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Research Article

Isolation and Identification of Antimicrobial Compounds from *Berberis aristata* Root Extract

Authors & Affiliation

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Abstract

Berberis aristata (Berberidaceae) is an influential medicinal plant and found in the different provinces of the world. It has compelling medicinal value in the traditional Indian system of medicine. More multitudinous secondary metabolites are contemporary within the plants, which helps to cure the disease. The chromatographic techniques have significant role in natural products chemistry and discovery of innovative compounds of pharmaceutical and biomedical importance. The aim of the present study is to evaluate sequestration of secondary metabolites using column-chromatographic techniques and this metabolites can be further secluded, identified by using LC-ESI-Q-TOF-MS system and also performed its antimicrobial activity. These metabolites were characterized for determination of medicinal properties. This study helps to identify the presence of secondary metabolic compounds in the plant.

Keywords: *Berberis aristata* root, column chromatography, LC-ESI-Q-TOF-MS, antimicrobial activity.

Introduction

Phytoconstituents obtained from medicinal plants are effective for treating various human ailments. The traditional medicine practice is widespread in India and medicinal plant metabolites are successfully used to treat various communicable and non-communicable diseases[1,2]. Phytoconstituents of medicinal plants are admirably coping number of deadly diseases such as cancer, hepatitis, AIDS etc. and considered safe future medicines [3]. The chemical exploration of these medicinal plants to discover hidden drug leads potential could be critical in the treatment of present and future human ailments.

Berberis aristata have its place in the Berberidaceae family (**Fig. 1,2**). Turmeric is indigenous to Himalayas in India. It is exorbitantly used in Ayurvedic medicine. It is commonly known as Zarishk and Daruhaldi[4,5]. It is mostly used for the medicaments piles, liver like diseases jaundice, diabetes and urinary problems. The plant contains multiple alkaloids like berberine, epiberberine, dehydrocaroline, oxycanthine, jatrorhizine and columbamine, karachine[6,7].

Fig. 1: *Berberis aristata Plant, stem and* root

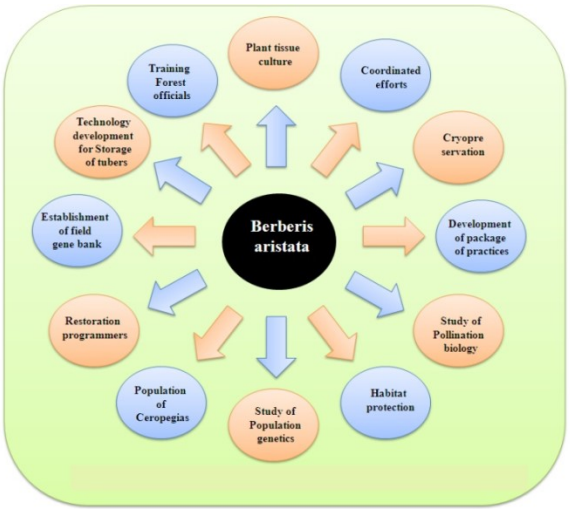


Fig. 2: Possible conversation routes for *Berberis aristata*

The isoquidoline alkaloids are the major bioactive constituents present in plant[8]. These alkaloids possess anti-inflammatory, immunestimulating, hypotensive, antiprozoal and antimicrobial activities[9]. The root bark decoction is ,used as a wash for ulcers and is said to enhance their aspects and encourage cicatrisation[10-16]. Metabolomics is non-selective, comprehensive analytical approach used to identify and quantify metabolites from biological samples. Metabolomics focuses on global detection of small molecules in samples with a molecular weight below 1500 Da [17]. A summary of the importance and applications of *Berberis aristata* Plant were provided in **Fig. 3**.

