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Research Article

IN VITRO ANGIOTENSIN-CONVERTING ENZYME INHIBITORY ACTIVITY OF ETHYLACETATE FRACTION OF *ASPIDOPTERYS INDICA*: PHYTOCHEMICAL PROFILING INTEGRATED BY HIGH-RESOLUTION LIQUID CHROMATOGRAPHY MASS SPECTROSCOPY AND *IN SILICO* APPROACH

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ABSTRACT

Objective: Ethyl acetate fraction of the endemic drug *Aspidopterys indica* (EAAI) was screened for its *in vitro* angiotensin-converting enzyme (ACE) inhibitory potential. High-resolution liquid chromatography mass spectroscopy high-resolution liquid chromatography mass spectroscopy (HR-LCMS) was used to identify the biologically active metabolites. It was subjected to an *in silico* docking approach to recognize their molecular-level interactions with the ACE.

Methods: Methanol extract was prepared using ultrasonic extraction and fractionated with ethyl acetate by vacuum liquid chromatography. ACE inhibition was tested *in vitro* by the Cushman-Chung method. Phytochemical profiling of the active fraction was analyzed by HR-LCMS. In addition, *in silico* molecular docking of identified compounds was performed by AutoDock Vina (PyRx 0.8) to assess the binding affinity to the ACE enzyme.

Results: The ethyl acetate fractionated residue from the methanol extract of *A. indica* was tested for ACE inhibition; the IC_{50} of EAAI was 117.59 µg/mL, and positive control captopril was 81.56 µg/mL. After comprehensive HR-LCMS analysis, a broad range of 26 remarkable metabolites were identified, including four terpenoids, three flavonoids, three glycosides, two alkaloids, two long-chain amino alcohols, three phenolic acids, one phenolic compound, and two proteins. A glycoside (beta-D-gentiobiosyl crocetin-8.6 kcal), and a flavonoid maritimetin had (−7.8 kcal) demonstrated high binding affinities for 1O86 in a docking study. The findings revealed that EAAI manifested significant ACE inhibition, though less potent than captopril. *In silico* studies revealed that beta-D-gentiobiosyl crocetin had a binding affinity notably similar to captopril.

Conclusion: The ACE inhibition of phytochemicals offers its usage in antihypertensive medications. The present study highlights the substantial potential of *A. indica* as an ACE inhibitor; it can provide further insights into the research of bioactive components that may align with antihypertensive action.

Keywords: *Aspidopterys indica*, Ethyl acetate fraction, High-resolution liquid chromatography mass spectroscopy, Angiotensin-converting enzyme inhibition, *In vitro*, *In silico.*

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INTRODUCTION

Hypertension is a serious medical condition marked by persistent elevation of blood pressure more than 140/90 mmHg. Prevalence of hypertension has been more widespread, especially in middle- and low-income countries, now afflicting 31.5% of the population and 1.04 billion people [1]. It is a common chronic disease in adults that may result from cumulative effects such as genetic predispositions, demographic characteristics, and external environmental conditions [2]. The renin-angiotensin system is a biological process that helps to regulate blood pressure; the enzyme renin catalyzes angiotensinogen into angiotensin I (Ang-I), constriction of blood vessels, and water retention [3]. Angiotensin-converting enzyme inhibitors are a group of drug classes routinely employed in treating hypertension. Some of the most widely used angiotensin-converting enzyme (ACE) drugs are benazepril, ramipril, enalapril, and captopril. ACE inhibitors exert their mechanism by inhibiting the conversion of Ang-I to angiotensin II (Ang-II) and inhibition of bradykinin conversion to inactive metabolites [4]. Nature-derived products offer remarkable features compared to chemical entities due to their notable features as a significant source of potent phytochemicals, and they are safe, effective, and have reduced side effects [5]. Natural sources are rich in bioactive molecules that are ACE inhibitors, including phenolics, alkaloids, glycosides, flavonoids, xanthones, terpenes, peptides, and tannins [6]. Recent studies revealed that terpenoids, flavonoids, and phenolic compounds from several plant isolates have gained considerable recognition as ACE inhibitors [7]. Computational techniques like molecular docking and an *in silico* approach can detect potential drug targets for bioactive compounds. Aerial parts of *Aspidopterys indica* were traditionally used to manage hypertension [8]. Catechin and isoorientin are compounds isolated [9], and a few compounds have been reported to have antioxidant and methanol fraction antihypertensive activity [10]. Besides ethnobotanical aspects, as noted, there is still a lack of information on the phytocompounds responsible for antihypertensive action. The main objective of the study is metabolite profiling of the ethyl acetate fraction of *A. indica*, and *in vitro* ACE inhibition and *in silico* approach of phytoconstituents, aiming to provide a scientific rationale for the therapeutic application of *A. indica* as an antihypertensive agent. The major components identified by high-resolution liquid chromatography mass spectroscopy (HR-LCMS) profiling were examined for ACE inhibitor activity.

