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162.141	3.38	15.41	-	1.53
3	Hexadecanoic acid, methyl ester	$C_7H_{14}O_5$	270.457	0.98	0.99	-	0.46
4	2,6,10-Dodecatrienal, 3,7,11-trimethyl	$C_{15}H_{24}O$	220.350	-	-	1.16	-
5	Beta.-Amyrone	$C_{23}H_{34}O_4$	372.505	-	-	-	0.22
6	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	2.81	7.41	-	-
7	Isopropyl palmitate	$C_{19}H_{38}O_2$	298.511	0.51	1.08	-	0.40
8	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	0.18	-	12.82	8.82
9	Eicosane	$C_{20}H_{42}$	282.556	-	0.61	-	-
10	Cholesterol chloroformate	$C_{28}H_{45}ClO_2$	449.12	-	-	2.07	-
11	Diosgenin acetate	$C_{29}H_{44}O_4$	456.66	-	-	6.98	1.26
12	Trans-2,3-dimethylthiane	$C_7H_{14}S$	130.24	-	-		9.02
13	Dicyclohexyl phthalate	$C_{20}H_{26}O_4$	330.424	-	-	0.81	-
14	3-Bromocholest-5-ene	$C_{27}H_{45}Br$	449.51	0.28	1.55	-	0.42
15	Physcion	$C_{16}H_{12}O_5$	284.267	3.23	4.66	-	-
16	Diosgenin benzoate	$C_{34}H_{46}O_4$	518.727	1.07	-	-	-
17	Cholesta-4,6-dien-3-ol,	$C_{27}H_{44}O$	384.64	0.59	1.69	-	1.31
18	Diosgenin	$C_{27}H_{42}O_3$	414.63	2.65	-	6.84	2.14
19	Myristyl palmitate	$C_{30}H_{60}O_2$	452.808	0.29	1.43	0.46	-
20	Trisdibutylphenyl phosphite	$C_{42}H_{63}O_3P$	646.92	0.31	1.34	-	1.02
21	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410.68	0.42	-	-	1.13

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22	7.beta.-Hydroxydiosgenin	$C_{29}H_{46}O$	410.686	0.34	-	-	-
23	Cholestenone	$C_{27}H_{44}O$	384.64	0.29	-	-	0.28
24	Tetracosamethyl-cyclododecasiloxane,	$C_{24}H_{72}O_{12}Si_{12}$	889.84	1.27	0.76	-	0.22
25	Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_4P$	662.93	0.76	-	-	2.93
26	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	741.54	0.75	-	-	-
27	Silane, 2-butenylmethoxymethylphenyl-	$C_{12}H_{18}OSi$	206.35	-	0.42	-	-
28	Carvone	$C_{10}H_{14}O$	150.221	-	0.88	-	0.28
29	2-Hydroxycyclopentadecanone	$C_{15}H_{28}O_2$	240.38	-	-	-	3.06
30	Stearic acid	$C_{18}H_{36}O_2$	284.48	-	-	-	0.98
31	4 [(Trimethylsilyl)oxy]cyclohexanol	$C_9H_{20}O_2Si$	188.342	-	0.99	-	-
32	Patchoulol	$C_{15}H_{26}O$	222.372	-	2.01	-	-
33	Neophytadiene	$C_{20}H_{38}$	278.515	-	0.58	1.99	0.66
34	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	593.232	-	0.32	-	-
35	Tetracosyl acetate	$C_{26}H_{52}O_2$	396.70	0.51	-	-	-
36	Heptadecane	$C_{17}H_{36}$	240.475	-	0.32	-	-
37	3-(3-Methylbutyl)thiophene-1,1-dioxide	$C_9H_{14}O_2S$	186.0	-	-	-	0.34
38	Silicone oil			-	0.66	-	-
39	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{34}O_2$	294.479	0.18	1.11	-	-
40	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.564	-	0.44	-	0.39