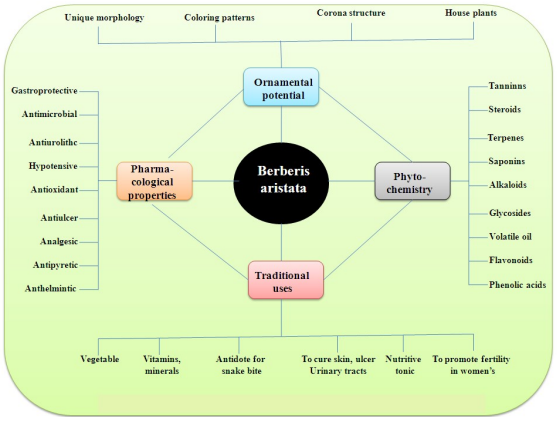


Fig. 2: Importance and implications of *Berberis aristata*Plant

Mass spectrophotometry (MS) imaging such as Q-TOF LC/MS is a tag free procedure also therefore, be able to employ deprived of previous knowledge of the analytes from biological samples [18, 19]. The metabolic fingerprinting from biological samples using Agilent 6550 iFunnel Q-TOF LC/MS System (6550 Q-TOF) has been reported earlier [20]. Therefore, in the present study authors isolated pharmacologically relevant plant secondary metabolites using high resolution mass spectrometry (HR-LCMS) and also test the antimicrobial activity of *berberis aristata* root.

Materials and Methods:

Berberis aristata roots (500 gm) were procured from local market of Aurangabad (MS) India. The roots were cleaned using distilled water then allowed to dry around room temperature (27 ºC). The root rhizome cut into small pieces and grind into powder using grinder mixer. Solvent extracted by adding dried powder (100 gm) in 500 ml ethanol (Rankem, USA) using Soxhlet apparatus for 8 h. The resulting extract (80 gm) filtered through Whatman filter paper no. 44 and evaporated to dryness at constant temperature then stockpiled at 4 ºC for later usage.

Extraction & fractionation for column chromatography:

In preliminary fractionation, dried plant material (80 gm) was gradually immersed with non-polar towards polar solvents (**Fig. 4**). Extraction of

dried powder using solvents such as n-hexane, $CHCl_3$, Ethyl acetate and CH_3OH in which methanolic sample extracts gave highest antimicrobial activity. By administering the linear gradient of methanol with chloroform to the methanolic extract, it was evaporated over and treated to column chromatography. Fraction 4 was the most antimicrobially active of the ten fractions (200 mL) sampled. As a result, same fraction was chosen for ESI-Q-TOF-MS analysis to identify metabolites.

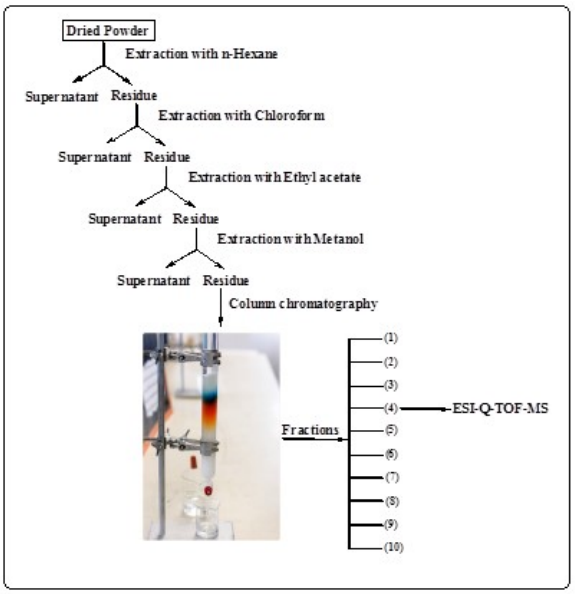


Fig. 4: Schematic representation of fractionation from *Berberis aristata*roots

Antimicrobial screening activity

Antimicrobial activity was investigated utilising disc diffusion method. Antimicrobial activity assays were performed on clinical isolates containing Gram-positive & Gram-negative bacteria obtained out from Department of Botany research laboratory. Micro dilution procedures employing fractions sequentially diluted in sterile nutrient broth were also employed to identify the minimal concentration roots isolates to control the bacteria. Ciproflaxin (2 µg/disc) and Fluconozale (10 µg/disc) were used as references drugs for bacteria and fungi respectively.

