HR-LC-MS Phytochemical Profiling of *A.cordata* Methanolic Fraction and Antihypertension in Doca Salt-Induced Hypertensive Rats

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Abstract.

The biochemical profile of A.cordata's methanolic fraction to assess antihypertensive potential .A high resolution LC-MS technique was used to identify A.cordata phytochemicals and appraise antihypertension in a Doca salt induced model in rats.

Chlorgenic acid, caffeic acid, ferulic acid, 4-Hydroxycinnamic acid, 4-methylumbelliferyl sulfate, 5,7-Dihydroxychromone were found. There is reduction in elevated levels of Kidney enzymes, liver enzymes as well as improvement in levels of antioxidant enzymes Phenolics and coumarins may contribute to *A.cordata*'s antihypertensive attributes

Key words: *A.cordata*, Methanolic fraction, HR-LC/MS Doca salt model induced Hypertension

Running Title: HR-LC-MS profile and Antihypertensive action of *A.cordata* Methanolic fraction.

Introduction:

Medicinal plants for thousands of years in India utilised for treatment various diseases. As a result of the presence of various chemical constituents. Plants are used for medicinal purposes in several countries and are the source of many potent and powerful drugs(1). Pharmacological researchers in search of novel bioactive compounds initially faced significant technological impediments in extracting, isolating, and characterising the compounds. Despite complex obstacles, researchers have been able to overcome methodological difficulties in finding metabolites from chemically diverged complex crude mixtures by continuing to study plant chemistry. This was made possible by researchers with the use of liquid chromatography-mass spectrometry techniques for untargeted phytochemical profiling(2). Hypertension is a serious medical condition that provokes the risk of a variety of health-related issues. It is a noncommunicable global disease with no initial symptoms and a high mortality rate; it is considered as the silent killer. Approximately 1.13 billion people worldwide suffer from hypertension, with the majority coming from low and middle-income countries(3) Treatment of hypertension necessitates a combination of drugs that act at various therapeutic sites and thus have adverse effects. which inturn leads to complications in the treatment of hypertension. Herbal drugs, on the other hand, that contain a therapeutically important combination of constituents have a variety of clinical issues(4)

Aspidopterys cordata (Heyne ex Wall) A.Juss of the Malphigiaceae family, Synonyms: Hiraea cordata, Herbacious Climbing Shrubs Found in the forests of Mahabubnagar,

Adilabad, Nizamabad, Medak, and Ranga Reddy districts(5),(6). This plant has a high concentration of phenols and flavonoids, as well as effective DPPH and Nitric oxide antioxidant activity(7). Since there isn't any evidence of previously reported phytochemicals, an attempt is made for phytochemical profiling of *A.cordata* methanolic fraction via HR-LC-MS, and antihypertensive activity was tested on Doca salt induced hypertensive rats.

Methodology:

Authentication and collection of Plant material:

Aspidopterys cordata was discovered at Kinnerasani Wild Life Sanctuary in the Telangana district of Bhadradri Kothagudem. Botanist Dr. K. Venkata Ratnam, Assistant Professor, Department of Botany, Rayalaseema University, Kurnool, has confirmed the plant; a plant specimen (RU/BD/VSN-092) has been submitted for future reference(7)

Plant Extraction:

Plant material aerial parts were collected, washed with water, dried in the shade, defatted with petroleum ether, and sonicated with Methanol at 40KHz for 45 minutes at 45^{o}C. After filtering, the supernatant solutions were decanted, concentrated in a Rotary evaporator, and stored in a desscicator(8)

Fractionating Methanolic Extract:

The methanolic extract was fractionated with increasing polarity solvents such as hexane, chloroform, ethylacetate, and methanol using vacuum liquid chromatography. (9). Methanolic fraction recovery was found to be good, so it was chosen for the study.

HR-LC-MS:

Tha anlalysis of metabolites obtained from methanolic fraction by IIT Saif, Mumbai using LC-ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel)(10).

Experimental animals:

In this study, male Wistar rats(180-200gm) were selected and were acclimatized, randomly grouped in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2˚C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed free access to water and fed with a standard commercial pelleted rat diet. The experimental procedures and protocols used in this study were reviewed and approved by the Institutional animal ethics committee (IAEC) before experimental studies (1447/PO/Re/S/11/CPCSEA-31/A).

Acute toxicity studies:

For acute toxicity studies of *A.cordata* OECD-425 guidelines were followed.