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41	Squalene	$C_{30}H_{50}$	410.73	-	1.12	3.21	0.19
42	Hexacosane	$C_{26}H_{54}$	366.71	-	1.08	-	-
43	Tetratetracontane	$C_{44}H_{90}$	619.204	-	0.41	-	-
44	Triphenyl phosphate	$C_{18}H_{15}O_4P$	326.28	0.36	-	-	-
45	Dicyclohexyl phthalate (Isocarnosol)	$C_{20}H_{26}O_4$	330.424	1.09	-	-	-
46	1H-purin-6-amine	$C_{12}H_{10}FN_5$	243.245	-	1.19	-	-
47	Gamma-sitosterol	$C_{29}H_{50}O$	414.718	-	3.77	-	9.86
48	Lupeol	$C_{30}H_{50}$	426.729	-	1.94	-	-
49	Digitoxigenin-4-en	$C_{23}H_{32}O_4$	372.505	-	-	-	0.39
50	Pentadecanoic acid, isopropyl ester	$C_{18}H_{36}O_2$	284.48	-	-	1.15	-
51	(Z)-Decyl icos-9-enoate	$C_{30}H_{58}O_2$	450.78	-	2.04	-	-
52	1,2,4-Trimethyl-3-nitrobicyclo [3.3.1] nonan-9-one	$C_{12}H_{19}NO_3$	225.288	-	-	0.49	-
53	Methyl 3-oxoheptanoate	$C_8H_{14}O_3$	158.197	-	-	-	1.21
54	2,6-di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	220.35	-	-	-	0.34
55	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256.424	-	-	0.78	-
56	Phthalic acid, heptyl trans-hex-3-enyl ester	$C_{21}H_{30}O_4$	346.46	-	-	1.21	-
57	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.44	0.15	0.25	4.74	1.25
58	Phthalic acid, cyclobutyl isobutyl ester	$C_{16}H_{20}O_4$	276.332	-	-	0.73	0.38
59	1-Octadecyne	$C_{18}H_{34}$	250.47	-	-	0.95	-
60	cis-9-Hexadecenal	$C_{16}H_{30}O$	238.415	-	7.28	-	-

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61	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296.48	-	-	9.93	-
62	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.434	-	-	2.67	0.55
63	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666.38	-	0.84	-	-
64	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	$C_{18}H_{26}O_3$	290.39	-	-	2.15	-
65	β-sitosterol	$C_{29}H_{50}O$	414.718	2.37	-	3.55	-
66	4-Methyl-1-octene	C_9H_{18}	126.239	-	-	-	3.21
67	Vitamin E	$C_{29}H_{50}O_2$	430.71	-	0.49	1.16	0.16
68	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.126	-	-	-	1.18
69	2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)	$C_{10}H_{14}O$	150.22	-	-	2.45	-
70	7, beta-Hydroxydiosgenin	$C_{27}H_{42}O_4$	430.0	-	-	1.37	0.24
71	Cyclohexanol, 3,5-dimethyl-	$C_8H_{16}O$	128.215	-	-	0.53	-
72	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O$	390.564	21.38	24.41	24.69	19.19
73	Acetin, 1-mono	$C_5H_{10}O_4$	134.13	-	-	-	3.17
74	Carvacrol	$C_{10}H_{14}O$	150.221	-	-	-	0.15
75	Sequirin C	$C_{17}H_{18}O_5$	302.32	0.67	-	-	-
76	Flavidin	$C_{15}H_{12}O_3$	240.258	5.09	-	-	-
77	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.177	-	-	-	1.78
78	Pyrogallol	$C_6H_6O_3$	126.111	-	3.84	-	-
79	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	-	-	-	0.15
80	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	0.74	0.60	-	0.43

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81	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	-	-	-	0.33
82	1-Octadecanol	$C_{18}H_{38}O$	270.50	-	-	-	0.08
83	1-Chlorooctane	$C_8H_{17}Cl$	148.674	-	-	1.00	-
84	Palmitoleyl oleate	$C_{34}H_{64}O_2$	504.884	-	2.44	-	-
85	Methyl stearate	$C_{19}H_{38}O_2$	298.511	-	-	1.99	-
86	cis-1,2-Cyclohexanedimethanol	$C_8H_{16}O_2$	144.211	-	-	1.47	-
87	Myristyl palmitate	$C_{30}H_{60}O_2$	452.808	-	2.66	-	-
88	22,23-Methylene-cholesterol	$C_{28}H_{46}O$	398.67	-	-	-	0.47
89	alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.71	0.26	-	-	-
90	Cyclopropa[5,6]-33-norgorgostan-3-ol, 3',6-dihydro-,(3. beta.,5.beta.,6.alpha.,22.xi.,23.xi.)	$C_{30}H_{50}O$	426.71	-	-	-	0.28
91	1,2-benzenedicarboxylic acid, dicyclohexyl ester	$C_{20}H_{26}O_4$	330.42	-	-	-	1.31
92	Methyl margarate	$C_{18}H_{36}O_2$	284.484	-	0.34	-	-
93	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.125	-	-	-	8.23
94	Stigmasterol	$C_{29}H_{48}O$	412.70	-	-	-	0.44
95	Chrysophanol	$C_{15}H_{10}O_4$	254.241	43.97	-	-	-