Equipment and conditions

At SAIF, IIT, Bombay, researchers identified metabolites from ethanolic extract. A LC-ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel) system comprising a G4220B pump, G4226A auto sampler, and G1316C, as well as a diode array detector (DAD), was used to examine the samples. The gradient system of 0.1 percent formic acid into water (A) then acetonitrile (B) at a flow rate of 0.3 ml/min was chosen as the elution solvent. The gradient system began with 95 percent A: 5 percent B and progressed to 5 percent A: 95 percent B in 50 minutes, before returning to the initial composition of 95 percent A: 5 percent B in 10 minutes and remaining at that composition for 5 minutes. ESI positive ionization mode was adopted for the MS analysis. The following were the MS basis settings: 3500 V capillary voltage, 250°C gas temperature, 13 L/min drying gas flow, 300°C sheath gas temperature, 11 sheath gas flow, 35 (psig) gas pressure for nebulizing, fragment or 175 V, Skimmer 65 V, Octopole RF Peak at 750 V, with mass range *m/z* 50-1000. The FWHM resolution was 40,000. To structure compliance, the Metlin database was employed.

Results and Discussion

The different concentration methanol extract of *Berberis aristata* roots shows antimicrobial activity against the tested organism in the order of *Bacillus subtilis* (9.5 mm), *Staphylococcus aureus* (10.5 mm), *Escherichia coli* (12 mm), *Pseudomonas aeruginosa* (8 mm). The maximum antibacterial activity was observed against *Escherichia coli*.

In case of fungi activity against tested organism was in the order *Aspergillus niger* (11.5 mm), *Aspergillus fumigatus* (8.5 mm), *Penicillium digitatum* (7 mm), *Penicillium notatum* (8 mm), *Fusarium oxysporum* (9.5 mm).The maximum antifungal activity was observed against *Aspergillus niger* (11.5 mm) as shown in (**Table 1**).

Table 1: *In vitro* antimicrobial activity of methanolic extracts of *Berberis aristata* roots

	Name of organism	Diameters of zone of inhibition (mm/ml)			
		50 µl	100 µl	150 µl	A
Bacteria	*Bacillus subtilis*	2.0	5.5	9.5	10.0
	Staphylococcus aureus	3.5	4.0	10.5	14.0
	Escherichia coil	4.0	7.5	12.0	17.0
	Pseudomonas aeruginosa	2.5	5.0	8.0	10.0

Fungi	*Aspergillus niger*	10.2	11	11.5	13.0
	Aspergillus fumigates	5.5	6.0	8.5	10.0
	Penicillium digitatum	3.0	5.5	7.0	9.0
	Penicillium notatum	4.5	6.5	8.0	9.0
	Fusarium oxysporum	7.0	8.5	9.5	12.0

Identification of metabolites by ESI-Q-TOF-MS

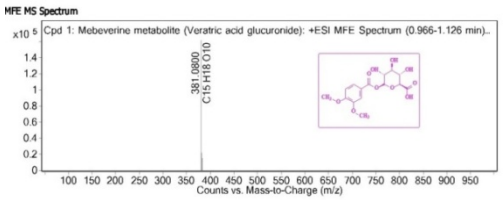
Metabolomics has become an indispensable tool for advancing our understanding of common and bioactive metabolites in plants [12]. The ESI-Q-TOF-MS generated metabolomic chromatogram of methanol extract of *berberis aristata* root is depicted in Table 2. Fatty acids, phenolics, organic compounds, alkaloids, pyrimidines, amino dipeptides, and tripeptides were discovered in the examination of metabolites (**Table 2**).

