RESEARCH ARTICLE

Isolation, Characterisation and *In-vitro* Antioxidant activities of Flavonoid Compounds from Methanolic fraction of *Aspidopterys indica*

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ABSTRACT

Aspidopterys indica belongs to the family Malphigiaceae and is used to treat hypertension and skin diseases. In the current investigation, methanolic extract was fractionated using vacuum liquid chromatography, and the methanolic fraction was subjected to column chromatography for isolation and characterized by fourier transform infrared spectroscopy (FTIR), Mass, proton nuclear magnetic resonance (^{1}H-NMR), and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy. The compounds isolated were assessed for their *in-vitro* DPPH antioxidant activity. Two compounds, AI-1 and AI-2, established after spectral analysis, were identified as catechin flavan-3-ol and isoorientin flavone C-glucoside. The results indicated that the compounds showed concentration-dependant DPPH scavenging action and IC_{50} of catechin 93.66 µg/mL, isoorientin 92.09 µg/mL. This study concludes that catechin and isoorientin with known antioxidant potential were isolated from the methanolic fraction of *A. indica,* which could effectively manage oxidative stress-related diseases.

Keywords: Isolation, Fractionation, Bioactive compounds, DPPH antioxidant activity.

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INTRODUCTION

Phytochemicals found in plants that cause distinct physiological responses in the human body are called secondary metabolites. These are usually nutrient substances but have protective and preventive properties and are known to cure diseases in homeopathic and herbal medicine. Searching for bioactive compounds is needed using newer phytochemical techniques.[1]

Due to enormous chemical diversity, natural products consistently gave a scope to discover new drug molecules as an extract, pure isolated compound, or standardized product. In accordance with the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their basic health needs.[2] The utilization of medicinal plants in Asia constitutes a long historical event of human communication in companion with the living world. Plants involved in conventional medicine contain various elements can be useful in the treatment of persistent and contagious infections.[3]

Plants accommodating agreeable biochemicals can meet the human body's needs by acting as natural antioxidants[4] and these are key chemical components that can prevent or delay cell damage. Plant-based antioxidants such as phenolic compounds such as flavonoids, tannins and lignins, and vitamins A, C, and E all play an essential role in the oxidation process by reacting with free radicals and protecting cells from free radical damage without causing side effects.[5-7] They can be used as natural antioxidants to prevent or regenerate cell damage in the human body. The primary mechanism of action is to boost the body's immune system by producing free radicals.[8] Numerous studies have shown a positive association between increased dietary intake of natural phenolic antioxidants, reduced mortality from ischemic heart disease and cancer, and increased life expectancy.[9] Flavonoids are often suggested as good sources for antioxidant therapy because of their potential role in supporting health and are naturally occurring polyphenolic compounds ubiquitous in photosynthetic cells.[4]

Aspidopterys indica W(Theob) is a slender climbing shrub belonging to Malphigiaceae.[10] The aerial parts show hypotensive action.[11] The whole plant treats skin diseases.[12] The plant has reported high phenolic and flavonoid content and promising *in-vitro* Antioxidant action.[13] So far, no phytocomponents have been isolated from this plant. Therefore, in this contemporary research, we attempted to isolate and

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characterize the phytochemicals in the methanolic extract of the aerial parts of *A. indica* and estimate the antioxidant capacity of the isolates.

MATERIALS AND METHODS

Collection of Plant Material and Authentication

The plant portions of *Aspidopterys indica* were gathered from Bhadradri, Kothagudem, Telangana District.

Extraction

The aerial portions of *A.indica* were collected, Washed with running water, shade-dried, and pulverized in an electric blender. Methanol is added plant sample and ultrasonicated at 40 kHz at 40°C for 45 minutes. Supernatants were concentrated in rota vapor, and concentrates were stored in a desiccator.[13]

Fractionationization

Silica-gel of mesh size (100–200) is loaded Sintered glass funnel with a G_1 grade fritted disk (90–150 µ); the methanolic extract is adsorbed onto silica gel saturated until it is dry and then introduced to adsorbent and solvents like n-hexane, chloroform, ethyl acetate, and methanol were passed from the top of the column, and mild vacuum of 20 to 70 mmhg is applied. The fractions were collected into the volumetric flask. Solvents were added until colorless fractions were obtained, and fractions were concentrated in a rota vapor.[14]

Aspidopterys indica methanolic fraction (40 g) was made to dissolve in little bit of methanol and later completely adsorbed onto (60–120) mesh silica gel. After the complete drying of silica gel, this adsorbed fraction was packed into a glass column. The column was eluted slowly by adding solvents increasing in polarity; 170 fractions were collected. Fractions were concentrated using Rota vapor. Thin layer chromatography (TLC), similar fractions were combined and shown in Table 1 (Figure 1).

