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RESEARCH ARTICLE
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Isolation and Characterisation of Bioactive Compounds from Methanolic Fraction of Aerial parts of Aspidopterys cordata

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ABSTRACT

Background: The Present communication deals with Isolation of active bio compounds from Methanolic fraction of A.cordata

Method: Aerial components were ultrasonically extracted with methanol, fractionated using vacuum liquid chromatography, packed into silica gel columns, and their structures were determined using 1HNMR, 13CNMR, mass, and FTIR spectroscopies.

Results: The compounds isolated were characterised and were confirmed to be Ursolic acid a pentacyclic triterpenoid and Fisetin, dietary bioflavonol.

Keywords: *Bioactive, Phytochemistry, Pharmacy.*

INTRODUCTION

Humankind has always been interested in natural compounds, plants, and animal sources throughout history. Various plant extracts from various plant parts have been commonly applied in folkloric medicine for both common and chronic illnesses. Alkaloids, steroids, tannins, glycosides, volatile and fixed oils, resins, phenols, and flavonoids are only a few active substances deposited in the various parts of plants. These active chemicals work together to provide the plant its positive or therapeutic effects.(Rasul, 2018)

Pure phytochemicals must be acquired through extraction and isolation before structure identification, bioactivity screening, and other processes since plant chemical ingredients are complicated. New technology and extraction techniques have emerged in recent years, accelerating the extraction and analysis of phytochemicals.(Feng et al., 2019)

A.cordata is a herbaceous Climbing Shrubs with White Tomentose in younger parts. Leaves with broadly ovate or orbicular, 5-9 x 3-6 cm, closely appressed hairs with glossy texture above and softy whitish pubescent beneath the leaf surface, the base is cordate, Margin entire, apex is acuminate, Samaras orbicular, pale-brown, glabrous, finely reticulate, three-winged seeds are globose. Flowering and Fruiting in September-February(T.Pullaiah, 2015)

(K.N.Reddy & C.Sudhakar Reddy, 2016; Udaya Chandrika P & Dr.Sunitha K, 2023) A.cordata was reported to have phytochemicals like Heptacosonoic acid, 14-methyl, Methyl ester- skin cancer protein (Kandasamy et al., 2012), 2-Dodecen-1-yl(-)succinicanhydride-Antineoplastic agents, Antioxidants, Antimicrobial(Rawal Jatin R & Sonawani Priya R, 2016), Heptadecenal- Antibacterial and Antioxidant(Faridha Begum et al., 2016) in hexane fraction. Undecanoic acid-Antibacterial (Nisar et al., 2013), Betacarotene-Antibacterial, Antioxidant(Basim et al., 2021) in ethyl acetate fraction. 1,3, Bis-T-Butylperoxy-pthalan- anti-tumor(Sreedevi et al., 2022) in chloroform fraction. HR-LC-MS of Methanolic fraction A.cordata showed presence of Ferulic acid-Antioxidant, anti-inflammatory, antiviral, antiallergic, antimicrobial, antithrombotic, anti-carcinogenic and hepatoprotective actions (Seo et al., 2021). Hydroxycinnamic acid-Antioxidant(Teixeira et al., 2013) Gallic acid-Antibacterial antifungal, antiviral, anti-inflammatory, antioxidant, anticancer, anti-diabetic effect, Chlorogenic acid- Antioxidant, anticancer, antitumour (Feng et al., 2019)Caffeic acid- Anticancer, antioxidant (Monteiro Espíndola et al., 2019; Udaya chandrika P & Dr.Sunitha K, 2023; Udaya Chandrika P & Dr.Sunitha K, 2023). These fractions are reported to have antihypertensive activity (Udaya Chandrika Pulla & Sunitha Katta, 2021). The main objective of the current communication was to isolate phytochemicals from the methanolic fraction of A.cordata.