Table 2: Major abundant metabolites of *berberis aristata*root

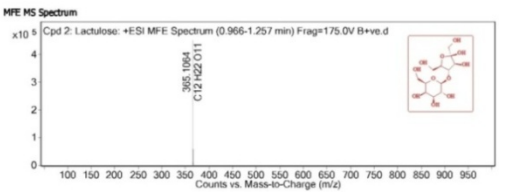
Sr. No.	Name	RT	Mass	Formula	M/Z	Ion	DB diff. (ppm)
a	Veratric acid glucuronide	0.982	358.09	$C_{15}H_{18}O_{10}$	381.08	$(M+Na)^+$	-1.01
b	Lactulose	1.000	342.11	$C_{12}H_{22}O_{11}$	365.10	$(M+Na)^+$	-2.5
c	Triparanol	1.002	143.09	$C_7H_{13}NO_2$	144.10	$(M+H)^+$	-2.67
d	Scopoline	1.014	155.09	$C_8H_{13}NO_2$	156.10	$(M+H)^+$	-2.6
e	Histidine	1.032	155.07	$C_6H_9N_3O_2$	156.07	$(M+H)^+$	-3.46
f	Dienestrol	4.681	266.13	$C_{18}H_{18}O_2$	289.12	$(M+Na)^+$	-23.67
g	3-(3-Indolyl)-2-oxopropanoic acid	7.113	203.05	$C_{11}H_9NO_3$	204.06	$(M+H)^+$	-1.02
h	7,8-Dihydroxyflavone	7.900	254.05	$C_{15}H_{10}O_4$	255.06	$(M+H)^+$	-0.97
i	Dihydrostreptomycin	9.227	567.28	$C_{21}H_{41}N_7O_{11}$	568.29	$(M+H)^+$	-5.91
j	Acetohexamide	9.339	324.11	$C_{15}H_{20}N_2O_4S$	347.10	$(M+Na)^+$	2.1
k	Protorifamycin I	9.676	639.31	$C_{35}H_{45}NO_{10}$	640.31	$(M+H)^+$	-8.93
l	Spaglumic acid	10.375	304.08	$C_{11}H_{16}N_2O_8$	305.09	$(M+H)^+$	18.63
m	2,4-Dihydroxy-3,4-dimethoxy-4-ethoxybenzophenone	10.376	318.17	$C_{17}H_{18}O_6$	319.11	$(M+H)^+$	-4.43
n	Elephatopin	10.378	360.12	$C_{19}H_{20}O_7$	361.13	$(M+H)^+$	-5.13
o	Piperine	11.200	285.13	$C_{17}H_{19}NO_3$	286.14	$(M+H)^+$	-1.19
p	Traumatic acid	13.033	228.13	$C_{12}H_{20}O_4$	229.14	$(M+H)^+$	0.03
q	Dextromoramide	13.597	392.25	C25H32N2O2	393.26	$(M+H)^+$	-1971
r	Propofol	13.86	178.13	$C_{12}H_{18}O$	179.14	$(M+H)^+$	0.36
s	3-deoxo-3-□-acetoxydeoxy dihydrogeduninof	17.632	512.28	$C_{30}H_{40}O_7$	535.27	$(M+Na)^+$	-6.79

The foremost plentiful metabolites recognized in methanol extract of *berberis aristata*root thru ESI-QTOF-MS examination were (a) Veratric acid glucuronide, (b) Lactulose, (c) Triparanol, (d) Scopoline, (e) Histidine, (f) Dienestrol, (g) 3-(3-Indolyl)-2-oxopro panoic acid, (h) 7,8-Dihydroxyflavone, (i) Dihydrostreptomycin, (j) Acetohexamide, (k) Protorifamycin I, (l) Spaglumic acid, (m) 2,4-Dihydroxy-3,4-dimethoxy-4-ethoxy benzophenone, (n) Elephatopin, (o) Piperine, (p) Traumatic acid, (q) Dextromoramide, (r) Propofol, (s) 3-deoxo-3-β-acetoxydeoxy dihydrogeduninof mass 358.09, 342.11, 143.09, 155.0, 155.07, 266.13, 203.05, 254.05, 567.28, 324.11, 639.31, 304.08, 318.11, 360.12, 285.13, 228.13, 392.25, 178.13 and 512.28 respectively (**Figure 5a-s**). **Table 2** displays the mass, retention time, molecular formula, as well as DB difference (ppm) of major as well as minor metabolites.