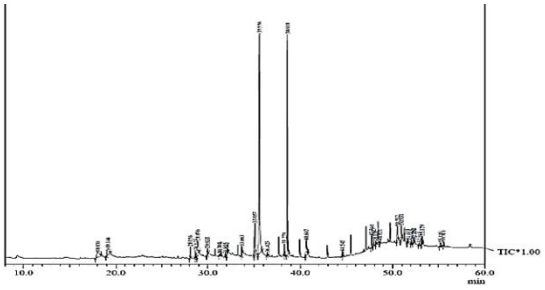


Figure 1: GC-MS chromatogram of rhizome extract of R. emodi.

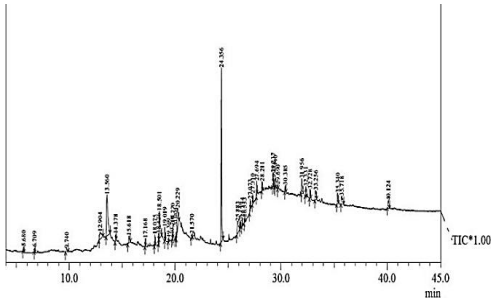


Figure 2: GC-MS chromatogram of fruit extract of R. emodi.

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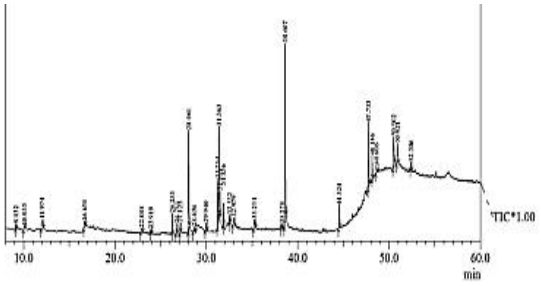


Figure 3: GC-MS chromatogram of leaf extract of R. emodi.

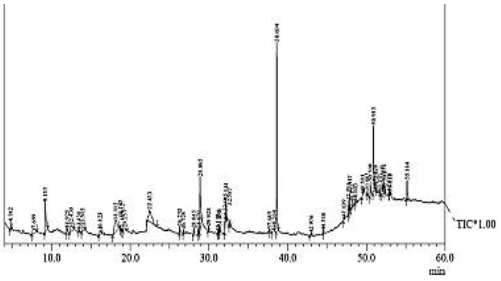


Figure 4: GC-MS chromatogram of callus extract of R. emodi.

GC–MS profiling of methanolic extracts of *Rheum emodi* collected from the Siran Valley revealed a chemically diverse metabolite spectrum with clear organ-wise variation. Overall, the dataset indicates three main chemical "themes" across the plant parts: (i) anthraquinone-type markers concentrated in the rhizome (and partially in fruit via physcion), (ii) lipid-derived fatty acids and their methyl/alkyl esters enriched mainly in leaves and to some extent fruit/callus, and (iii) phytosterols/triterpenoids and isoprenoids (e.g., squalene, tocopherols) appearing differentially across organs. Such compartmentalization is biologically meaningful because different tissues have different ecological roles: the rhizome acts as a storage and defense organ, leaves serve as a photosynthetic tissue rich in membrane lipids and antioxidants, fruits accumulate protective and signaling compounds, and callus (in vitro tissue) often shows altered secondary metabolism due to absence of natural environmental cues and tissue differentiation[14–17].

A striking outcome of the rhizome profile is the dominance of chrysophanol (43.97% area), making it the most abundant identifiable metabolite in the rhizome extract. Chrysophanol is a hallmark anthraquinone in *Rheum* spp., and its enrichment strongly supports the traditional and pharmacognostic preference for rhizome as the most medicinally relevant plant part. Anthraquinones in *Rheum* are widely associated with antimicrobial, anti-inflammatory, laxative, hepatoprotective and anticancer-related bioactivities; therefore, the high chrysophanol signal provides a plausible chemical basis for the ethnomedicinal use of *R. emodi* in liver disorders, gastrointestinal complaints and inflammatory conditions. In the same direction, physcion (3.23% in rhizome; 4.66% in fruit) further strengthens the anthraquinone signature. Physcion is structurally related to chrysophanol and emodin-type

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compounds and is frequently linked with antioxidant and cytotoxic/anticancer properties reported for *Rheum* anthraquinones. The presence of physcion in fruit but absence of chrysophanol there suggests that fruit tissues may either accumulate different anthraquinones at lower concentrations or selectively store certain derivatives[18-20].