METHODOLOGY

Plant Collection and Authentication

Aspidopterys cordata was gathered from Kinnerasani Wild Life sanctuary, Bhadradri Kothagudem District Telangana, was confirmed by Botanist Dr. K. Venkata Ratnam, Assistant Professor, Department of Botany, Rayalaseema University, Kurnool; leaf specimen has submitted (RU/BD/VSN-092) for future reference.(P. Udaya Chandrika & Dr.K.Sunitha, 2022)

Extraction

A. cordata were individually collected, cleaned, dried in the shade, coarsely ground, and extracted on an ultrasonicator at 40 kHz for 45 minutes at 40°C. After the supernatants were separated, filtered, and dried under vacuum, the extract concentrations were kept at a constant level in a desiccator. (P. Udaya Chandrika & Dr.K.Sunitha, 2022)

Fractionization by Vacuum Liquid Chromatography

The fractions are collected into the volumetric flask after the methanolic extracts of both plants are prepared as a slurry, adsorbed on to silica gel, and introduced over the adsorbent. The solvent (n-hexane) is poured from the top of the column and a moderate vacuum of 20–70 mm hg is applied. The solvent is gradually added until the resulting fraction is colourless. The procedure is repeated with Methanol, ethyl acetate, and chloroform. Rota vapour is used to concentrate the fractions once they have been separately collected. Analytical tests were conducted on the additional residues that were collected following separation utilising columns.(Maurya et al., 2018)

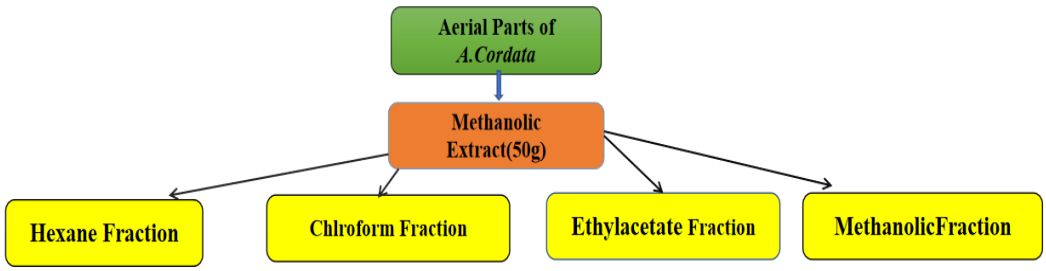

FIG 1: Scheme for Fractionizing A. cordata Methanolic Extract

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Isolation of compounds from A.cordata

Methanolic fraction based on its phytochemical profiling(Udaya chandrika P & Dr.Sunitha K, 2023) was packed in a silica gel column chromatography (silica gel 100-200 mesh size) The solvents were allowed to flow in the order of increasing polarity.

