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Extraction, Isolation and Characterization of Bioactive from Bergenia Ciliata and Caesalpinia Bonducella

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Abstract
Introduction: India is very rich in biodiversity. From the ancient times our ancestors have focused on various traditional medicinal herbs to treat various ailments. This research focuses on two important medicinal plants namely Bergenia ciliata and Caesalpinia bonducella. Various bio-actives are reported to have some medicinal effects before, like flavonoids namely 3-O-galloylcatechin and 3-O-galloylepicatechin isolated from Bergenia ciliata has shown anti Alzheimer activity. This research focuses on isolation of such bio-actives which can lead to future potential drugs. The authors strongly believe that such isolated bio actives can be a source of potent medication in the future of pharma innovation leading to good health and wellbeing of individuals.
Methods: Powdered seeds of Caesalpinia bonducella and powdered rhizomes of Bergenia ciliata were first extracted separately through Soxhlet extraction which was then followed by preliminary TLC, column chromatography, UV, FT-IR, NMR and Mass to isolate and characterize bio-actives.
Results: The percentage yield of Caesalpinia bonducella extract was found to be 4% and that of Bergenia ciliata was found to be 15.10%. In UV spectroscopy the wavelength of isolated fraction (E) of Bergenia ciliata extract were found to be two peaks at 297 and 414 nm and isolated fraction (e) of Caesalpinia bonducella extract was found to be one peak at 316 nm. These isolated fractions were obtained from column chromatography and were then further characterized using FT-IR, NMR and Mass.
Conclusion: The authors have successfully isolated and characterized 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one and 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopentaphenanthren-3-ol as bio actives from Bergenia ciliata and Caesalpinia bonducella respectively.

Keywords
Bergenia ciliata; Caesalpinia bonducella; Bio actives; Traditional medicines; Good health and wellbeing; Industry, innovation and infrastructure

1. Introduction
The medicinal herbs are a hub of treatment to various ailments. The pharmaceutical industries are focusing more on traditional herbs nowadays. Each plant consists of a variety of phytoconstituents in it. Each phytoconstituent is believed to offer some or the other therapeutic effect. But the main challenge with these medicinal plants is the extraction of these phytoconstituents like the method for extraction, solubility of extracts, quantity of bio actives isolated and so on. This research focuses on two important medicinal plants namely Bergenia ciliata and Caesalpinia bonducella. The Bergenia ciliata commonly known as pashanbhed is known to have phytoconstituents like bergenin, phenolic compounds, alkaloids, saponins etc.

and Caesalpinia bonducella commonly known as sagargota is known to have phytoconstituents like α-caesalpin, β-caesalpin, terpenoids, homoplantagenin etc.[1] This research focuses on the Soxhlet extraction, UV, NMR, Mass, FT-IR, techniques for isolation of bio actives.

2. Materials and methods

2.1. Collection

The dried and powdered seeds of Caesalpinia bonducella and dried and powdered rhizomes of Bergenia ciliata were collected from local ayurvedic herbs dealer G Y Hakim and Sons, Raopura, Vadodara-390001, Gujarat, India.[2]

2.2. Authentication

The dried seeds of Caesalpinia bonducella and the dried rhizomes of Bergenia ciliata were authenticated at the department of botany, Maharaja Sayajirao University, Vadodara-390002, Gujarat, India.

2.3. Extraction

The dried and powdered seeds of Caesalpinia bonducella and rhizomes of Bergenia ciliata were first defatted with petroleum ether and then extracted with ethyl acetate and methanol respectively using Soxhlet apparatus. After the extraction process, the extract of samples was filtered and concentrated to dryness. The extracts were collected in air tight container.[3]

2.4. Qualitative phytochemical estimation of extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in petroleum ether, ethyl acetate extracts of Caesalpinia bonducella and petroleum ether and methanol extracts of Bergenia ciliata using standard procedures.[4]

2.5. Qualitative chromatographic analysis

2.5.1. Preliminary Thin Layer Chromatography (TLC)

The solvent system developed for preliminary TLC of methanol extract of Bergenia ciliata in which the maximum spots were visible was toluene: ethyl acetate: acetic acid (6.2:4:0.4) mobile phase with standard flavonoid and ethyl acetate extract of Caesalpinia bonducella in which the maximum spots were visible was toluene: ethyl acetate: acetic acid (8:4.2:0.4) mobile phase with standard sterol respectively.[5]