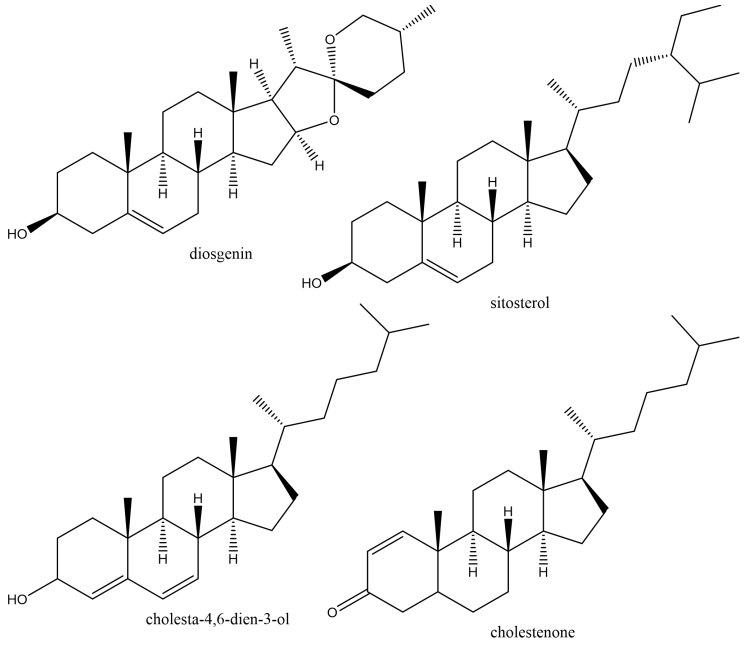


Figure 5: steroidal/triterpenoid constituents Obtained From rhizome Extract

Beyond anthraquinones, the rhizome also exhibited moderate representation of steroidal/triterpenoid constituents (e.g., diosgenin 2.65%, β-sitosterol 2.37%, cholesta-4,6-dien-3-ol 0.59%, cholestenone 0.29%, and several related sterol derivatives). Diosgenin is particularly noteworthy because it is a well-known steroidal sapogenin often discussed in the context of anti-inflammatory and metabolic effects, and it also serves as a precursor in semi-synthetic steroid production. In your results, diosgenin appears not only in rhizome but also as a major feature in leaves (6.84%) and callus (2.14%), along with derivatives such as diosgenin acetate (leaves 6.98%; callus 1.26%) and 7β-hydroxydiosgenin (leaves 1.37%; callus 0.24%). This distribution suggests that steroidal metabolism is not confined to the rhizome alone; rather, it may be active in aerial tissues and even in undifferentiated callus to some degree, possibly due to baseline sterol biosynthesis pathways required for membrane structure and growth[21-22].

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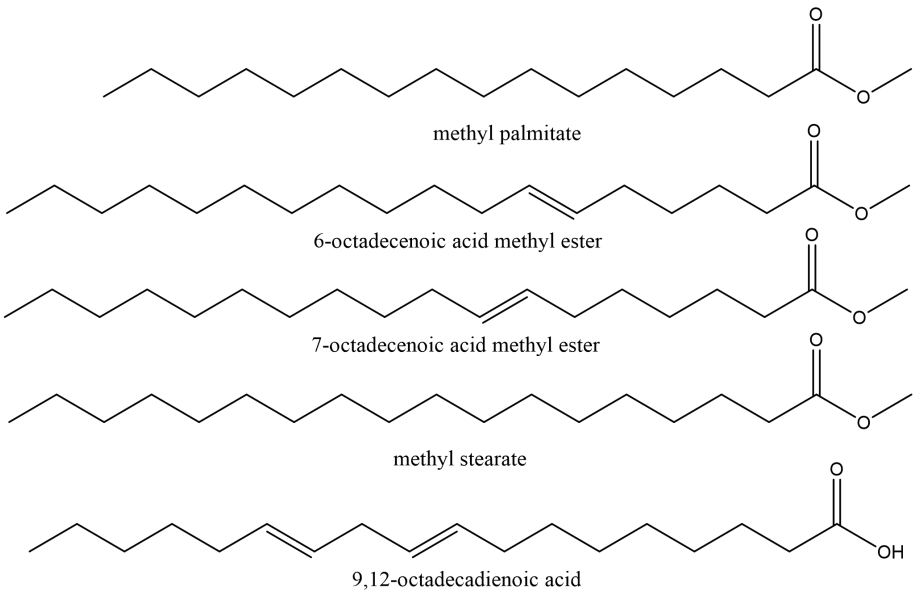