In-vitro antioxidant activity

Antioxidant activity was determined for the Isolated compounds AI-1 and AI-2.

DPPH antioxidant activity

From 0.004% w/v DPPH solution, 1-mL was taken and added to 3 mL of extract/standard solutions of different concentrations varying from (5–150 µg/mL) mixed and incubated for around 30 minutes at room temperature. In 1-mL DPPH in 3 mL methanol was used as the negative control. The absorbances were measured at 517 nm in triplicates. Ascorbic acid is used as standard. IC_{50} is the concentration at which 50% of free radical inhibition is seen.[13,17]

$$\% \text{ Scavenging activity} = \frac{A_o - A_s}{A_s} \times 100$$

A_o absorbance of Negative control reaction mixture

A_s absorbance of Extract/Standard and DPPH reaction mixture

Graph plotted against %scavenging of AI-1, AI-2, and ascorbic acid at varying concentrations from the trend line and regression equation IC_{50} values were calculated.

Statistical Analysis

All results were statistically analyzed by one-way ANOVA, significance level of $p < 0.005$, using Graph Pad Prism 9.5.1 software. Experimental data was collected in triplicate and represented as mean ± S.D.

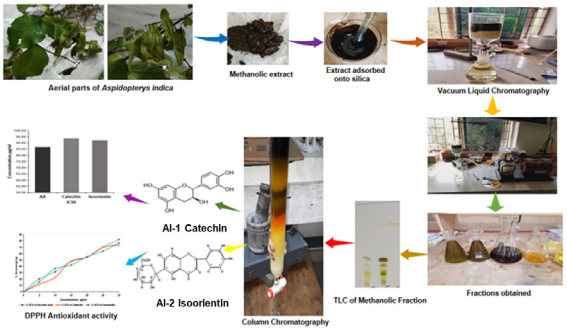

Figure 1: Illustrative representation of Isolation of *A. indica*[15]

RESULTS AND DISCUSSION

Identification of Compounds

Two compounds were obtained from methanolic fraction; ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry for further structural confirmation.

Structural Elucidation of Compound Ai-1

The light brown residue from fraction 16 yielded AI-1 (32 mg) with U.V. max 279 nm, molecular formula $C_{15}H_{11}0_6$. Mass spectra revealed a base peak at m/z 291.083 and fragment peaks as recorded in Figure 2. FTIR spectrum exhibited absorption peaks at 3449 (O-H), 2552 (C=C), 1423 (C-C), 1059 (C-O-C) as shown in Figure 3.

^{1}H-NMR spectral data of compound AI-1

The ^{1}H NMR spectrum showed M.P. ranged from 175–180 °C. In Figure 4 ^{1}H NMR spectrum showed δ 6.63 (1H, d, C-2'),

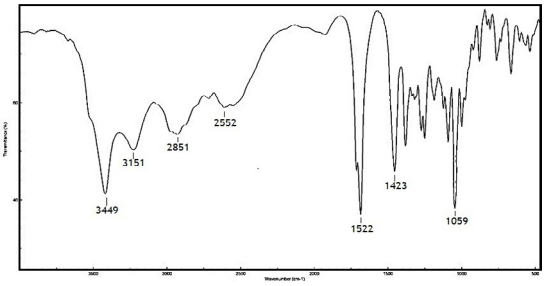

Figure 2: FTIR spectra of AI-1[18]

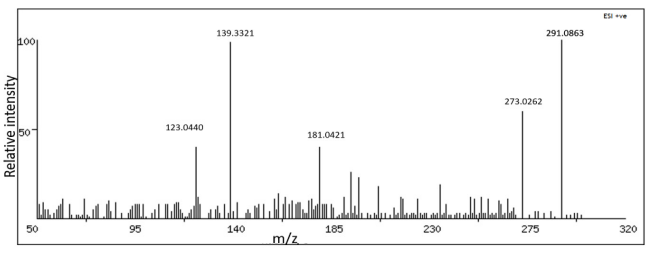

Figure 3: Mass spectrum of AI-1[19]