2.5.2. Column chromatography

Toluene: ethyl acetate: acetic acid (6.2:4:0.4) for Bergenia ciliata (BC) extract and toluene: ethyl acetate: acetic acid (8:4.2:0.4) for Caesalpinia bonducella (CB) extracts were taken as mobile phase for column chromatography. Normal phase column chromatography was performed using silica gel (60-120 mesh). The fractions/elutes collected were concentrated and TLC was performed to identify the presence of single compound.[6]

2.6. Spectroscopic characterization

2.6.1. UV-visible spectroscopy

The isolated fraction (E & e) of BC and CB were scanned from 200 to 800 nm wavelength using UV-visible spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded.[7]

2.6.2. FT-IR spectroscopy

FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer. The isolated fraction (E & e) of BC and CB were mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The samples pellets were placed into the sample holder and FT-IR spectra were recorded in the range 400-4000 cm-1 in FT-IR spectroscopy.[8]

2.6.3. NMR spectroscopy

1H NMR spectra were recorded on Jeol Resonance NMR. Tetramethylsilane (TMS) was used as an internal standard. NMR spectroscopy was performed for the isolated fraction (E & e) of BC and CB to identify the structure of the compound present in the isolated fraction. Chemical shifts were shown in δ values (ppm) with TMS as an internal reference.[9]

2.6.4. Mass spectroscopy

The mass spectrometer used for the identification of the molecular weight of isolated fraction (E & e) of BC and CB was micrOTOF-MS.[10]

3. Results

3.1. Percentage yield

The percentage yield of petroleum ether extract of CB was found to be 2.950% and that of ethyl acetate extract was found to be 4%. The percentage yield of petroleum ether extract of BC was found to be 0.823% and that of methanol extract was found to be 15.10%.

3.2. Solubility determination of extracts

The extract of Caesalpinia bonducella was freely soluble in ethanol and methanol; insoluble in water; soluble in ethyl acetate and acetone; slightly soluble in DMSO and partially soluble in petroleum ether and chloroform. The extract of Bergenia ciliata was sparingly soluble in water and ethyl acetate; freely soluble in ethanol and methanol; soluble in DMSO, acetone and chloroform and insoluble in petroleum ether.

3.3. Qualitative phytochemical analysis of extracts of Caesalpinia bonducella and Bergenia ciliata

The petroleum ether and ethyl acetate extracts of Caesalpinia bonducella and petroleum ether and methanol extracts of Bergenia ciliata showed presence of carbohydrates, alkaloids, terpenoids, flavonoids, tannins, phenolic compounds, saponins, proteins, amino acids and glycosides.

3.4. Preliminary TLC preparation for the estimation of active constituents

Table 1: TLC of methanol extract of Bergenia ciliata

S. no.	Solvent system	No. of spots	Color of spots at wavelength (365 nm)	Color of spots at wavelength (254 nm)	Rf value (extract)	Rf value (std. flavonoid)
`1.	Toluene: ethyl acetate: acetic acid (6.2:4:0.4)	09	Blue (std.) Dark blue (BC) Dark blue Dark blue Blue Florescence Blue Florescence Florescence	Green (std.) Green (BC) Green Green Green Light green Light green Light green -	- 0.18 0.27 0.32 0.45 0.59 0.66 0.82 0.89	0.67

Table 2: TLC of ethyl acetate extract of Caesalpinia bonducella

S. no.	Solvent system	No. of spots	Color of spots at wavelength (365 nm)	Color of spots at wavelength (254 nm)	Rf value (extract)	Rf value (std. sterol)
`1.	Toluene: ethyl acetate: acetic acid (8:4.2:0.4)	13	Light blue (std 365) Purple (CB) Blue Florescence Florescence Florescence Light pink	Green (std 254) Dark green (CB) Dark green Green Green Green	- 0.09 0.18 0.26 0.32 0.48 0.51 0.56	0.58

			Light pink Light blue Dark florescence Light pink Purple Florescence	Green Green Dark green Light green Green Green Light green	0.59 0.71 0.80 0.84 0.91	

The Rf values of BC and CB extracts were found to be 0.66 and 0.59 which was matching with standard flavonoid and sterol that was found to be 0.67 and 0.58.