METHODS

Plant authentication and collection

Aerial parts of *A. indica* were collected from the kinnerasani wild life sanctuary, Bhadradri, Kothegudem district, Telangana, India. A sample was placed in the herbarium of the Botanical Survey of

India, Hyderabad, with a specimen voucher of BSI/DRC/2019-2020/Tech/838.

Extraction

A. indica aerial parts were collected, cleaned under running tap water, dried under shade, powdered coarsely, and extracted using methanol by ultra-sonication at 40 kHz for 45 min at 45°C. Extract underwent filtration, concentrated under vacuum, and then kept at room temperature in a desiccator until further use.

Fractionation

About 50 g of silica gel 60 (0.04–0.063 mm) was packed over a sintered glass funnel with a diameter of 6 cm and a height of 8 cm. The slurry of methanol extract of 5 g was adsorbed over silica gel and introduced into the column. Elution was carried out using a gradient of solvents with increasing polarity. Fractionation was initiated with hexane under a mild pressure of 20–70 mm of Hg of vacuum, yielding three hexane fractions, which were collected until the eluate became colorless. Process was continued sequentially with chloroform (three fractions), ethyl acetate (EA, four fractions), and finally with methanol (five fractions). Each fraction of 100 mL was collected under vacuum, resulting in a total of 15 fractions. The individual fractions were analyzed by thin-layer chromatography (TLC) with solvent ratio of n-hexane: EA (2:8) and spray reagent of anisaldehyde-sulfuric acid for visualization. Fractions with similar TLC profiles were pooled.

Among the pooled fractions, EA with intermediate polarity was selected for further study as it displayed more intense TLC bands corresponding to phenolic and flavonoid compounds and showed higher antioxidant activity in preliminary assays. Therefore, it was designated as an active fraction and considered for further study. A detailed fractionation scheme is depicted in Fig. 1.

***In vitro* ACE inhibitory activity**

The ACE inhibitory action of ethyl acetate fraction of *A. indica* (EAAI) was performed using the Cushman-Cheung method [11]. The ACE converts Hippuryl-Histidyl-Leucine (HHL) into Hippuric acid (HA). Using a UV-vis spectrophotometer, HA concentration was noted at 228 nm to define the action of ACE. ACE inhibition was related to the decrease in the concentration of HA generated. Test solution of 50 µL of *A. indica* fraction or standard along with phosphate buffer (200 mL with pH 8.3), sodium chloride of 0.2 M, and HHL of 6.5 mM mixed with 100 µL of ACE solution and incubated at a temperature of 37°C for half an hour. Absorbance was monitored at 228 nm, and the ACE inhibitory action estimated by,

$$\% \text{ ACE Inhibition} = \frac{A_a - A_b}{A_a - A_c} \times 100$$

A_a - Absorbance of ACE with HHL devoid of sample
A_b - Absorbance of ACE along with HHL with sample or standard
A_c - Absorbance of HHL devoid of sample (control)/ACE.

Metabolic profiling of ethyl acetate fraction by HR-LCMS

HR-LCMS, Model G6550 iFunnel QTOF (Agilent Technologies) equipped with electron spray ionization in positive mode. Solvent system A: Water in 0.1% formic acid, and Solvent B: Acetonitrile or methanol in 0.1% formic acid, with a 0.3 mL/min flow rate. The elution was carried out by a gradient solvent system (A: B v/v) at 95:5 (0–1 min), 0:100 for the next 30 min, and 100:0 for 31–35 min at 1200 bar pressure. The m/z range of 150 to 1000 Daltons was scanned by quadrupole coupled time of flight mass analyzer. Mass resolution of 0.01% was used to detect the phytocompounds in the EAAI [10,12,13]. The identification of compounds using HR-LCMS was performed to ensure accuracy and reproducibility. Accurate mass measurements were made using a QT of mass analyzer, as it is a reliable approach to ensure reproducibility and accuracy, and provides precise m/z values, which provide the predicted molecular formulas with mass errors typically <5 ppm and also generates fragmentation spectra, which offer structural information that allows the prediction of compound elucidation. The MS/MS spectra obtained were compared with digital databases such as METLIN, PubChem, and MassBank. Retention time (Rt) was also noted. However, Rts were not taken alone as conclusive; an integrated approach by combining accurate MS/MS fragmentation analysis, mass measurement, and Rt comparison with reference standards, reference spectra, and relevant literature strengthens the identification of tentative compounds with confidence [14].