Table 1: Column chromatography details of Methanolic fraction of *A. indica*[16]

S.NO	*Fraction*	*Solvent system*	*Ratio*	*Color of eluent*	*Color of spots*
1	F1-F5	Hexane	100	No	No visual spots
2	F6-F10	$CHCl_3$: Hexane	1:99	No	No visual spots
3	F11-F15	$CHCl_3$:Hexane	5:95	No	No visual spots
4	F16-F20	$CHCl_3$: Hexane	10:90	No	No visual spots
5	F21-F25	$CHCl_3$: Hexane	15:85	No	Yellow
6	F26-F30	$CHCl_3$:Hexane	20:80	Yellow	Yellow
7	F31-F38	$CHCl_3$: Hexane	25:75	Yellow	Yellow
8	F39-F45	$CHCl_3$: Hexane	40:60	Yellow	No visual spots
9	F46-F55	$CHCl_3$:Hexane	50:50	Orange	Orange spots
10	F56-F60	$CHCl_3$: Hexane	60:40	Orange	Orange spots
11	F61-F65	$CHCl_3$: Hexane	70:30	Orange	Orange spots
12	F66-F70	$CHCl_3$:Hexane	80:20	Orange	No visual spots
13	F71-F75	$CHCl_3$: Hexane	85:15	-	No visual spots
14	F76-F80	$CHCl_3$: Hexane	90:10	Brown	Light brown spots
15	F81-F85	$CHCl_3$	100	Brown	Pale brown spots
16	F86-F90	EA: CHCl3	10:90	Light brown	Compound AI-1
17	F91-F95	EA: $CHCl_3$	15:85	Brown	No visual spots
18	F96-F100	EA: $CHCl_3$	20:80	-	No visual spots
19	F101-F105	EA: $CHCl_3$	25:75	Pale green	No visual spots
20	F10F-F110	EA: $CHCl_3$	30:70	Light green	Green spots
21	F111-F115	EA: $CHCl_3$	40:60	Light green	Green spots
22	F116-F120	EA: $CHCl_3$	50:50	-	No visual spots
23	F117	EA: $CHCl_3$	60:40	-	No visual spots
24	F118-F120	EA: $CHCl_3$	80-20	-	No visual spots
25	F121-F125	EA	100	-	No visual spots
26	F126-F128	MeOH: E.A.	5:95	Dark green	No visual spots
27	F129-F130	MeOH: E.A.	10:90	Dark green	No visual spots
28	F131-F135	MeOH: E.A.	20:80	No visual spots	No visual spots
29	F136-F140	MeOH: E.A.	25:75	Dark brown	Brown spots
30	F141-F145	MeOH: E.A.	30:70	Dark brown	Brown residue 2
31	F146-F150	MeOH: E.A.	35:65	Gritty brown	Brown spots
32	F151-F155	MeOH: E.A.	40:60	Gritty brown	Brown spots
33	F156-F160	MeOH: E.A.	50:50	Gritty brown	Compound 1 AI-2
34	F161-F165	MeOH: E.A.	60:40	-	No visual spots
35	F166-F170	MeOH: E.A.	70:30	-	No visual spots

6.59 (1H, d, C-5'), 6.66 (1H, m, C-6'), 4.86 (1H, d, C-2), 4.88 (1H, d C-3), 3.04 (1H, dd, C-3a, equatorial), 2.72 (1H, dd, C-3b, axial) these two double doublets confirms the absence of carbonyl functional group on fourth position. The broad peak at 1.82 represents the hydroxyl group attached to the C ring at the 3rd position. Similarly, the peaks at 6.23 and 6.24 represent the molecule's polyhydroxy nature. The signals at the range of 6ppm also represent the aromatic ring system upon a molecule.

^{13}C-NMR Spectrum of Compound AI-1

As shown in Figure 5, similarly, the ^{13}C-NMR spectrum of the compound indicated 15 carbon signals with no free methyl groups and methoxy groups with δ values 81.21 (C-2), 69.79 (C-3), 28.97 (C-4), 154.84 (C-5), 95.76 (C-6), 156.09 (C-7), 95.72 (C-8), 155.06 (C-9), 99.68 (C-10), 131.26 (C-1'), 115.32 (C-2'), 145.17 (C-3'), 145.21 (C-4'), 115.93 (C-5'), 119.50 (C-6'). The quaternary carbon (C-9) linked to the oxygen atom and the carbons bearing hydroxyl groups exhibit higher ppm values in the ^{13}C-NMR spectrum (Figure 6).