3.5. Column chromatography

Table 3: Rf values of all collected fractions of BC after column chromatography

Sr. no.	Fraction	Solvent system	No. of spots	Color of spots at wavelength (365nm)	Rf value (extract)	Rf value (std. flavonoid)
1.	A1	Toluene: ethyl acetate: acetic acid (6.2:4:0.4)	-	-	-	0.67
2.	A2		-	-	-	
3.	B		-	-	-	
4.	C		01	Fluorescence	0.82	
5.	D		-	-	-	
6.	E		01	Blue	0.66	
7.	F		01	Blue	0.58	
8.	G		01	Blue	0.46	
9.	H		02	Light blue Blue	0.44 0.38	
10.	I		02	Light blue Blue	0.32 0.20	

Table 4: Rf values of all collected fractions of CB after column chromatography

Sr. no.	Fraction	Solvent system	No. of spots	Color of spots at wavelength (365nm)	Rf value (extract)	Rf value (std. sterol)
1.	a	Toluene: ethyl acetate: acetic acid (8:4.2:0.4)	-	-	-	0.59
2.	b		01	Fluorescence	0.84	
3.	c		02	Light pink Dark pink	0.56 0.98	
4.	D		02	Fluorescence Purple	0.56 0.98	
5.	E		01	Blue	0.60	
6.	F		-	-	-	
7.	G		01	Fluorescence	0.76	
8.	H		03	Fluorescence Pink Dark pink	0.74 0.76 0.82	
9.	I		04	Fluorescence Fluorescence Fluorescence	0.76 0.80 0.86	

				Fluorescence	0.91	
10.	J		01	Fluorescence	0.74	
11.	K		02	Pink Fluorescence	0.56 0.60	
12.	L		01	Fluorescence	0.55	
13.	m1		01	Fluorescence	0.52	
14.	m2		01	Fluorescence	0.52	
15.	N		-	-	-	

The Rf value obtained after performing the TLC confirmed the presence of active constituent in fraction (E) of BC methanolic extract and fraction (e) of CB ethyl acetate extract by comparing with standard flavonoid and standard sterol (Table 3 and 4).

3.6. Spectroscopic characterization

3.6.1. Active constituents' estimation by UV-spectroscopy

UV spectra of the isolated fractions (E) of BC was recorded in solvent as toluene: ethyl acetate: acetic acid (6.2:4:0.4) (Figure 1) and isolated fractions (e) of CB extracts was recorded in solvent as toluene: ethyl acetate: acetic acid (8:4.2:0.4) (Figure 2) over a scanning range of 200-800 nm. The wavelength of isolated fraction (E) of BC extract were found to be two peaks at 297 and 414 nm and isolated fraction (e) of CB extract was found to be one peak at 316 nm.

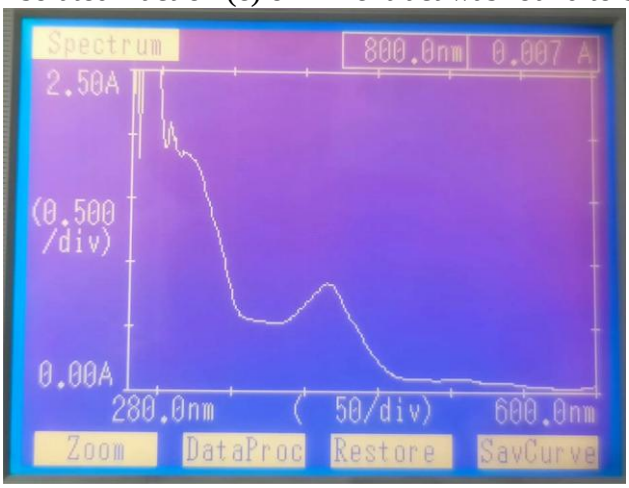


Figure 1: Active constituents' estimation by UV- Spectra of (E) fraction of BC extract after column chromatography

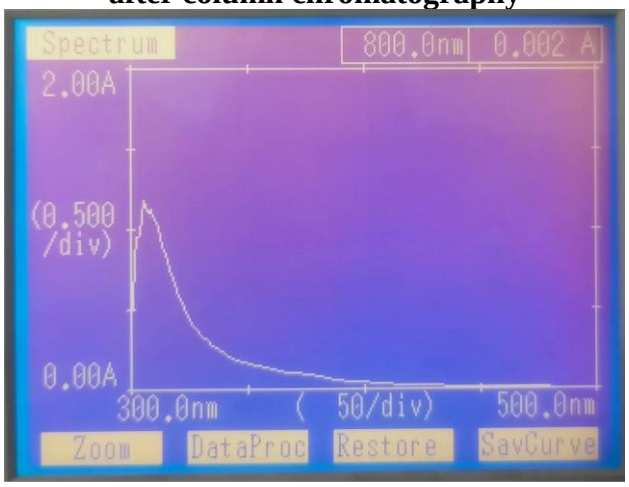


Figure 2: Active constituents' estimation by UV- Spectra of (e) fraction of CB extract after column chromatography