a



B



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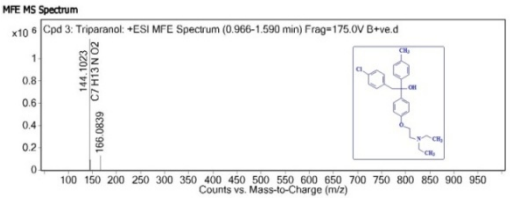

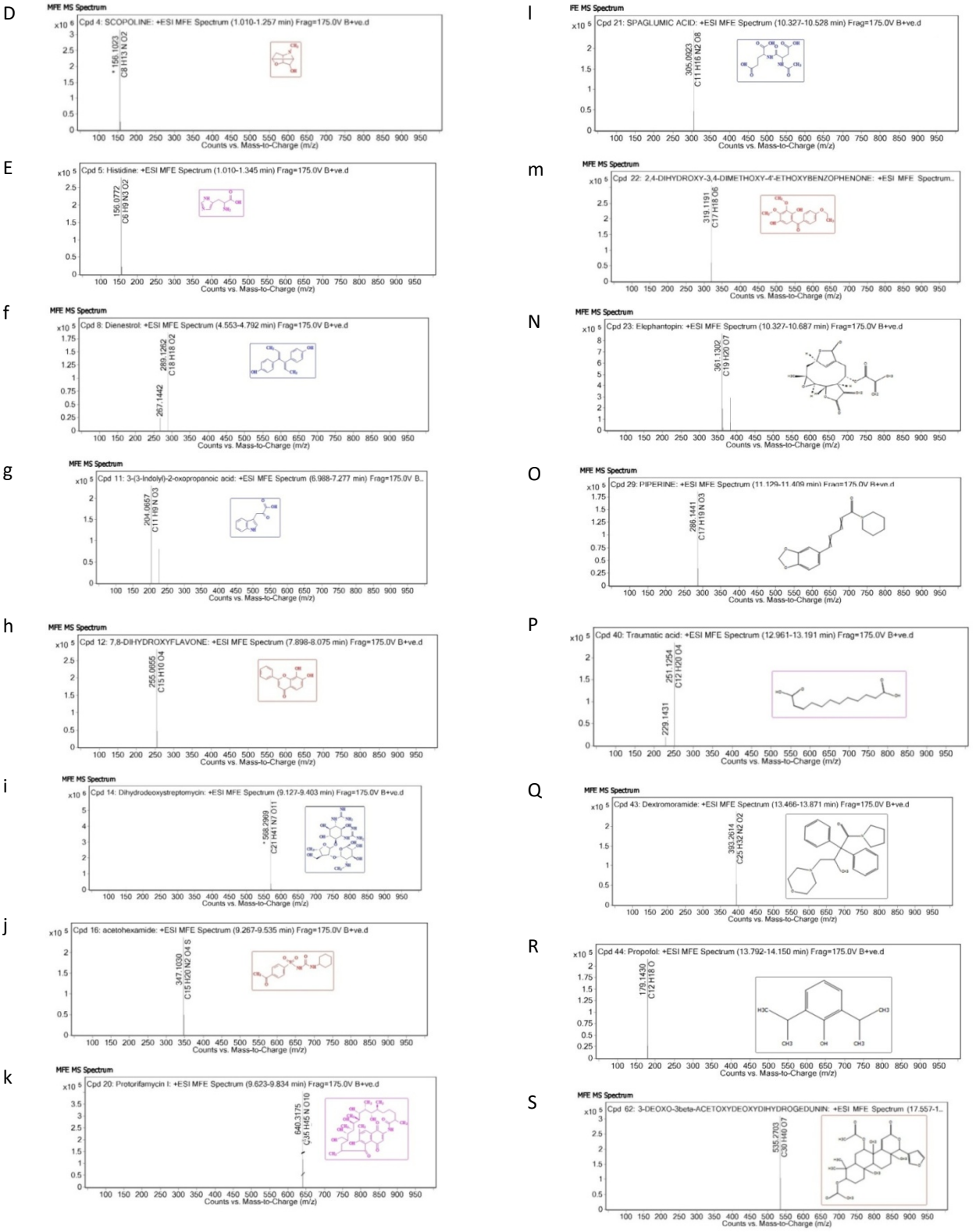

Fig. 5: Major abundant metabolite ESI-Q-TOF-MS spectra in *berberis aristata*root (a) Veratric acid glucuronide, (b) Lactulose, (c) Triparanol, (d) Scopoline, (e) Histidine, (f) Dienestrol, (g) 3-(3-Indolyl)-2-oxopro panoic acid, (h) 7,8-Dihydroxyflavone, (i) Dihydro-streptomycin, (j)

Acetohexamide, (k) Protorifamycin I, (l) Spaglumic acid, (m) 2,4-Dihydroxy-3,4-dimethoxy-4-ethoxybenzophenone, (n) Elephatopin, (o) Piperine, (p) Traumatic acid, (q) Dextromoramide, (r) Propofol, (s) 3-deoxo-3- *β*-acetoxydeoxydihydrogedunin.

These nineteen major metabolites discovered in *Berberis aristata* root are reported to have featured chemical and structural properties with therapeutic values as investigated from several sources earlier reviewed here. The findings of current studies were compared with those earlier reports in the treatment of constipation and hepatic encephalopathy [21-22]. (Pubchem CID: 11333).

Triparanol was the first synthetic cholesterol-lowering drug (Pubchem CID: 6536). Dienestrolis is used to treat menopausal symptoms[23]. (Pubchem CID: 667476).

7,8-Dihydroxyflavone (Tropoflavin) has been found to act as a potent and selective small-molecule agonist of tropomyosin receptor kinase B, the core gesticulating receptor of neurotrophin a neurotrophic factor[24] that is brain-derived. Tropoflavin can pass across the blood-brain barrier[25-26] and is orally accessible. (Pubchem CID: 1880). Dihydrostreptomycin: It is derivative of streptomycin that has a bactericidal property. It's a semi-synthetic aminoglycoside antibiotic used in the treatment of tuberculosis (Pubchem CID: 439369). Acetohexamide (trade name Dymelor) is used to treat diabetes mellitus type 2. (Pubchem CID: 1989).