Grouping of animals:

Group 1	UNTZD Control
Group 2	UNTZD + DOCA
Group 3	UNTZD + DOCA+Captopril (30mg/kg)
Group 4	UNTZD + DOCA+ AIMF (200mg/kg)
Group 5	UNTZD +DOCA+ AIMF (400mg/kg)

***Invivo* DOCA-salt-induced hypertension in rats:**

The administration of a synthetic mineralocorticoid derivative, DOCA, in combination with salt loading in the diet to young adult Wistar rats following surgical removal of one kidney induces hypertension with cardiovascular remodelling characteristic of human volume-overload induced hypertension, especially hypertrophy, fibrosis, conduction abnormalities and endothelial dysfunction.

Nephrectomy was conducted on anesthetised mice (zoletil injection I.p 25mg/kg and zolazepam 25 mg/kg) by performing a small cut, and the left kidney was excised, accompanied by the joining left renal artery, vein with ureter. The cut was sutured, and adequate post-operative treatment was continued for seven days. The animals additionally

got 1% solution of sodium chloride everyday through drinkable water and subcutaneously of (DOCA)-salt (20 mg/Kg body weight) into 0.4ml DMF twice weekly for five weeks(11)

Measurement of blood pressure:

The tail-cuff method was used for assessing mean heart rate weekly to ascertain the impact of the treatment upon that groups(12). Animals were anaesthetized using thiopental sodium (45mg/kg body weight, i.p.) after five weeks of therapy, and their hearts were separated for biochemical measures.

Estimation of Biochemical parameters:

Blood is collected from the retero orbital plexus, sera is extracted via cold centrifuged at 2,000 rpm for 5 minutes, and serum levels are determined. Reitman and Frankel, method were used to measure AST, ALT and ALP(13). Jeffe kinetic technique(14), Dioxime method(15), and Urease PAP method(16) were used to calculate the concentrations of Creatinine Urea, Uric acid in plasma.

Homogenates were prepared using cardiac tissue on ice in the proportion of 4 g tissue with 16 ml of phosphate buffer at pH 7.5 and were agitated at 20000 rpm for 15 minutes at 40 degrees Celsius until being frozen at -50 degrees Celsius until analysis. Antioxidants components GSH, CAT, and SOD were estimated using the Ellman(17), Clairbone(18), and Fridovich methods(19), MDH (20)respectively.

Statistical analysis:

Statistical analyses were carried out using SPSS version 10.0 and a one-way variance analyses (ANOVA), followed by Duncan's multiple range test (DMRT). P0.05 was used as the significance level.

Results:

Table.1: HR-LC-MS phytochemical profiling of *A.cordata* Methanolic Fraction

S.NO	Compound Name	Formula	m/z	Base peak	RT
1	5,7-Dihydroxychromone	$C_9H_6O_4$	177.0201	197.809	0.814
2	4-Hydroxycinnamic acid	$C_9H_8O_3$	163.0407	197.8091	1.108
3	Gallic acid	$C_7H_6O_5$	169.0147	197.8086	1.218
4	Caffeic acid	$C_9H_8O_4$	179.0357	197.8091	1.533
5	4-Hydroxycinnamic acid	$C_9H_8O_3$	163.0407	197.8091	1.879
6	2,6-Dihydroxyphenylacetate	$C_8H_8O_4$	167.0356	353.0907	2.729
7	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0908	353.0908	2.78
8	Ferulic acid	$C_{10}H_{10}O_4$	193.0522	197.8094	3.083
9	Resorcinol	$C_6H_6O_2$	109.0302	197.8087	5.1
10	Ferulic acid	$C_{10}H_{10}O_4$	193.0522	197.8094	9.018
11	4-Methylumbelliferyl sulfate	$C_{10}H_8O_6S$	301.0014	197.8093	10.038
12	Resorcinol	$C_6H_6O_2$	109.0302	197.8087	12.67
13	2,6-dihydroxybenzoic acid	$C_7H_6O_4$	153.0199	197.8086	12.704
14	4-Hydroxycinnamic acid	$C_9H_8O_3$	163.0407	197.8091	12.71
15	Debromohymenialdisine	$C_{11}H_{11}N_5O_2$	290.0897	128.0355	13.027
16	Debromohymenialdisine	$C_{11}H_{11}N_5O_2$	290.0899	128.0358	17.39
17	Dihydroferulic acid 4-O-glucuronide	$C_{11}H_{20}O_{10}$	371.101	137.0256	18.639
18	Mecarbinzid	$C_{13}H_{16}N_4O_3S$	353.0904	191.0564	19.429
19	Mecarbinzid	$C_{13}H_{16}N_4O_3S$	353.0909	191.057	19.436
20	Mecarbinzid	$C_{13}H_{16}N_4O_3S$	353.0908	191.0563	19.829
21	Quinacridone	$C_{20}H_{12}N_2O_2$	371.1023	371.1002	20.12
22	Edulisin I	$C_{28}H_{26}O_8$	535.1593	134.8949	20.31
23	Edulisin I	$C_{28}H_{26}O_8$	535.1595	134.896	20.39
24	Lauryl hydrogen sulfate	$C_{12}H_{26}O_4S$	265.1508	265.1506	20.48