3.6.2. Active constitutes estimation By FTIR – Spectroscopy

In IR Spectra of isolated fraction (E) of BC methanolic extract showed that -OH group Strong, Broad peak appeared at 3455.60 cm-1, C-H stretching peaks of Alkane at 2925.40 & 23765.47 cm-1 and C-C stretching peak of Alkane at 1106.09 cm-1 (Table 5, Figure 3).

Table 5: FTIR- Spectrum Frequency Range of the isolated fraction (E) of BC methanolic extract

Sr. No.	Fraction	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound Class
1	E	3550-3200 (cm^{-1})	3455.60	Strong, Broad	O-H stretching	Hydroxyl Group
		3000-2840 (cm^{-1})	2925.40	Medium	C-H stretching	Alkane
		2400- 2000 (cm^{-1})	2365.47	Strong	C-H stretching	Alkane
		1400- 1100 (cm^{-1})	1106.09	Weak	C-C stretching	Alkane

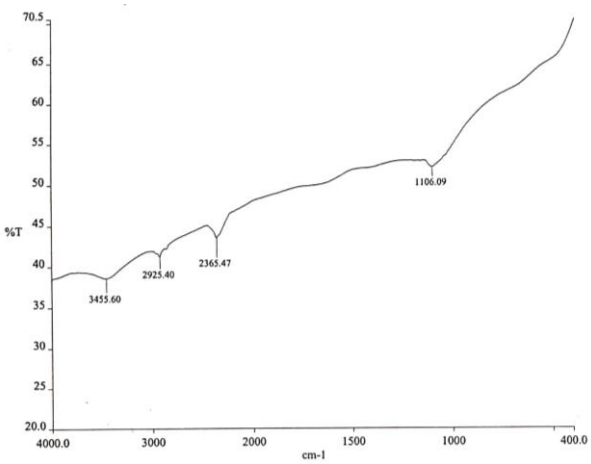


Figure 3: IR spectra of the isolated fraction (E) of BC methanolic extract

In IR Spectra of isolated fraction (e) of CB ethyl acetate extract showed that OH group Strong, Broad peak appeared at 3439.35 cm-1, C-H stretching peaks of Alkane at 2925.22, 2874.92 & 2375.08 cm-1. C-O stretching peak of Carbonyl group at 1675.07 cm-1, OH bending peak of Alcohol at 1399.16 cm-1and C-C stretching peak of Alkane at 1103.80 cm-1 (Table 6, Figure 4).

Table 6: FTIR- Spectrum Frequency Range of the isolated fraction (e) of CB ethyl acetate extract

Sr. No.	Fraction	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound Class
1	e	3550-3200 (cm^{-1})	3439.35	Strong, Broad	O-H stretching	Hydroxyl Group
		3000-2840 (cm^{-1})	2925.22	Medium	C-H stretching	Alkane
		3000-2840 (cm^{-1})	2874.92	Medium	C-H stretching	Alkane
		2400- 2000 (cm^{-1})	2375.08	Strong	C-H stretching	Alkane

		2000- 1600 (cm^{-1})	1675.07	Medium	C-O stretching	Carbonyl group
		1420-1330 (cm^{-1})	1399.16	Medium	O-H bending	Alcohol
		1400- 1100 (cm^{-1})	1103.80	Weak	C-C stretching	Alkane