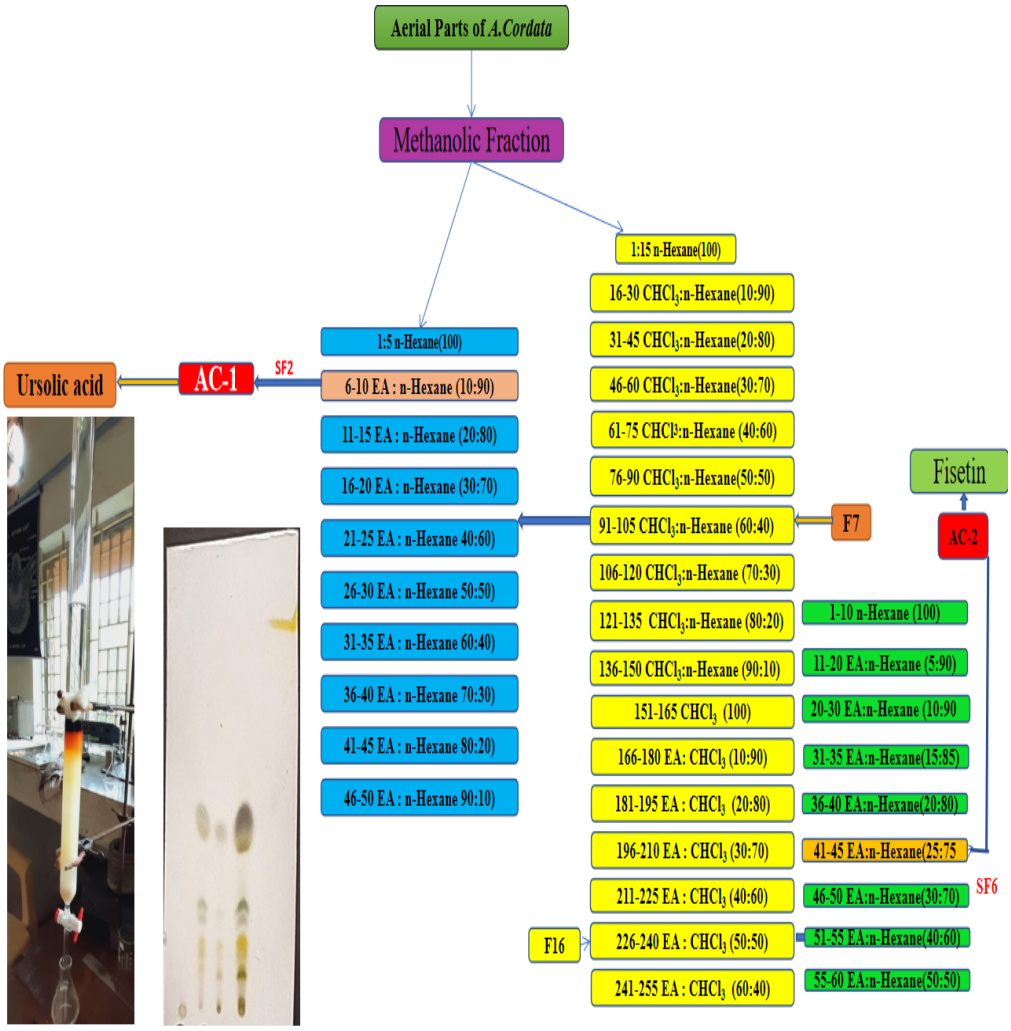

FIG 2: Schematic representation of Isolation of compounds from A.cordata Methanolic Fraction

Fractions (91-105) collected from the above column (main column) were pooled and numbered as fraction No.7 packed into another column and subfraction F2, with solvent ratio n-Hexane: Ethyl acetate (70:30) confirmed the AC-I. Fraction No.16 was packed in column Sixty fractions were collected. Sub Fraction-6 (41-45 sub fractions) at solvent ratio EA: n-Hexane (25:75) yielded a compound AC-2. was further subjected to recrystallization and yellow amorphous powder AC-2 was further characterized by ^{1}H-NMR, ^{13}C-NMR and Mass spectrometry.

RESULTS AND DISCUSSION

Spectral Data of Compound-AC-1

TLC of AC-1

Based on the spots obtained on TLC chromatogram similar fractions were combined.

Mobile phase: Ethyl acetate : n-Hexane(20:80)

Spray Reagents: Anisaldehyde sulphuric acid reagent

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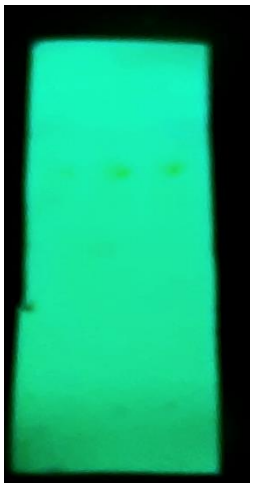

FIG 3: TLC chromatogram of Isolated AC-1

1H NMR spectroscopy of Compound AC-1

^{1}H NMR (400 MHz, CDCl3) has given signals at δ 5.25 (s, 1H), 4.12 (s, 1H), 2.19 (s, 1H), 1.99 (s, 1H), 1.70(s, 1H), 1.66 (d, J = 12.8 Hz, 1H), 1.58 (s, 1H), 1.53 (s, 1H), 1.46(s, 1H), 1. 43(s, 1H), 1.35 (s, 1H), 1.29 (d, J = 11.8 Hz, 3H), 1.16 (s, 1H), 1.11 (s, 1H), 1.04(s, 1H), 0.96 (t, J = 15.3, 6.8 Hz, 3H), 0.78 (t, J = 10.0 Hz, 3H). It exhibited seven singlets characteristic of tertiary methyl groups (δ H 1.99, 1.66, 1.58, 1.53, 1.35, 1.16 and 1.11.)