Table.2: Biological activities of few compounds

Ferulic acid-Antioxidant, anti-inflammatory, antiviral, antiallergic, antimicrobial, antithrombotic, anti-carcinogenic and hepatoprotective actions.(21)
Hydroxycinnamic acid- Antioxidant(22)
Gallic acid- Antibacterial antifungal, antiviral, anti-inflammatory, antioxidant, anticancer, anti-diabetic effect(23)
Chlorogenic acid- Antioxidant, anticancer, antitumour(24)
Caffeic acid- Anticancer, antioxidant(25)

Fig.1 Chromatograms of Major Phytocompounds of HR-LC-MS of *A.cordata*

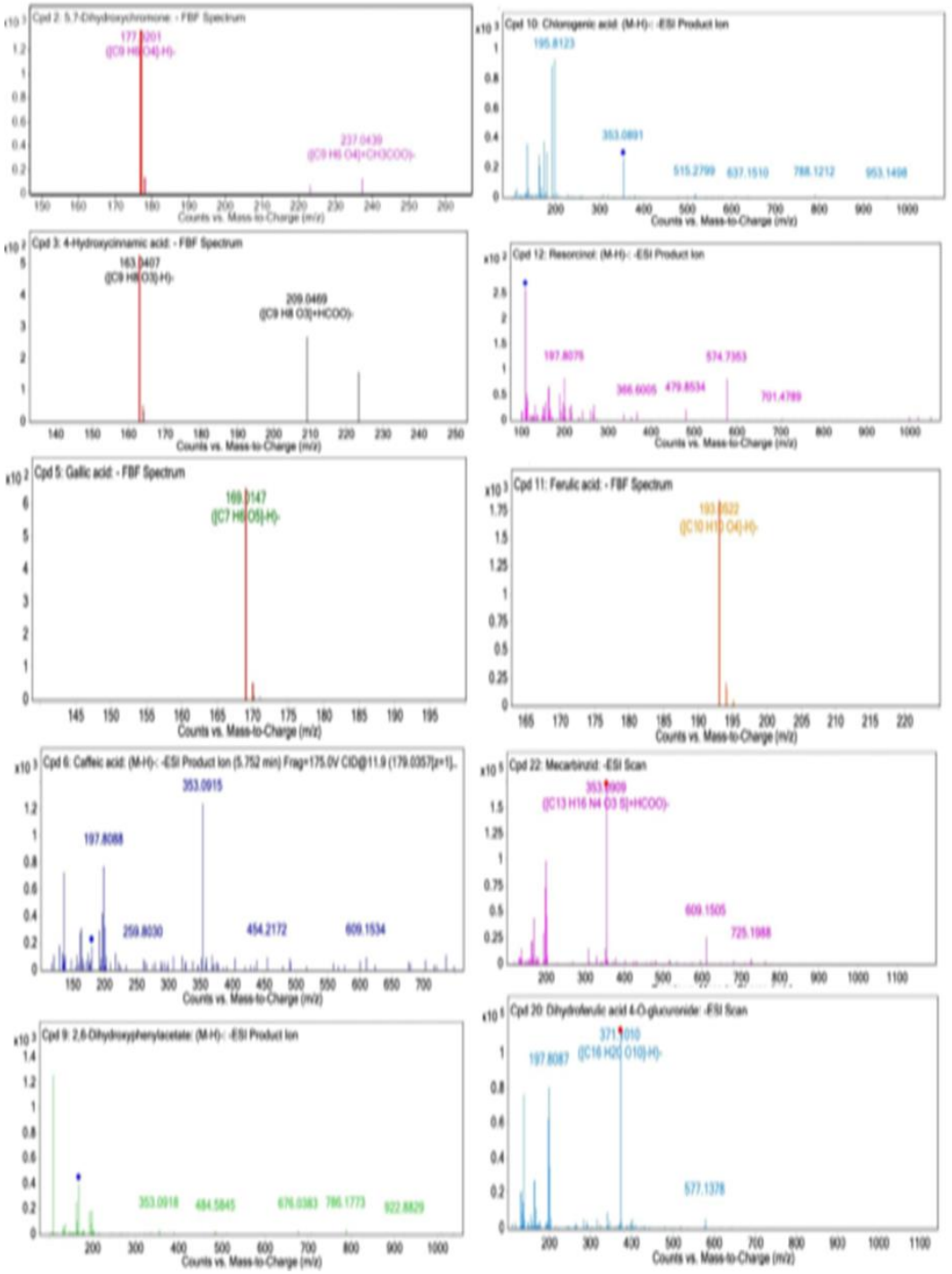

Table.3 Effect of Methanolic fractions of *A.cordata on* serum Liver enzymes in uninephrectomized DOCA-salt hypertensive rats

Treatment groups	AST IU/L	ALT IU/L	ALP IU/L
G1	72.21±1.22	29.32±1.04	81.23±2.01
G2	132.87±1.77$^{\#\#}$	71.33±0.44$^{\#\#}$	142.54±1.52$^{\#\#}$
G3	75.21±1.03**	31.33±0.48**	81.33±1.22**
G4	88.22±0.33*	38.22±1.33*	117.21±1.38*
G5	79.22±0.88**	31.22±0.37**	81.22±1.22**

Values are expressed as Mean±SEM (n=6)
The statistical significance was determined using one-way analysis of variance (ANOVA) and the Dunnett multiple comparisons test, performed using SPSS software**.**
P values: # P< 0.05 or * P< 0.05 (Significant), ##P< 0.01 or **P< 0.01 (Highly significant)

Table.4 Effect of Methanolic fractions of *A.cordata* on Serum Kidney parameters in uninephrectomized DOCA-salt hypertensive rats

Treatment Groups	Urea(mg/dL)	Uric acid (mg/dL)	Creatinin(mg/dL))
G1	18.32±1.32	1.36±0.23	0.82±0.43
G2	51.45±1.6$^{\#\#}$	3.98±1.35$^{\#\#}$	3.12±2.01$^{\#\#}$
G3	19.23±2.15**	1.53±1.22**	0.98±1.82**
G4	33.23±0.34*	2.18±0.34**	2.01±1.34*
G5	27.34±0.34*	1.95±0.98*	1.45±0.43*

Values are expressed as Mean±SEM (n=6)