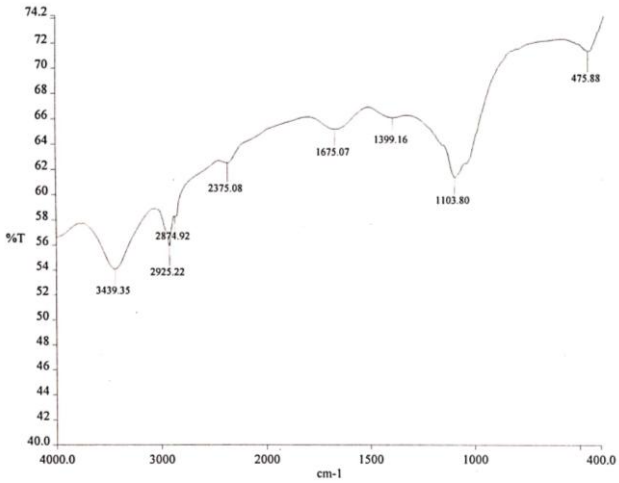


Figure 4: IR spectra of the isolated fraction (e) of CB ethyl acetate extract

3.6.3. ^{1}H NMR – Spectroscopy

^{1}H NMR spectra of isolated fraction (E) of BC methanolic and fraction (e) of CB ethyl acetate extract was recorded on NMR Spectrometer. Tetramethylsilane used as an internal standard. The signals are denoted with the symbols s, d, t, and m for singlet, doublet, triplet, and multiplet, respectively.

3.6.3.1. ^{1}H NMR spectra of the isolated Fraction (E) of BC

In ^{l}H-NMR spectra isolated fraction (E) of BC methanolic extract showed H-1 proton appeared at 3.69 (d) ppm, H-1 proton appeared at 5.28 (d) ppm, H-1 proton appeared at 6.14 (d) ppm, H-1 proton appeared at 6.14 (d) ppm, H-1 proton appeared at 6.39 (d) ppm, H-1 proton appeared at 6.39 (d) ppm, H-1 proton appeared at 6.87 (dd) ppm, H-1 proton appeared at 6.89 (dd) ppm, ^{l}H-1 proton appeared at 7.98 (dd) ppm and H-1 protons appeared at 8.00 (dd) ppm (Figure 5).

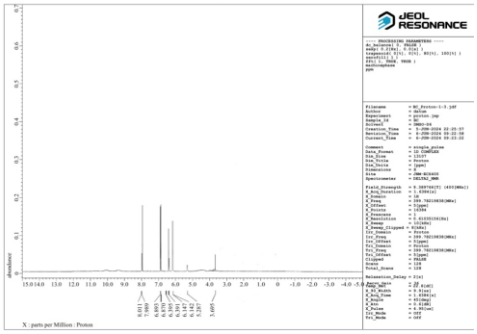


Figure 5: ^{l}H-NMR spectra of the isolated compound (Fraction E) of BC methanolic extract

3.6.3.2. ^{1}H NMR spectra of the isolated Fraction (e) of CB

In ^{l}H-NMR spectra isolated fraction (e) of CB ethyl acetate extract showed that H-19 protons appeared at 0.60-1.10 ppm (0.63 (td), 0.70 (td), 0.80 (td), 0.82 (td), 0.83 (d), 0.83 (d), 0.85 (t), 0.97 (s), 0.99 (d)), H-6 protons appeared at 1.14-1.30 ppm (1.24 (tq), 1.28 (quint), 1.28 (quint), 1.28 (s)), H-18 protons appeared at 1.30-1.98 ppm (1.33 (ddd), 1.34 (o), 1.42 (tqd), 1.45 (dtd), 1.45 (ddd), 1.52 (dt), 1.57 (ddd), 1.57 (dddd), 1.59 (dddd), 1.75 (ddd), 1.79 (ddd), 1.82 (dtd), 1.91 (ddt), 1.94 (ddd)), H-3 protons appeared at 2.06-3.37ppm (2.16 (ddd), 2.46 (dd), 3.25

(ddd)), H-1 proton appeared at 3.29 ppm (dd), 4.24 ppm (dtd), 4.25 ppm (dd) and 5.08 ppm (dd) (Figure 6).

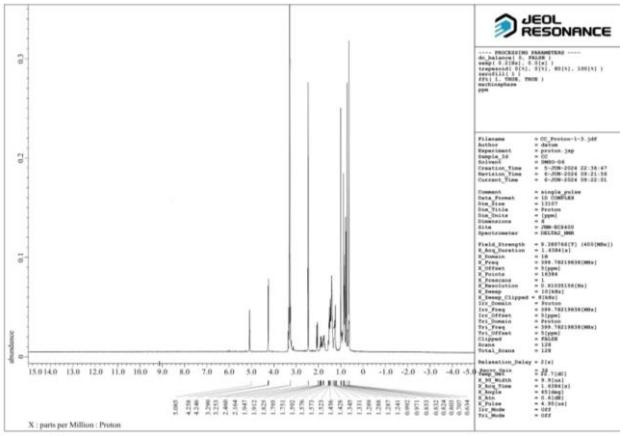


Figure 6: ^{1}H-NMR spectra of the isolated compound (Fraction e) of CB ethyl acetate extract

3.6.4. Mass – Spectroscopy

3.6.4.1. Mass spectra of the isolated Fraction (E) of BC Methanolic extract

Mass spectra of isolated Fraction (E) of BC Methanolic extract showed molecular ion [M^{+}] peaks at mlz 302.1410 which obtained 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one compound in which presence of carbons (C_{15}), Hydrogens (H_{10}) and Oxygen (O_7). Finally, the molecular formula of isolated Fraction (E) of BC Methanolic extract was found to be $C_{15}H_{10}O_7$ according to their fragments (Figure 7,8).