Figure 6: Fatty acid methyl esters and unsaturated fatty acid derivatives Obtained From leaves Extract

Leaves, in contrast to the rhizome, were dominated by fatty acid methyl esters and unsaturated fatty acid derivatives, which is consistent with the lipid-rich nature of leaf tissues. For example, leaves contained methyl palmitate (12.82%), 6-octadecenoic acid methyl ester (Z) (9.93%), 7-hexadecenoic acid methyl ester (Z) (2.67%), methyl stearate (1.99%), and a prominent unsaturated fatty acid signal (9,12-octadecadienoic acid (Z,Z) 4.74%). Such compounds commonly represent membrane lipid components, cuticular wax precursors, and storage lipids, and their abundance in leaves is expected from a physiological standpoint. Importantly, unsaturated fatty acids (e.g., linoleic-type signals) are frequently discussed in relation to anti-inflammatory and cardiometabolic relevance due to their involvement in eicosanoid pathways and antioxidant balance. While GC–MS peak area does not equate to absolute bioactivity, the leaf profile strongly supports the idea that leaves may contribute antioxidant and anti-inflammatory potential, complementing the rhizome's anthraquinone-rich pharmacological identity[23-24].

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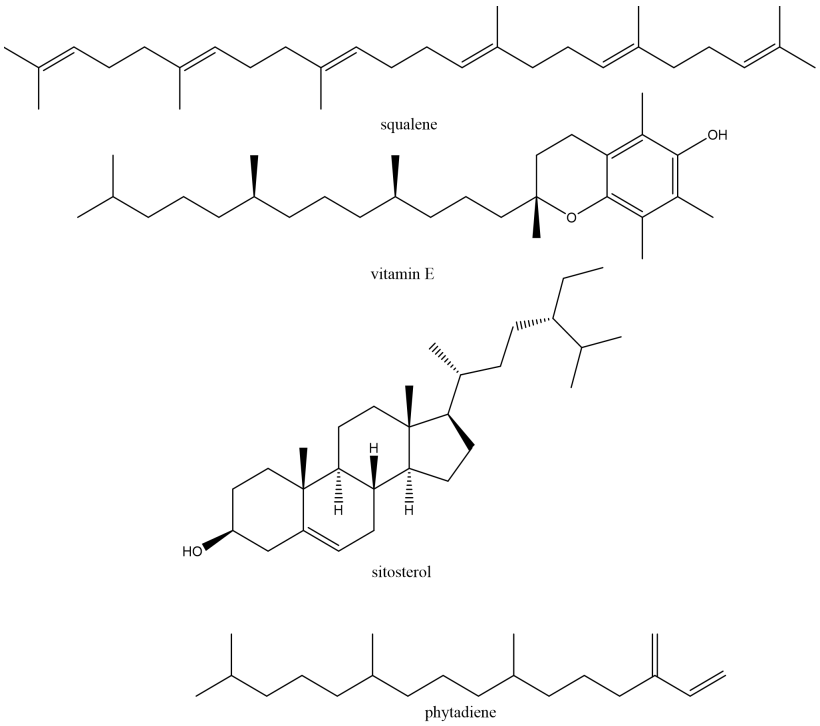


Figure 7: Antioxidant compounds Obtained From Fruit Extract

Leaves also showed a notable sterol/isoprenoid antioxidant profile: squalene (3.21%) and vitamin E (1.16%) were detected, and β-sitosterol (3.55%) appeared as well. Squalene is an isoprenoid hydrocarbon widely recognized for antioxidant behavior and roles in sterol biosynthesis; tocopherols (vitamin E) are classic lipid-phase antioxidants that protect membranes from oxidative stress. Their appearance in leaves aligns well with the ecological need to protect photosynthetic tissues from reactive oxygen species generated during high light exposure and environmental stress typical of high-altitude Himalayan regions. Interestingly, neophytadiene (1.99%) was also present in leaves, a diterpene hydrocarbon often reported in plant extracts and sometimes associated with anti-inflammatory or antimicrobial tendencies in the broader phytochemical literature[25-27].