***In silico* studies**

The phytocompounds identified in HR-LCMS metabolic profiling were subjected to *in silico* studies utilizing AutoDock Vina (PyRx 0.8) to recognize amino acid interactions with the potential target

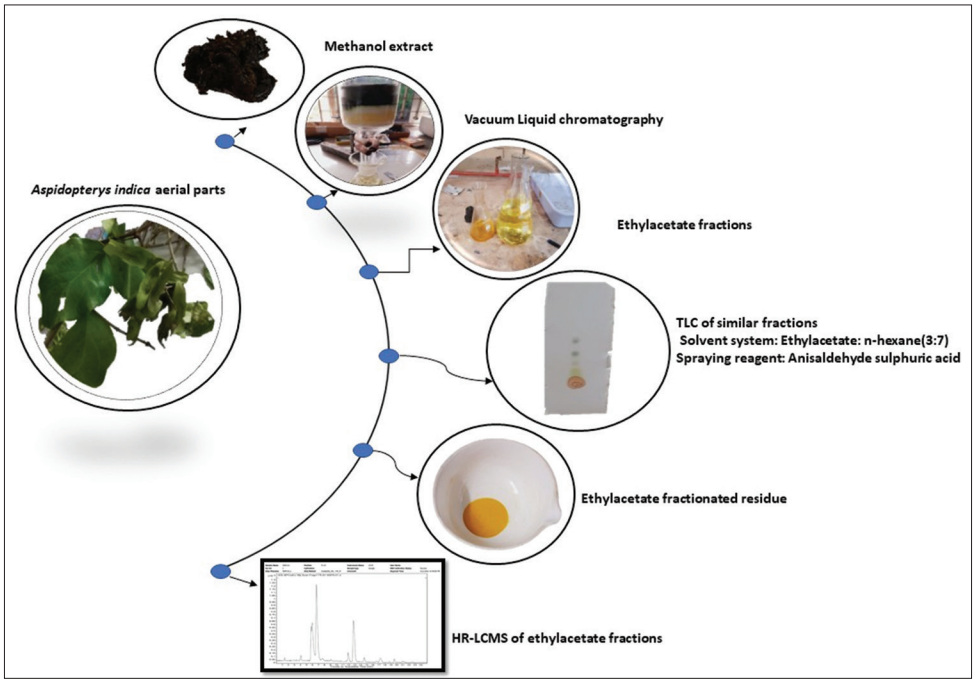

Fig. 1: Schematic representation of the vacuum liquid chromatography process used to fractionate the methanol extract of *Aspidopterys indica* with ethyl acetate

protein, human angiotensin-converting enzyme (protein data bank [PDB]: 1O86) [15]. Target protein PDB file was downloaded from https://www.rcsb.org and refined, excluding water molecules and incorporating polar hydrogens. Using the AutoDock tool of PyRx (a virtual application), the protein was loaded in PDB format and later converted into a PDB Partial charge (Q), Atom type (T) (PDBQT) file using the option macromolecule. Using open babel in the PyRx application, the ligand compounds' 2D structure in structure data file format was subjected to energy minimization, and PDBQT files were generated. AutoDock PDBQT files of both the macromolecule and the ligand were chosen using Vina Wizard. A grid box with lattice dimensions of X, Y, and Z (42.30, 25.82, 37.88) A° and run Vina docking, to identify the docking sites and amino acid interactions of the ligand to protein [16]. The protein-ligand complexes with optimal binding energies were identified, and the resulting poses were analyzed in Discovery Studio Visualizer 2025.

Statistical analysis

In vitro assay was performed in triplicate (n=3), and the values were expressed as mean±standard deviation. IC_{50} was calculated using a linear regression equation. Results were analyzed by one-way analysis of variance with SPSS version 10.0 software. The average values were significant at $p<0.01$.

RESULTS AND DISCUSSION

Fractionated residue of ethyl acetate

Resulting fractions were concentrated using a rotary evaporator and stored in a desiccator. Thin-layer chromatogram of the fraction was developed using a solvent system of EA: n-hexane (3:7) with a spray reagent of vanillin sulfuric acid. Fractions showing similar spots were combined, and a residue of 5g was obtained and kept in a desiccator for further analysis.