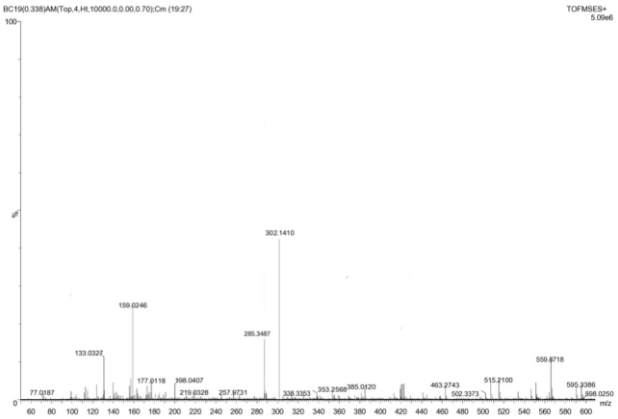


Figure 7: Mass spectra of the isolated fraction (E) of BC Methanolic extract

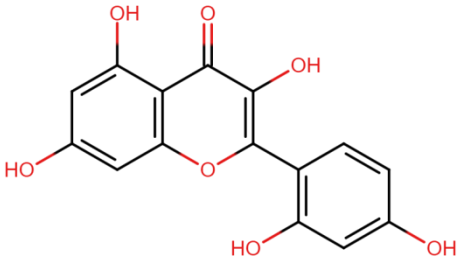


Figure 8: 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one

3.6.4.2. Mass spectra of the isolated Fraction (e) of CB Ethyl acetate extract

Mass spectra of isolated Fraction (e) of CB Ethyl acetate extract showed molecular ion [M^{+}] peaks at mlz 414.6906 which obtained 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopentaphenanthren-3-ol compound in which presence of carbons (C_{29}), Hydrogens (H_{50}) and Oxygen (O). Finally, the molecular

formula of isolated Fraction (e) of CB Ethyl acetate extract was found to be $C_{29}H_{50}O$ according to their fragments (Figure 9,10).