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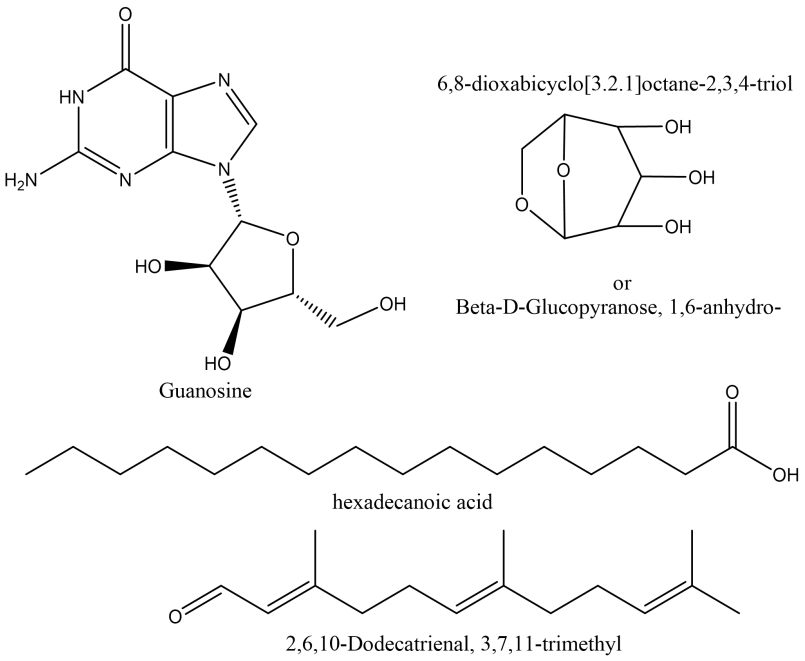


Figure 8: Phytomolecules Obtained From Fruit Extract

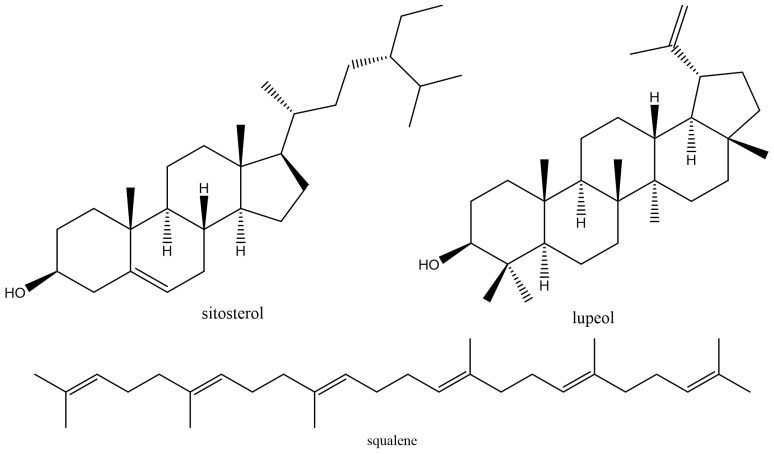


Figure 9: phytosterol/triterpenoid-related compounds

Fruit extract displayed an intermediate chemical pattern. Compared with leaves, fruit contained fewer lipid methyl esters at high proportions but had certain notable features: β-D-glucopyranose, 1,6-anhydro- (15.41%) suggests a carbohydrate-derived compound (often associated with thermal/derivatization products of sugars), while n-hexadecanoic acid (7.41%) and cis-9-hexadecenal (7.28%) indicate fatty acid and aldehyde-type chemistry that can contribute to aroma, defense, and signaling. Fruit also contained physcion (4.66%), linking fruit chemistry with the anthraquinone biosynthetic capability of the species. Furthermore, fruit showed a

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relatively strong presence of phytosterol/triterpenoid-related compounds such as gamma-sitosterol (3.77%), lupeol (1.94%), and squalene (1.12%). Lupeol is a pentacyclic triterpenoid widely discussed for anti-inflammatory and antimicrobial potential, while patchoulol is a sesquiterpene alcohol classically associated with fragrance and biological activity. The coexistence of sterols and terpenoids in fruit may reflect the fruit's need for chemical defense against microbial attack and herbivory, as well as the generation of volatiles for ecological interactions[28].