***In vitro* ACE inhibitory assay**

ACE, a metallopeptidase, contributes significant role in the control of hypertension and is identified as a potential target in the management of hypertension. Its primary action is to convert inactive Ang-I to Ang-II, a potent vasoconstrictor, to stimulate aldosterone secretion. ACE degrades the vasodilator, bradykinin, and inactivates other peptides like enkephalin, tachykinins like substance P, and neurotensin [17]. ACE inhibitors are recommended as first-line therapy for managing patients with cardiovascular diseases [18]. Antihypertensive activity of the ethyl acetate fraction of *A. indica* was evaluated by *in vitro* ACE inhibitor assay. With the increase in concentration, % of ACE inhibition is increased and represented in Fig. 2. The highest inhibition was at 150 µg/mL, as 63.42±0.32%. The IC_{50} of EAAI was found to be (117.59±1.25 µg/mL), whereas captopril exhibited an IC_{50} (81.56±2.12 µg/mL). EAAI exhibited good ACE inhibitory effect; however, its potency was significantly lower than captopril ($p<0.01$. Studies indicate that phytocompounds such as phenolics [19], flavonoids [20], and terpenoids [7] can inhibit ACE by forming complexes through interactions at the active site of the enzyme.

IC_{50} was calculated using linear regression analysis of concentration-inhibition curves, and values were reported as mean±SD (n=3).

HR-LCMS

HRLC-MS of the ethyl acetate fraction was carried out to identify the phytochemical composition of *A. indica*. Twenty-six major phytocompounds were identified based on mass, Rt, as mentioned in Table 1, and chromatograms are illustrated in Figs. 3 and 4. The compounds were characterized depending on accurate mass matching with database, literature, applying the mass error tolerance of ≤5 ppm, only 18 compounds were found within the threshold and were considered for further interpretation.

The HR-LCMS data revealed the presence of a diverse range of phytochemicals, including alkaloids, phenolic compounds, glycosides, flavonoids, and terpenoids. Various remarkable compounds with prominent biological activities were identified, including flavonoids such as rutin that exhibits ACE inhibitory [21], antidiabetic, hypercholesterolemia [22], antioxidant, antitumor, and anti-inflammatory action [23], as well as reduction of blood pressure [24]; maritimetin with promising antioxidant [25], phenolic acid like caffeic acid exhibiting cardioprotective, anti-inflammatory, antioxidant [26] and vasorelxant [27] effects; gallic acid showing antioxidant, antineoplastic, anti-inflammatory effects [28]; glycosides such as myrcetin-7-rhamnoside with antioxidant, hepatoprotective, cardioprotective [30]; beta-D-gentiobiosyl crocetin with free radical scavenging [31]; and terpenoids such as austinol showing antibacterial action [32]. The HR-LCMS method provides significant advantages in phytochemical profiling, and its exceptional sensitivity and high resolution make it highly efficient in identifying metabolic constituents. The phytochemical profiling through HR-LCMS identified the presence of diverse phytochemicals such as phenolics, flavonoids, and terpenoids. These groups of phytocompounds are widely recognized for their biological actions, possess significant antioxidant and ACE inhibitory potential, mitigate oxidative stress [33], and thus help regulate blood pressure [34-36]. Meanwhile, terpenoids are associated with vasodilation and ACE inhibition through several mechanisms [7]. The presence of these bioactive compounds in EAAI provides compelling evidence that observed ACE inhibitory effects might be due to the synergistic effects of these natural compounds.

***In silico* approach**

The ligands of different chemical classes were identified from HR-LCMS analysis of the EAAI. A docking study was conducted using AutoDock Vina, following standard procedures [36]. Ever since, the human angiotensin-converting enzyme (1O86), which function as crucial role in modulating blood pressure by means of renin-angiotensin system, has been selected as the prime target protein for screening ACE inhibition [37-40]. Captopril was taken as a reference ligand. The 2D representation of active sites and interactions of ligands is represented in Figs. 5 and 6. The binding score of compounds is described in Table 2. Glycoside (beta-D-gentiobiosyl crocetin −8.6 kcal) demonstrated the best binding energy, a flavonoid constituent maritimetin had −7.8 kcal significant binding affinity, quercetin (−7.7 kcal), rutin (7.3 kcal), and moderate binding affinities were indicated for 2(N)-methyl-norsalsolinol, an alkaloid (−6.2 kcal), phenolic acid, oryzalic acid B (−6.8 kcal), gallic acid (−6.1 kcal), and austinol (−5.8 kcal). Captopril revealed a binding affinity of −8.8 kcal. Molecular docking studies revealed beta-D-gentiobiosyl crocetin highlighted remarkable binding efficiency toward the potential protein target.

Pubchem Compound ID (CID)

Among the compounds identified in EAAI by HRLCMS within mass error of ≤5 ppm, β-gentiobiosyl crocetin, gallic acid, quercetin, maritimetin, rutin, and caffeic acid, as major, demonstrated the highest binding energies. Mechanistically, they interact with the ACE active site and implicate significant ACE inhibition, preventing conversion of Ang I to Ang II and reducing vasoconstriction.