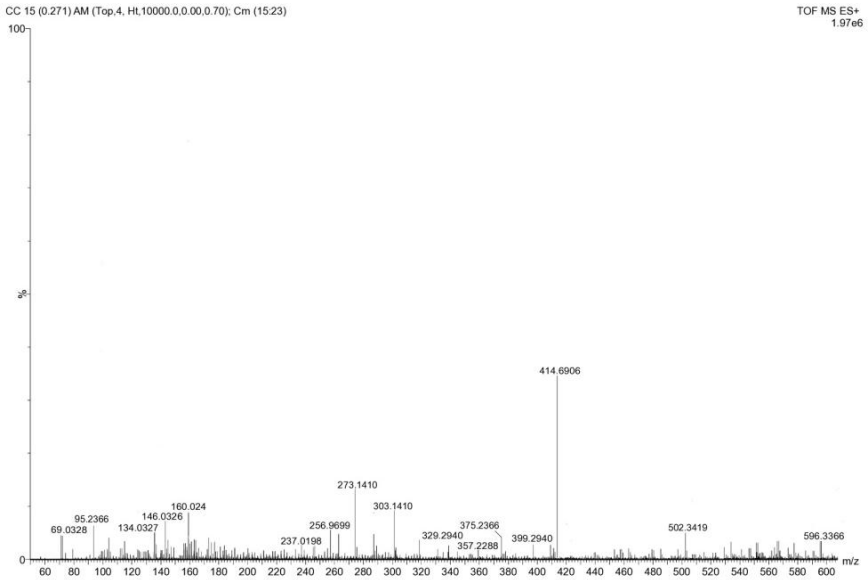


Figure 9: Mass spectra of the isolated fraction (e) of CB Ethyl acetate extract

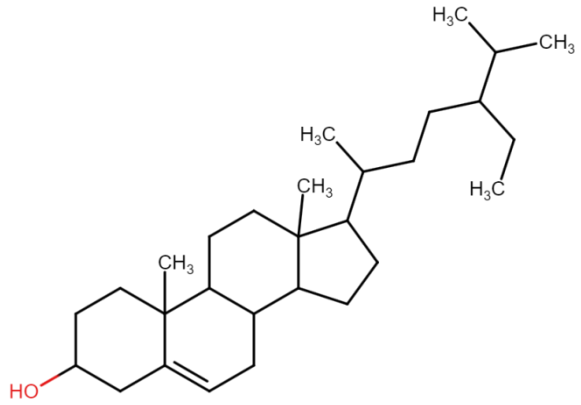


Figure 10: 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopentaphenanthren-3-ol

4. Discussion

In the Preliminary TLC of Methanol extract of Bergenia ciliata and ethyl acetate of Caesalpinia bonducella extracts were performed on different solvent systems. TLC performed in Toluene: Ethyl Acetate: Acetic acid (6.2:4:0.4) and in Toluene: Ethyl Acetate: Acetic acid (8:4.2:0.4) that were clearly visible bands of BC and CB Extracts with different Std. Flavonoids and Sterol. The Rf values of BC and CB Extracts were found to be 0.66 and 0.59 with different Std. Flavonoids and Sterol were found to be 0.67 and 0.58. **(Table 1 & 2).** So that Toluene: Ethyl Acetate: Acetic acid (6.2:4:0.4) and Toluene: Ethyl Acetate: Acetic acid (8:4.2:0.4) were taken as mobile phase for column chromatography. Active constitutes are isolated from column chromatography with the mobile phase of Toluene: Ethyl Acetate: Acetic acid (6.2:4:0.4) for BC to obtained Fractions 01-02 (A1 & A2), 03 (B), 04 (C), 05 (D), 06 (E), 07 (F), 08 (G), 09 (H) and 10 (I). Active constitutes are isolated from column chromatography with the mobile phase of Toluene: Ethyl Acetate: Acetic acid (8:4.2:0.4) for CB to obtained Fractions 01 (a), 02 (b), 03 (c), 04 (d), 05 (e), 06 (f), 07 (g), 08 (h), 09 (i), 10 (j), 11 (k), 12 (l), 13-14 (m1 & m2) and 15 (n). Rf value resulted after performing the TLC estimation is also done for the confirmation of active constituents in fractions E of BC and fractions e of CB with mobile phase Toluene: Ethyl Acetate: Acetic acid (6.2:4:0.4) and Toluene: Ethyl Acetate: Acetic acid (8:4.2:0.4) by comparing with different Std. Flavonoids and Sterol **(Table 3 & 4).** The collected

fractions were taken properly and the UV spectrum was determined. UV spectra of the isolated fraction (E) of BC **(Fig 1)** and fraction (e) of CB **(Fig 2)** were recorded over a scanning range of 200-800 nm and λmax of fraction (E) of BC and fraction (e) of CB were determined. The wavelength of isolated fraction (E) of BC extract were found to be two Peaks at 297 and 414 nm isolated fraction (e) of CB extract was found to be one Peak at 316 nm.

In IR spectra of isolated fraction (E) of BC methanolic extract showed that -OH group Strong, Broad peak appeared at 3455.60 cm-1, C-H stretching peaks of Alkane at 2925.40 & 23765.47 cm-1 and C-C stretching peak of Alkane at 1106.09 cm-1 **(Fig 3, Table 5).**

In IR spectra of isolated fraction (e) of CB ethyl acetate extract showed that OH group Strong, Broad peak appeared at 3439.35 cm-1, C-H stretching peaks of Alkane at 2925.22, 2874.92 & 2375.08 cm-1. C-O stretching peak of Carbonyl group at 1675.07 cm-1, OH bending peak of Alcohol at 1399.16 cm-1and C-C stretching peak of Alkane at 1103.80 cm-1 **(Fig 4, Table 6).**

In ^{1}H-NMR spectra isolated fraction (E) of BC methanolic extract showed H-1 proton appeared at 3.69 (d) ppm, H-1 proton appeared at 5.28 (d) ppm, H-1 proton appeared at 6.14 (d) ppm, H-1 proton appeared at 6.14 (d) ppm, H-1 proton appeared at 6.39 (d) ppm, H-1 proton appeared at 6.39 (d) ppm, H-1 proton appeared at 6.87 (dd) ppm, H-1 proton appeared at 6.89 (dd) ppm, ^{1}H-1 proton appeared at 7.98 (dd) ppm and H-1 protons appeared at 8.00 (dd) ppm (**Fig 5**).