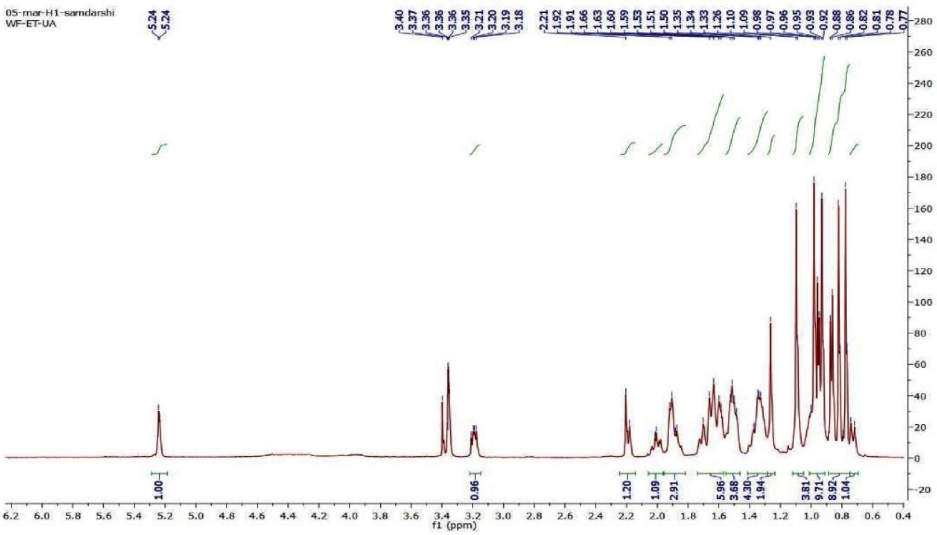

FIG 4: ^{1}H NMR spectra of Isolated compound AC-1

^{13}C-NMR Spectroscopy AC-1

^{13}C NMR (101 MHz, CDCl3) δ 180.42(C-28) 138.17(C-13), 125.49 (C-12), 78.20(C-3) 56.88(C-5), 53.10(C-18), 48.70 (C-17), 47.01(C-9), 42.53(C-14), 40.75(C-8), 39.20(C-19), 38.90(C-4), 38.40(C-20), 37.70(C-10), 37.48(C-1), 36.98(C-22), 33.38(C-7), 30.52(C-21), 29.40(C-15), 26.38(C-2), 26.10(C-26), 24.30(C-16), 24.57(C-11), 22.17(C-23,24), 20.59(C-30), 18.40(C-6), 17.41(C-27), 16.41(25), 16.20(C-29).