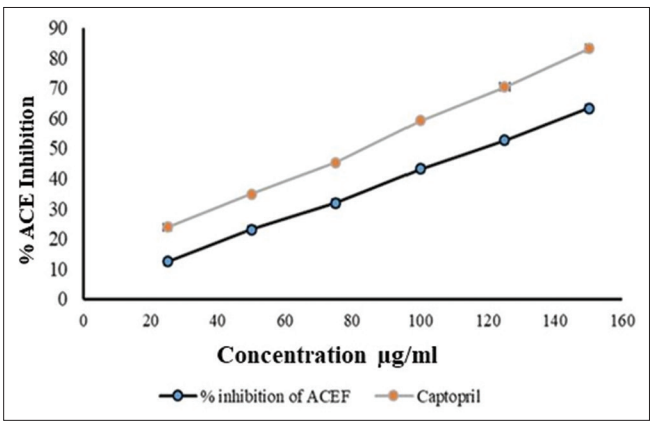

Fig. 2: *In vitro* angiotensin-converting enzyme inhibitory action of ethyl acetate fraction of methanol extract of *Aspidopterys indica*

Table 1: Different phyto compounds identified in the ethyl acetate fraction of *A. indica* by HR-LCMS

S. No.	Compound name	Formula	Proposed formula	Mass	RT	Observed m/z	Calculated m/z	Error (ppm)	Category
1	Prolyl-Arginine	$C_{11}H_{21}N_5O_3$	$C_{11}H_{21}N_5O_3$	271.1639	1.62	294.153	272.1717	1.99	dipeptide
2	2(N)-methyl-norsalsolinol	$C_{10}H_{13}NO_2$	$C_{10}H_{13}NO_2$	179.0939	1.879	180.1009	180.1013	4.02	alkaloid
3	Gallic acid	$C_7H_6O_5$	$C_7H_6O_5$	170.0219	2.017	169.0147	169.0142	−2.21	Phenolic acid
4	N-cis-Caffeoyltyramine	$C_{17}H_{17}NO_4$	$C_{17}H_{17}NO_4$	299.1204	2.87	300.1276	300.1231	−15.39	Phenolic amide
5	Arabinopyranobiose	$C_{10}H_{18}O_9$	$C_{10}H_{18}O_9$	282.0938	3.083	305.083	305.0843	4.54	Disaccharide
6	6-Methylquinoline	$C_{10}H_9N$	$C_{10}H_9N$	143.0725	3.348	144.0797	144.0808	7.28	alkaloids
7	3-Hydroxycoumarin	$C_9H_6O_3$	$C_9H_6O_3$	162.0306	4.145	163.0379	163.0390	6.63	Phenolic
8	Rutin	$C_{27}H_{30}O_{16}$	$C_{27}H_{30}O_{16}$	610.1515	5.701	611.1588	611.1607	3.04	Flavonoid
9	Caffeic acid	$C_9H_8O_4$	$C_9H_8O_4$	180.043	5.732	179.0357	179.0350	−3.85	Phenolic acid
10	Quercetin	$C_{15}H_{10}O_7$	$C_{15}H_{10}O_7$	302.0417	5.93	303.0489	303.0500	3.13	Flavonoid
11	Myricetin 7-rhamnoside	$C_{12}H_{20}O_{12}$	$C_{12}H_{20}O_{12}$	464.0936	6.006	465.101	465.1009	4	Glycoside
12	Maritimetin	$C_{15}H_{10}O_6$	$C_{15}H_{10}O_6$	286.0467	6.535	287.054	287.0528	4.07	Flavonoid
13	Oryzalic acid B	$C_{20}H_{30}O_5$	$C_{20}H_{30}O_5$	350.2096	9.018	373.1982	373.1985	−0.75	Phenolic acid
14	Phytosphingosine	$C_{18}H_{39}NO_3$	$C_{18}H_{39}NO_3$	317.2919	10.78	318.2991	318.2981	3.41	alcohol
15	Armillarin	$C_{24}H_{30}O_6$	$C_{24}H_{30}O_6$	414.2032	12.797	415.2106	415.2096	2.4	Terpenoid
16	Isosyringinoside	$C_{23}H_{34}O_{14}$	$C_{23}H_{34}O_{14}$	534.1857	10.38	557.1747	557.1652	17.14	Glycoside
17	Sphinganine	$C_{18}H_{39}NO_2$	$C_{18}H_{39}NO_2$	301.2973	12.41	302.3047	302.3039	2.57	alcohol
18	Phenyl butyryl glutamine	$C_{15}H_{20}N_2O_4$	$C_{15}H_{20}N_2O_4$	292.1428	12.71	293.52	293.1501	−1.82	Amino acid
19	Austinol	$C_{25}H_{30}O_8$	$C_{25}H_{30}O_8$	458.1926	12.79	481.1810	481.1825	3.22	Terpenoid
20	Triamcinolone diacetate	$C_{25}H_{31}FO_8$	$C_{25}H_{31}FO_8$	478.1993	13.027	501/1889	501.1879	1.98	-
21	Sorbitan palmitate	$C_{22}H_{42}O_6$	$C_{22}H_{42}O_6$	402.2951	17.46	425.2844	425.2814	7.43	-
22	Sorbitan stearate	$C_{24}H_{46}O_6$	$C_{24}H_{46}O_6$	430.3266	18.639	453.3158	453.3129	6.62	-
23	beta-D-gentiobiosyl crocetin	$C_{32}H_{44}O_{14}$	$C_{32}H_{44}O_{14}$	652.2734	18.735	653.281	653.2813	−0.51	Glycoside
24	Octadecyl fumarate	$C_{22}H_{40}O_4$	$C_{22}H_{40}O_4$	368.2925	19.429	391.2818	391.2817	0.31	Fatty acid
25	D8'-Merulinic acid A	$C_{24}H_{38}O_4$	$C_{24}H_{38}O_4$	390.2747	19.829	413.264	413.2639	6.03	Terpenoid
26	Bornyl butyrate	$C_{14}H_{24}O_2$	$C_{14}H_{24}O_2$	224.176	26.694	247.1653	247.1635	7.21	Terpenoid