Characterization of Compound AI-2

Fractions that yielded the same chromatogram were combined 33rd fraction (56–60 subfractions), producing light brown powder AI-2 with molecular formula $C_{21}H_{20}O_{11}$: melting

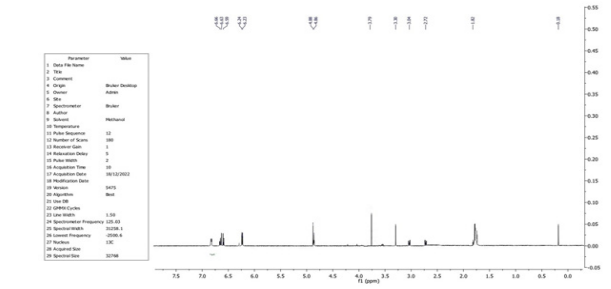

Figure 4: AI-1 ^{1}HNMR spectra[18]

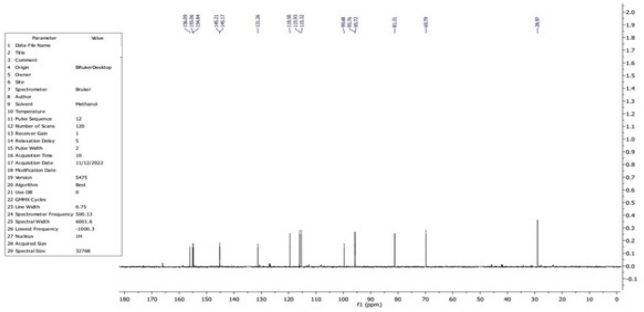

Figure 5: AI-1 ^{13}C-NMR spectra[18]

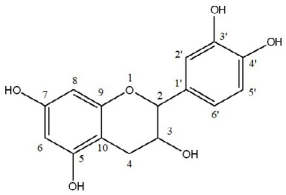

Figure 6: The compound identified as catechin from ^{1}H-NMR and ^{13}C-NMR[20]

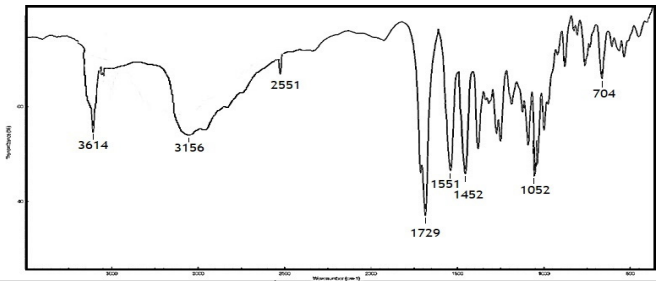

Figure 7: FTIR spectrum of AI-2[18]

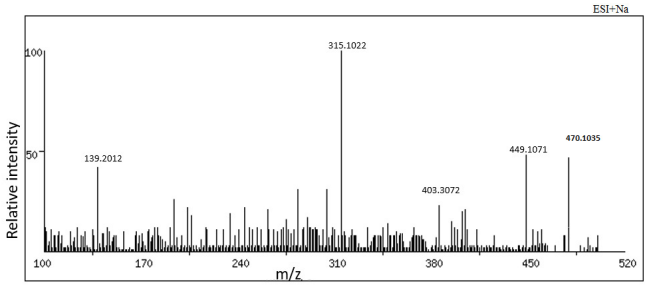

Figure 8: Mass spectra of AI-2[19]

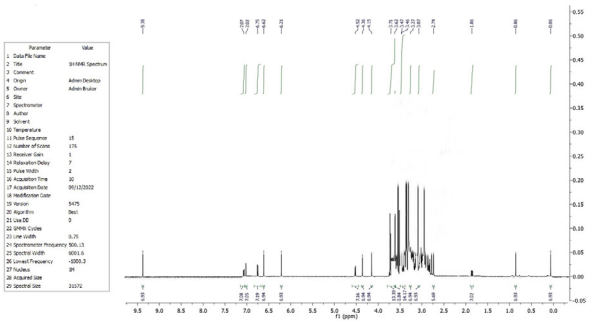

Figure 9: AI-2 ^{1}H-NMR spectra[18]