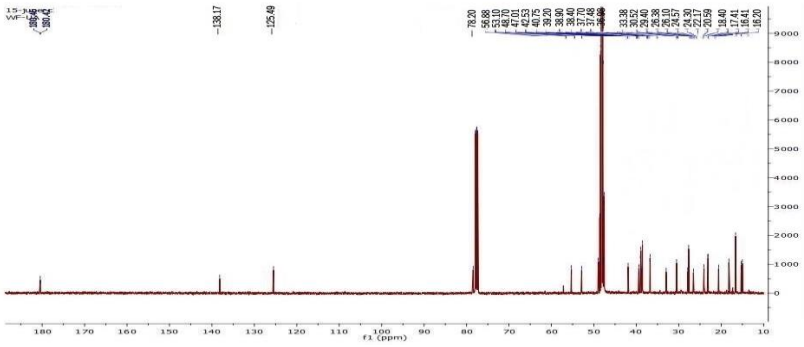

FIG 5: ^{13}C NMR spectra of Isolated compound AC-1

Mass spectra of AC-1

Mass spectra showed the characteristic peak at m/z=455

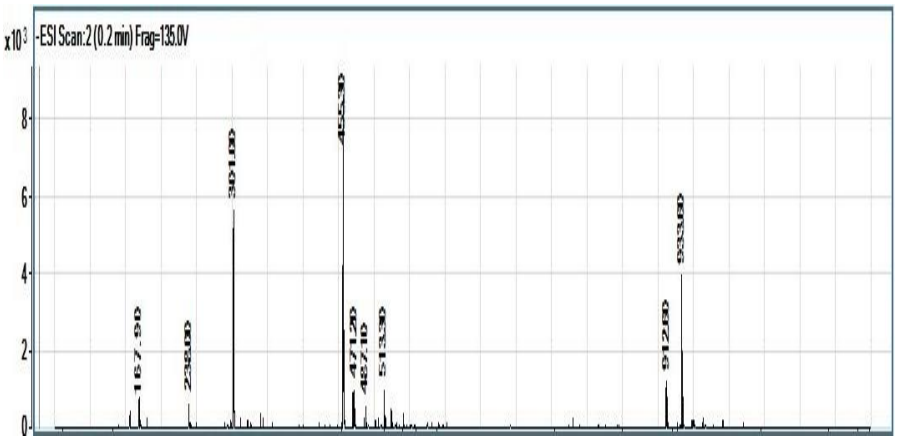

FIG 6: Mass spectra showed the characteristic peak at m/z=455

The FTIR spectra revealed the presence of functional groups tabulated in Table.1

TABLE 1: Functional groups detected in FT-IR Spectral data of AC-1

Wave number cm^{-1}	Functional group
3491	O-H
2979	C-H
2517	carboxylic acid
1550	C=C
1446	C=O
1372	$-CH_2$
1021	$-CH=CH_2$
878	=C-H

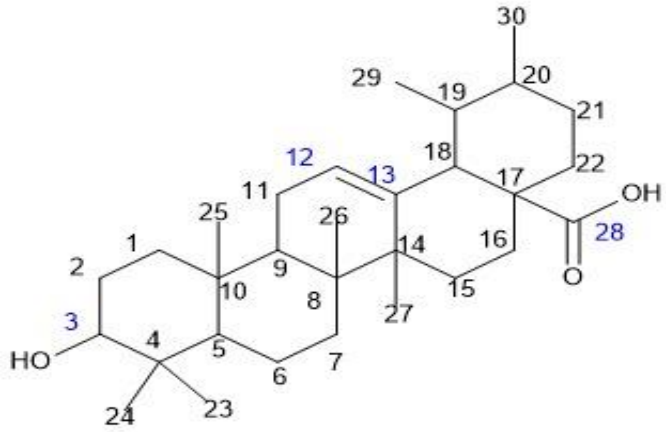

FIG 6: Structure of Ursolic acid

Characteristics of AC-1

It is white amorphous solid, chemical name given as 3β-hydroxy-urs-28-oic acid, Molecular formula $C_{30}H_{48}0_3$, Melting point 285^0C, UV λ max 223nm, Molecular wight 455.

From the spectral data, the structure of the isolate compound was established by comparing with the existing literature and it is found that the isolated compound is Ursolic acid.

Spectral Data of Compound-AC-2

Fraction No.16 and was packed in column chromatography (silica gel 60-120 mesh). The solvents were allowed to flow in the order of increasing polarity. Out of Sixty fractions collected, Sub Fraction-6 (41-45 sub fractions) was further subjected to recrystallization and yellow amorphous powder AC-2 was further characterized by ^{1}HNMR, ^{13}CNMR and Mass spectrometry.

^{1}H NMR spectroscopy of AC-2

^{1}H NMR (400 MHz, $CDCl_3$) has given signals at δ 7.57 (d, 1H) indicates the absence of a hydroxyl group and presence of an adjacent proton. Multiplets at the region from **6.55 to 6.57** indicates the alternative protons. Broad peaks at the region from 3-4 and 0-0.5 indicates the presence of hydroxyl groups and signals at the range of 6ppm also represents the aromatic ring system in the molecule.