HR-LCMS: High-resolution liquid chromatography mass spectroscopy

Table 2: Structural insights of molecular docking studies of ligands with target proteins

S. No.	PubChem Compound ID	Ligand name	Binding energy (Kcal/mol)	Hydrogen bonding	Hydrophobic interactions
1	37764	2(N)-methyl-norsalsolinol	−4.3	-	ASN A.406, PHE A.570, LYS A.118, GLY A.404, GLUA.403, PRO A.407, GLU A.411, HIS A.410, ARG A.522
2	5281292	Maritimetin	−7.8	THR A.282, ASN A.205	VAL A.380, VAL A.379, GLU A.376, ASP A.453, SER A.284, LEU A.375, LYS A.449
3	689043	Caffeic acid	−6.5	GLY A.404	PRO A.407, GLU A.403, HIS A.410, HIS A.387, ARG A.522, GLU A.411, PHE A.391, ALA A.356
4	5280805	Rutin	−7.3	ASN A.205, ASN A.209, VAL A.471, TYR A.481, ARG A.209, GLY A.474	GLN A.206, VAL A.201, THR A.477, ILE A.476. VAL A.214, ASP A.215, PHE A.215, LYS A.479
5	76594575	Myrcetin 7-rhamnoside	−4.8	PHE A.570	MET A.223, LYS A.118, ASN A.406, GLY A.404, HIS A.410, PRO A.407
6	5280343	Quercetin	−7.7	THR A.301, MET A.299, THR A.302	ASN A.374, ASN SER A.284, A.285, ASN A.285, GLU A.376, VAL A.379, LYS A.449, ASP A.300, MET A.450, LYS A.454
7	56955927	Austinol	−6.8	TYR A.523, TYR A.520, ALA A.354, HIS A.353	HIS A.387, GLN A.281, LYS A.511, HIS A.583, HIS A.513, VAL A.380, GLU A.411, VAL A.518, HIS A.383, PHE A.512,
8	131752211	Oryzalic acid B	−5.3	LYS A.449, SER A.284	SER A.422, LEU A.375, LYS A.454, ASP A.453, MET A.450, VAL A.379, GLU A.376, THR A.301, ASN A.374, ASN A.285
9	10461942	beta-D-Gentiobiosyl crocetin	−8.6	TYR A.213, ALA A.356	GLY A.212, ASP A.121, ASN A.406, GLU A.403, ARG A.522, LYS A.117, SER A.522
10	370	Gallic acid	−6.1	ALA A.356, GLU A.403	GLU A.411, ARG A.522, HIS A. 410, VAL A.518, TRP A.357, PHE A.391, HIS A.387, HIS A.410