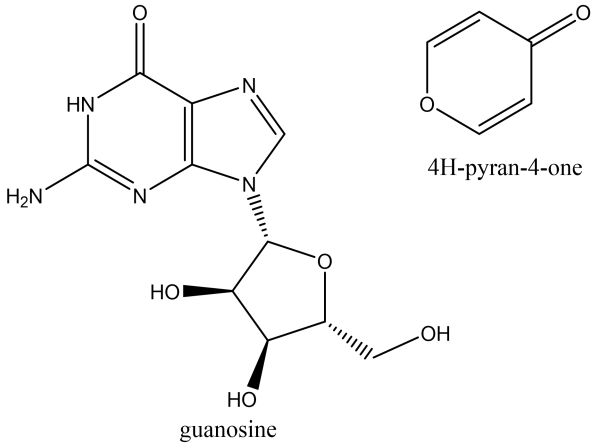


Figure 10: Phyto molecules extracted from Callus

Callus extract, as expected, showed a distinct pattern that can be interpreted through the lens of in vitro physiology. Callus contained guanosine (7.96%) and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (8.23%), as well as methyl palmitate (8.82%) and gamma-sitosterol (9.86%). The appearance of guanosine is especially interesting in callus because nucleotide and nucleoside pools often reflect active cell division and metabolic turnover. Similarly, pyranone derivatives can be related to sugar degradation or Maillard-type products depending on extraction and analytical conditions, but their prominence in callus may reflect different carbon metabolism under tissue culture conditions. Callus also carried diosgenin (2.14%) and diosgenin acetate (1.26%), suggesting that some steroidal biosynthesis remains active even in dedifferentiated tissue. However, callus lacked the strong anthraquinone signature observed in rhizome (e.g., chrysophanol), which aligns with the general observation that specialized secondary metabolism often requires tissue differentiation, developmental programming, and/or environmental stimuli that are reduced or absent under in vitro culture[28].

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A crucial part of interpreting GC–MS phytochemical profiles is recognizing non-plant contaminants and artefacts. In your dataset, bis(2-ethylhexyl) phthalate (DEHP) appears as a dominant peak across all plant parts (rhizome 21.38%, fruit 24.41%, leaves 24.69%, callus 19.19%). Additionally, dibutyl phthalate (rhizome 0.74%, fruit 0.60%, callus 0.43%) and other phthalate esters are present. Phthalates are widely known as plasticizers and are common laboratory contaminants introduced via plastic ware, tubing, solvent bottles, caps, or even GC septa. Because DEHP is high and relatively consistent across all tissues, it is more parsimonious to interpret it as extrinsic contamination rather than a true plant metabolite. Similarly, the presence of cyclosiloxanes (e.g., cyclodecasiloxane, cyclooctasiloxane, cyclononasiloxane, tetracosamethyl-cyclododecasiloxane) and "silicone oil" signals also strongly suggests contamination from column bleed, septum, syringe, vial septa, or silicone-based materials rather than genuine phytochemistry. In high-quality phytochemical discussions, it is good scientific practice to explicitly acknowledge these peaks as probable artefacts and focus biological interpretation primarily on well-established plant-derived classes such as anthraquinones, sterols, fatty acids, terpenoids, and nucleosides[29].

When the likely contaminants are set aside, the biologically meaningful picture becomes clearer: the rhizome is anthraquinone-rich and defense-oriented, leaves are lipid/antioxidant-rich and membrane-protective, fruits show a mixed sterol–terpenoid–lipid profile with selective anthraquinone presence, and callus expresses growth/metabolic markers (nucleosides) plus baseline sterols and fatty acid esters but lacks the rhizome's specialized anthraquinone accumulation. This organ-specific partitioning is consistent with the earlier qualitative phytochemical screening where anthraquinones were positive in rhizomes and leaves but absent/weak in fruits and callus. Here, GC–MS complements that qualitative work by identifying specific candidates (e.g., chrysophanol, physcion, diosgenin, β-sitosterol, lupeol, squalene, tocopherols, fatty acid esters) that can explain the broader "+ / −" phytochemical outcomes.