The statistical significance was determined using one-way ANOVA followed by the Dunnett multiple comparisons test in SPSS software.
P values: # P< 0.05 or * P< 0.05 (Significant), ##P< 0.01 or **P< 0.01 (Highly significant)

Table.5 Effect of Methanolic fractions of *A.cordata* on antioxidant enzymes in uninephrectomized DOCA-salt hypertensive rats

Treatment groups	SOD (U/gm Protein)	CAT(U/gm Protein)	GSH(µM/gm Protein)	MDH(n.Moles/gm Protein)
G1	32.72±0.11	15.56±0.33	220.65±0.33	2.32±0.32
G2	25.34±1.11$^{\#\#}$	8.53±1.33$^{\#\#}$	195.77±1.37$^{\#\#}$	6.12±1.32$^{\#\#}$
G3	31.81±1.27	13.33±1.23**	212.24±1.25**	2.89±0.33**
G4	30.08±0.34*	11.28±1.74**	209.33±1.24**	3.23 ± 0.93
G5	30.11±0.66**	12.39±0.33**	210.97±1.66**	3.04 ±0.11**

Values are expressed as Mean±SEM (n=6)
The statistical significance was determined using one-way analysis of variance (ANOVA) and the Dunnett multiple comparisons test in SPSS software**.**
P values: # P< 0.05 or * P< 0.05 (Significant), ##P< 0.01 or **P< 0.01 (Highly significant)

Discussion:

The biochemical Profiling of *A.cordata* methanolic fraction shows the presence of Ferulic acid, Chlorogenic acid, Caffeic acid, 5,7-Dihydroxychromone, 4-methylumbelliferyl sulfate, 4-Hydroxycinnamic acid were reported to have several actions as reported in table.2. Diastolic and systolic blood pressure reduction (12). The high dose of Methanolic fraction lowered elevated levels of kidney enzymes and liver enzymes like and enhanced antioxidant levels. The phenolic, polyphenolic, and coumarin componds could be responsible for antihypertensive action of *A.cordata*

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Authors Contribution:

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