Quercetin exerts antihypertensive action by reducing oxidative stress, inhibiting ACE activity, vascular smooth muscle relaxation, and normalizing endothelial function, modulation in gene expression and cell signaling [41]. β-gentiobiosyl crocetin, a carotenoid, facilitates endothelium-dependent vascular relaxation [42-43]. Rutin inhibited ACE and angiotensin 2 type 1 receptor (ATR1) [44]. Gallic acid has protective action against cardiovascular diseases, enhancing antioxidant action, inhibition of lipid peroxidation, and reduction of serum cardiac marker enzymes, and regulation of vascular parameters [45]. Maritimetin, a phenol compound, forms hydrogen bonds and hydrophobic interactions and stabilizes the ACE-ligand complex [46]. Caffeic acid hydrogen bond specifically interacts with GLY A.404 in the active site of ACE and enhances inhibition [47]. The bioactive constituents within a mass error of ≤5 ppm and significant docking scores suggest that their significant synergistic effect contributes to *in vitro* ACE inhibition. The IC_{50} of EAAI remains higher

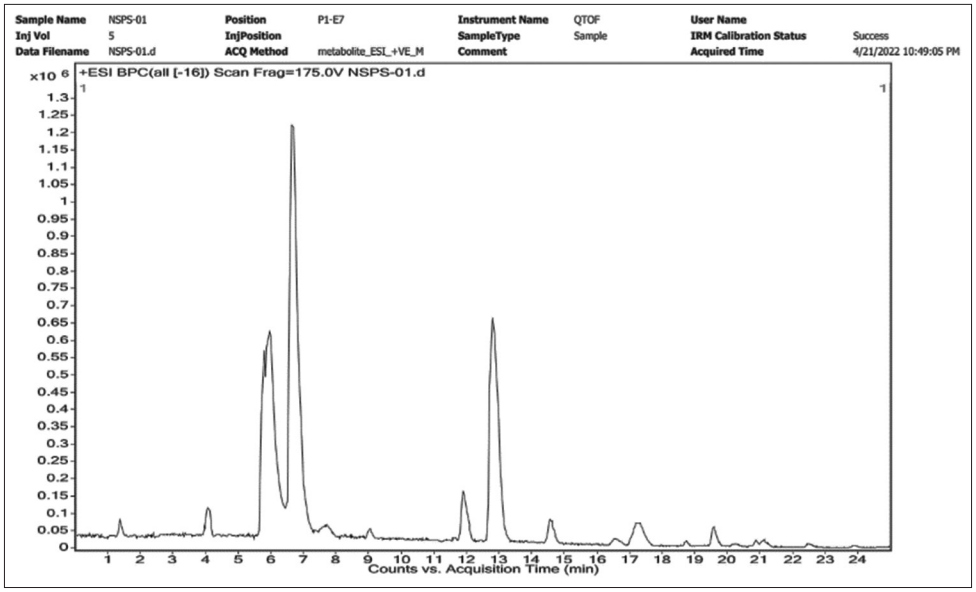

Fig. 3: Base peak chromatogram of the ethyl acetate fraction of *Aspidopterys indica* recorded in positive electron spray ionization mode

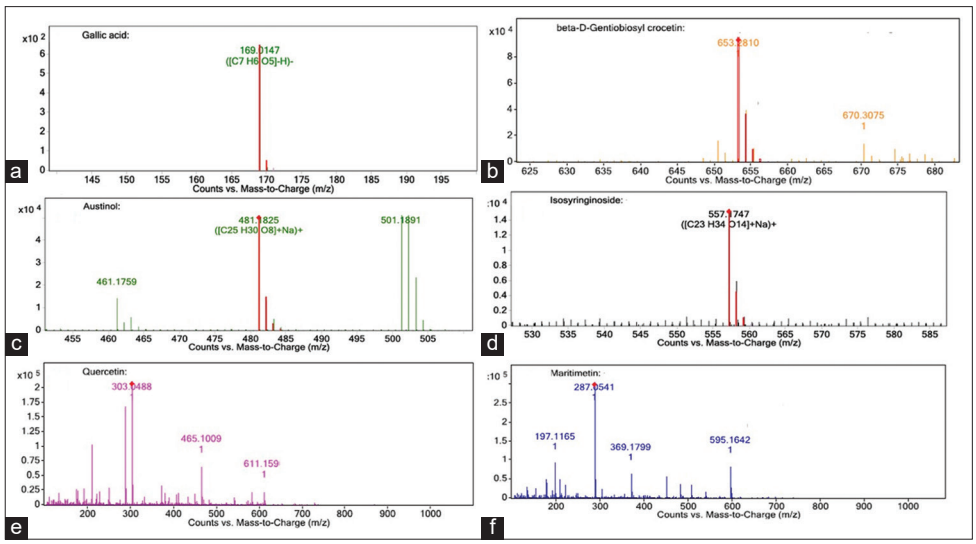

Fig. 4: Chromatogram profiles of compounds identified in endemic drug *Aspidopterys indica*. The ion chromatograms corresponding to (a) gallic acid, (b) beta-D-gentiobiosyl crocetin, (c) austinol, (d) isosyringinoside, (e) quercetin, and (f) maritimetin