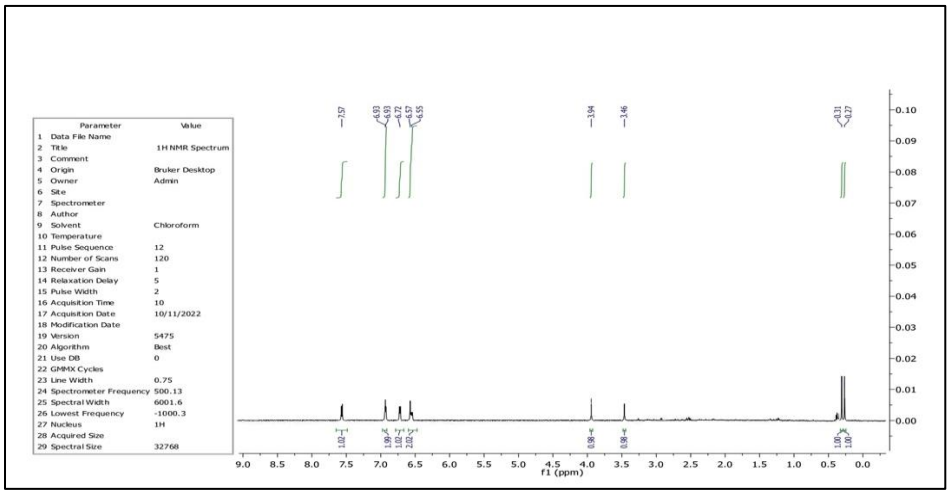

FIG 7: ^{1}HNMR spectra of Isolated compound AC-2

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^{13}C NMR spectra of AC-2

^{13}C NMR (101 MHz, $CDCl_3$) δ values 147.71 (C-2), **137.10 (C-3), 172.15 (C-4),** 128.09 (C-5), 114.70 (C-6), **161.45 (C-7),** 102.63 (C-8), 157.54 (C-9), 114.49 (C-10), 121.81 (C-1'), 115.98 (C-2'), 145.29 (C-3'), 148.35 (C-4'), 116.29 (C-5'), 121.63 (C-6').