From a pharmacological perspective, the rhizome's chrysophanol dominance provides a direct chemical rationale for prioritizing rhizome in traditional medicine

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and for using rhizome markers in quality control. Meanwhile, leaves and fruits—although not matching rhizome in anthraquinone abundance—contain several constituents commonly associated with antioxidant and anti-inflammatory relevance (tocopherols, squalene, unsaturated fatty acids, sterols, and terpenoids). This is important because it suggests a potential value of non-rhizome parts, especially for applications where antioxidant support or nutraceutical potential is emphasized. However, it must also be noted that GC–MS primarily captures volatile/semi-volatile and derivatizable components; many polar phenolics, glycosides and higher-molecular-weight constituents may be under-represented in GC–MS unless derivatization is performed. Therefore, it is possible that leaves and fruits contain additional phenolic antioxidants that would be better resolved by LC–MS/MS or HPLC-based profiling[30].

Finally, your results have meaningful implications for future work. First, the presence of marker anthraquinones (particularly chrysophanol and physcion) supports developing a chemical fingerprint for *R. emodi* material from the Siran Valley for authentication and quality control. Second, given the high representation of contaminants (phthalates and siloxanes), it would strengthen the study to include method blanks (solvent blank, Soxhlet blank, vial blank) and to minimize plastic contact (use glass wherever possible, PTFE-lined caps, clean syringes/liners). Third, the callus data highlight that in vitro cultures may not naturally reproduce the rhizome-like anthraquinone profile; if the goal is to enhance anthraquinone production in callus, targeted strategies such as elicitation (e.g., methyl jasmonate, salicylic acid), precursor feeding, or optimized culture conditions could be considered. In summary, the GC–MS profile provides strong evidence that *R. emodi* rhizome is the most anthraquinone-rich and pharmacognostically significant organ, with chrysophanol as the dominant constituent and physcion as an additional key compound. Leaves and fruits contribute complementary sterol–terpenoid–lipid chemistry with antioxidant implications, whereas callus shows a metabolism shaped by tissue culture conditions. Together, these findings not only support ethnomedicinal claims but also offer a foundation for part-wise utilization, chemical standardization, and subsequent bioactivity-guided investigations—particularly relevant for *R. emodi* from the Himalayan ecology of the Siran Valley.

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Conclusion

The present study provides a comparative phytochemical and GC–MS based chemical characterization of *Rheum emodi* (Wall.) collected from the Siran Valley, Pakistan, using methanolic extracts of rhizome, fruit, leaves and callus. Preliminary qualitative screening confirmed organ-wise variation in major metabolite groups, with the rhizome exhibiting the richest phytochemical profile and showing clear positivity for anthraquinones, while leaves showed strong occurrence of phenolics/tannins and flavonoids, indicating potential antioxidant relevance. These observations were further supported by GC–MS profiling, which revealed distinct chemical fingerprints for each plant part. Among all tissues, the rhizome displayed the most prominent bioactive signature, dominated by the anthraquinone chrysophanol (43.97% area) along with physcion (3.23%), supporting the traditional medicinal importance of rhizome as the primary therapeutic part of *R. emodi*. The presence of phytosterols and steroidal constituents such as β-sitosterol, diosgenin, and related derivatives further strengthens the pharmacological potential of this species, particularly in relation to anti-inflammatory and metabolic health applications. Leaves were characterized by higher representation of fatty acid methyl esters and unsaturated lipid derivatives, together with antioxidant-associated compounds such as squalene and vitamin E, highlighting leaves as a potential supplementary source of bioactive constituents. Fruits exhibited a moderate profile with selective anthraquinone presence (notably physcion) and detectable terpenoids/triterpenoids such as lupeol, suggesting possible protective and biofunctional roles. Callus showed a comparatively reduced spectrum, with dominance of primary/metabolic markers (e.g., guanosine) and selected sterol/lipid constituents, indicating that in vitro tissues may not fully mimic specialized metabolite accumulation found in differentiated organs. Overall, the findings establish a baseline chemical fingerprint of *R. emodi* from Siran Valley and validate rhizome-centered traditional use while also indicating chemical value in aerial parts. Future work should focus on minimizing potential analytical contaminants, confirming key compounds using authentic standards, quantifying major markers, and validating biological activities through targeted in vitro and in vivo assays.

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Authors Contributions

Nadia Jabeen conducted GC–MS analysis. Aiman Laraib Syed assisted in plant processing, phytochemical screening, and data compilation. Syed Mubashar Hussain Gardazi interpreted results, and finalized the manuscript.

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