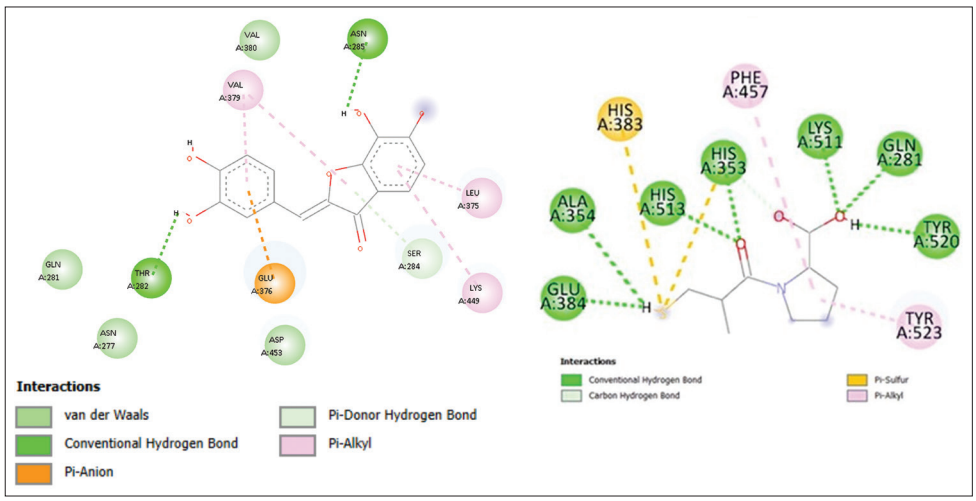

Fig. 5: 2D interactions of maritimetin and captopril with 1O86

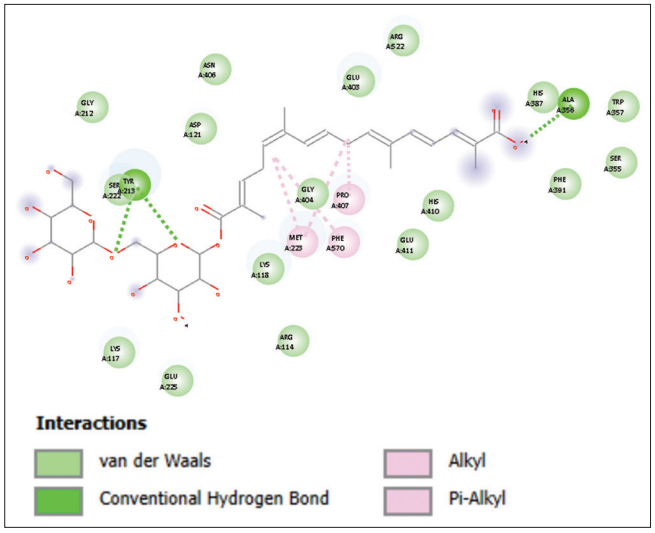

Fig. 6: 2D interactions of with beta-D-gentiobiosyl crocetin1O86

than that of captopril due to several factors. Bioavailability of isolated compounds may be poor due to poor absorption and low solubility [48]. Moreover, the interactions, synergistic effects, and metabolic transformation of these bio-compounds may produce metabolites with ACE inhibition, contributing to a higher IC_{50} for EAAI [49, 50].

CONCLUSION

Aerial parts of *Aspidopterys indica* were ultrasonicated to obtain a methanol extract, and further, it was fractionated using ethyl acetate by vacuum liquid chromatography. The results of *in vitro* studies of EAAI manifested good ACE inhibition compared to captopril. The fraction employing the HRLCMS technique identified active bio-chemicals with promising therapeutic efficacy – an effective strategy for recognizing the secondary metabolites in natural sources. The present study identified that EAAI contains distinct phytocompounds such as terpenoids, flavonoids, glycosides, and phenolic acids. The *in silico* studies revealed the possible interactions at the active sites of the ACE target and binding affinities of phytochemicals, supporting their ACE inhibitory potential. Beta-D-gentiobiosyl crocetin, maritimetin, quercetin, and rutin have a high affinity toward the target protein (PDB ID: 1O86) involved in ACE inhibition, similarly to captopril. These findings support the antihypertensive potential of *A. indica*. Future research studies could focus on *in vivo* experimental studies to evaluate therapeutic efficacy and safety profiles of identified phytochemicals.

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AUTHORS' CONTRIBUTIONS

Authors have participated in collection of data, analysis, drafting, and revision of the content.

CONFLICTS OF INTEREST

The authors proclaim no conflicts of interest.

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