In ^{1}H-NMR spectra isolated fraction (e) of CB ethyl acetate extract showed that H-19 protons appeared at 0.60-1.10 ppm (0.63 (td), 0.70 (td), 0.80 (td), 0.82 (td), 0.83 (d), 0.83 (d), 0.85 (t), 0.97 (s), 0.99 (d)), H-6 protons appeared at 1.14-1.30 ppm (1.24 (tq), 1.28 (quint), 1.28 (quint), 1.28 (s)), H-18 protons appeared at 1.30-1.98 ppm (1.33 (ddd), 1.34 (o), 1.42 (tqd), 1.45 (dtd), 1.45 (ddd), 1.52 (dt), 1.57 (ddd), 1.57 (dddd), 1.59 (dddd), 1.75 (ddd), 1.79 (ddd), 1.82 (dtd), 1.91 (ddt), 1.94 (ddd)), H-3 protons appeared at 2.06-3.37ppm (2.16 (ddd), 2.46 (dd), 3.25 (ddd)), H-1 proton appeared at 3.29 ppm (dd), 4.24 ppm (dtd), 4.25 ppm (dd) and 5.08 ppm (dd) (**Fig 6**).

Mass spectra of isolated Fraction (E) of BC Methanolic extract showed molecular ion [M^{+}] peaks at mlz 302.1410 which obtained 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one compound in which presence of carbons (C_{15}), Hydrogens (H_{10}) and Oxygen (O_{7}). Finally, the molecular formula of isolated Fraction (E) of BC Methanolic extract was found to be $C_{15}H_{10}O_{7}$ according to their fragments (**Fig. 7 & 8**)

Mass spectra of isolated Fraction (e) of CB Ethyl acetate extract showed molecular ion [M^{+}] peaks at mlz 414.6906 which obtained 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopentaphenanthren-3-ol compound in which presence of carbons (C_{29}), Hydrogens (H_{50}) and Oxygen (O). Finally, the molecular formula of isolated Fraction (e) of CB ethyl acetate extract was found to be $C_{29}H_{50}O$ according to their fragments (**Fig. 9 & 10**)

5. Conclusion

The Various examination of the methanol extract of Bergenia ciliata belonging to the family Saxifragaceae and ethyl acetate extract of Caesalpinia bonducella belonging to the family Fabaceae were effectively carried out. From this physical, chemical and spectral investigation were confirmed the presence of 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one in Fraction (E) of methanol extract of Bergenia ciliata and 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl 2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopentaphenanthren-3-ol in Fraction (e) of ethyl acetate extract of Caesalpinia bonducella.

Research Highlights

Research question

What bioactive compounds can be isolated from Bergenia ciliata and Caesalpinia bonducella and how can they be structurally characterized using chromatographic and spectroscopic techniques?

What is the current knowledge?

- ✓ Isolated bio-actives from plants have proven to be effective in treating and managing various ailments like cancer, diabetes mellitus, arthritis and other disorders.
- ✓ Bergenia ciliata commonly known as pashanbheda is used to dissolve kidney and bladder stones. It has bergenin as the primary active compound.
- ✓ Caesalpinia bonducella commonly known as sagargota is used to treat fever and inflammatory conditions. It has caesalpinin, bonducellin as the main active constituents.

What is new here?

- ✓ The solvent system developed for preliminary TLC of methanol extract of Bergenia ciliata in which the maximum spots were visible was toluene: ethyl acetate: acetic acid (6.2:4:0.4) mobile phase with standard flavonoid and ethyl acetate extract of Caesalpinia bonducella in which the maximum spots were visible was toluene: ethyl acetate: acetic acid (8:4.2:0.4) mobile phase with standard sterol respectively.
- ✓ Spectroscopic data like UV, FT-IR, NMR and Mass for isolated bio-actives.
- ✓ Isolation procedure for 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one and 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopentaphenanthren-3-ol as bio actives from Bergenia ciliata rhizomes and Caesalpinia bonducella seeds respectively.

Author Contributions

Prajakta Uday Kulkarni contributed to conceptualization, methodology, validation, investigation, resources, data curation, writing-original draft preparation and project administration. Dr. Vimal Kumar contributed to writing-review and editing, visualization and supervision. All authors have read and agreed to the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical approval

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