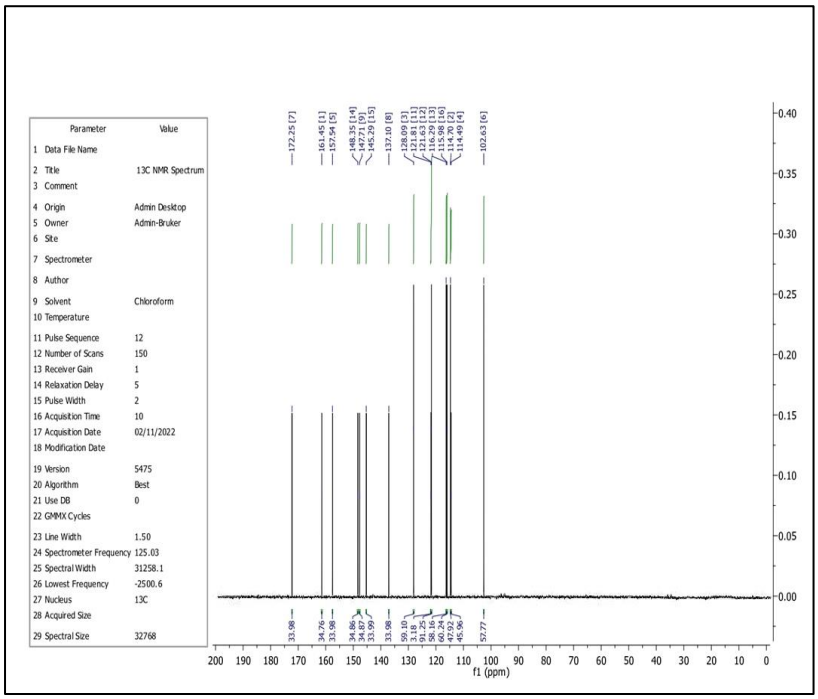

FIG 8: ^{13}C NMR spectra of Isolated compound AC-2

Mass spectra of AC-2

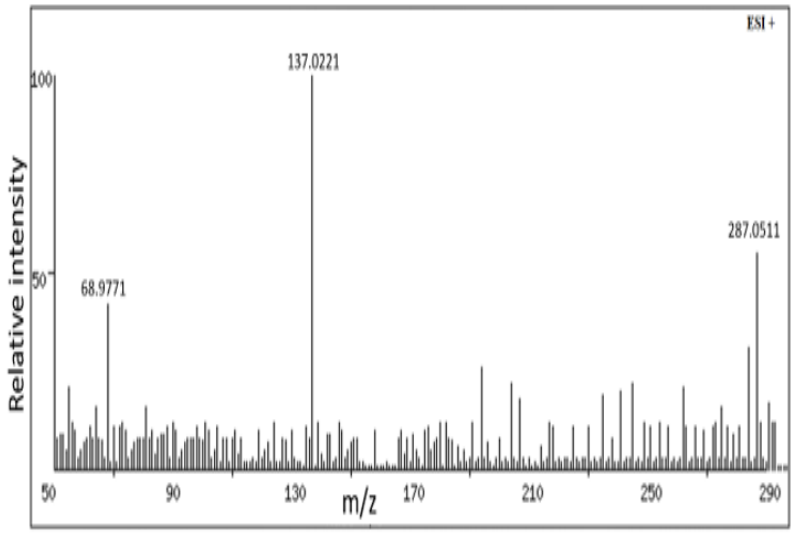

FIG 9: Fig Mass Spectral data of AC-2

Mass spectra showed the characteristic peak of Fisetin at m/z=287 (ESI +ve)

FT-IR Spectra of AC-2

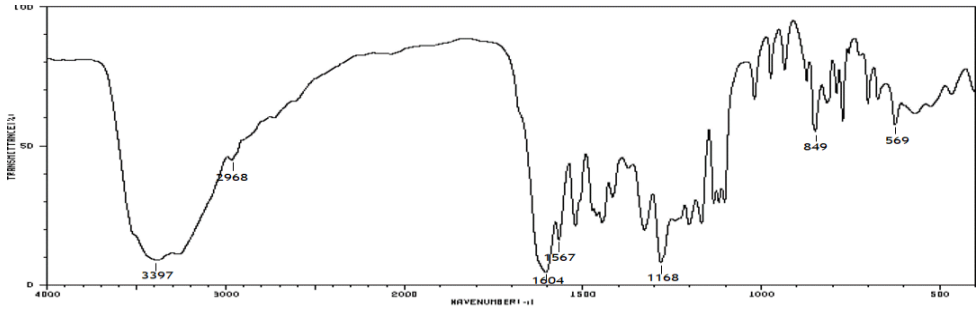

FIG 10: FTIR spectrum of AC-2

Interpretation of FTIR spectral data of AC-2 were denoted in table.2

TABLE 2: Functional groups detected in AC-2

Wave number cm^{-1}	**Functional group**
3397	O-H
2968	C-H
1604	C=O and Ar C=C
1567	C-C
1168	C-O
849	=CH-H

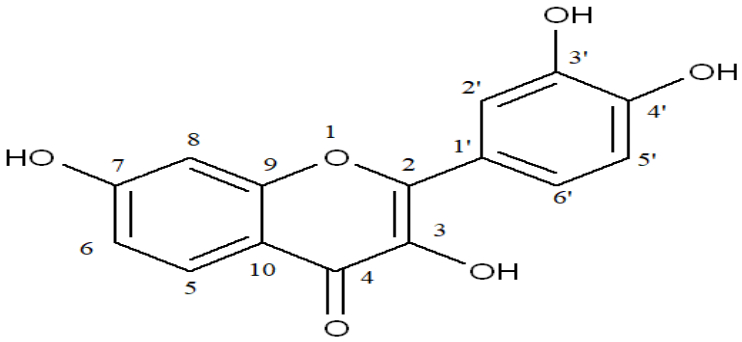

FIG 11: Structure of Fisetin

Characteristics of Fisetin

The compound obtained was in form of yellow amorphous powder, chemical name 2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one, Molecular formula of $C_{15}H_{11}O_6$, Melting point was found to be 299°C, Molecular wight 286.23,

UV λ max 248nm

From the spectral data, the structure of the isolate compound was established by comparing with the existing literature and it is found that the isolated compound is Fisetin.

CONCLUSION

Fisetin, a bioactive flavonoid, and ursolic acid, a pentacyclic triterpenoid, were extracted from the methanolic fraction of aerial portions of A. cordata.

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