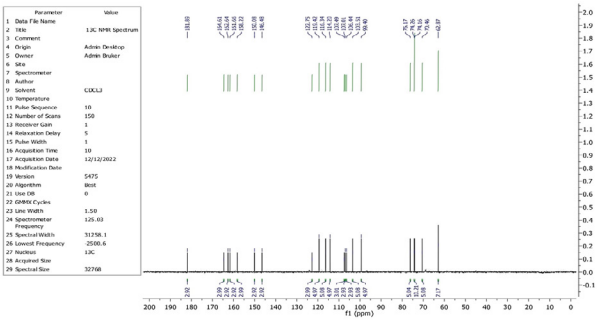

Figure 10: Compound AI-2 ^{13}C-NMR spectra[18]

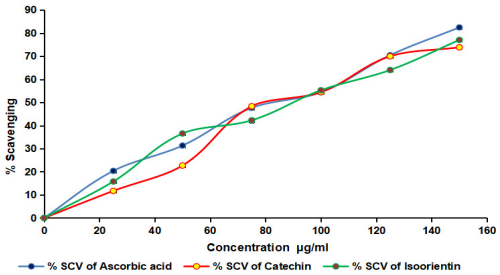

Figure 11: %Scavenging activity of catechin, isoorientin at varying concentrations, and ascorbic acid as positive control[13]

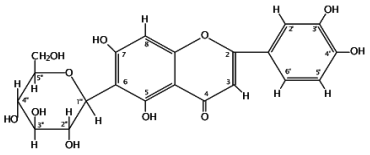

Figure 12: Structure of isoorientin[21]

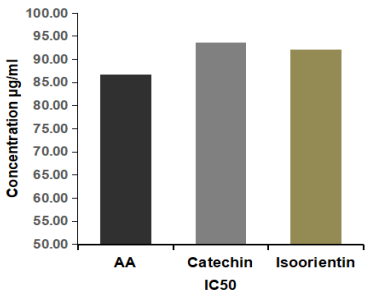

Figure 13: IC_{50} of DPPH scavenging activity of ascorbic acid, catechin, and isoorientin[13]

point 175°C and U.V. λ_{max} 350 nm. As shown in Figure 7, I.R. spectra were interpreted as 3156 aromatic C-H, 2551 C=C, 1729 C=O, 1551 -H-C=C-H, 1052 at C-O. Figure 8 manifested the characteristic m/e base peak at 449.1071.

^{1}H-NMR spectra of compound AI-2

The ^{1}H-NMR spectrum indicated in Figure 9 showed δ (2.5–3.7) multiplet means Anomeric carbon in the sugar 6.75 (d,1H) (C-5'), 7.07 (d,1H)(C-6,), 6.21 (s, 1H)(C-8), 7.02 (d, 1H)(C-2')

^{13}C-NMR spectra of compound AI-2

Similarly, the ^{13}C-NMR spectrum indicated in Figure 10 of the compound indicated 15 carbon signals with no free methyl groups 164.61 (C-2), 103.51 (C-3), 181.89 (C-4), 158.22 (C-5), 107.01 (C-6), 162.64 (C-7), 99.40 (C-8), 161.66 (C-9), 106.44 (C-10), 122.75 (C-1′), 114.20 (C-2′), 146.48 (C-3′), 150.08 (C-4′), 116.34 (C-5′), 119.42 (C-6′), 107.49 (C-1″), 76.17 (C-2″), 74.16 (C-3″), 70.46 (C-4″), 74.26 (C-5″), 62.87 (C-6″) indicates methylene group.

DPPH Antioxidant Activity

The scavenging activity of the isolated compounds showed a concentration-dependent activity; higher concentration, higher scavenging (Figure 11). Structure of isoorientin is shown in Figure 12. There is no significant difference between the isolated compounds and the standard. IC_{50} of ascorbic acid 86.75 µg/mL, catechin 93.66 µg/mL, isoorientin 92.09 µg/mL (Figure 13).

CONCLUSION

The generation of ROS is a consequence of oxidative stress.[22,23] Catechin and isoorientin were isolated from the methanol fraction of A: Catechin polyphenolic compound flavan-3-ol, Isoorientin flavone C glucoside with known antioxidant activity.[24-27] The methanol fraction showed significant DPPH scavenging activity. It has been discovered that the main components of the methanol fraction are flavonoids. The results demonstrate that *A. indica* can be a rich source of bioactive compounds and can be further tested for pharmacological effects *in-vivo***.**

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