Rifamycin I is a mycobacteria-specific antibiotic that is intended to cure tuberculosis, leprosy, & mycobacterium avium complex (MAC) infections[27]. (Pubchem CID: 6324616). Spaglumicacidis used as an antiallergic medication in eye drops and nasal preparations[28]. (Pubchem CID: 188803). Elephatopinit has been used for stomach ailment, hepatitis, nephritis, and bronchitis in folk medicine [29]. Piperine, it has been used in some forms of traditional medicine[30], (Pubchem CID: 638024). Traumatic acid: Traumatic acid is a potent wound healing agent in plants ("wound hormone")[31]. (Pubchem CID: 5283028). Propofol used for status epilepticus if other medications have not worked[32]. (Pubchem CID: 4943). Moreover, there was some recent report with medicinal valuables from root extract of *Plocama pendula*[33].

Conclusion

To our knowledge, it's the first work to provide thorough metabolite profiling of a methanolic excerpt of *Berberis aristata* root utilising the HR-LCMS method. According to our findings, B. *aristata* root is a supplier of key compounds that contribute phytopharmacology activity, such as antioxidant action, phytochemical research, & antibacterial activity.

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Authors' Contributions

PVR and AD: data collection, analysis, conceived, designed and performed the experiments; STA and RV: wrote and formatted the manuscript for publication; All the authors have read the final manuscript and approved the submission.

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Conflict of Interest

All the authors declare no potential conflicts of interest.

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