Prosopis cineraria (L.) Druce Fruit pods extract act as a source of Pharmaceuticals and having protein binding properties

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Abstract

Fruit pods of *Prosopis cineraria* was extracted with hot methanol and fractionated into different solvents. These extracts/fractions were used to evaluate various phytochemicals, total phenolic content, total flavonoids content, mineral content and protein binding capacity from pod part. Various phytoconstituents like saponins, tannins, carbohydrates, flavonoids, alkaloids, cardiac glycosides, fat and fixed oil and terpenoids have been screened from methanolic extract of *Prosopis cineraria* pods. Phytochemical investigation resulted the isolation of 1-heptatricontanol, 3, 8, 8-trimethoxy-3-piperidyl-2, 2' bimaphthalene-1, 1', 4, 4'-tetrone, β-sitosterol and catechin. Total phenolics content was highest in methanol fraction (844.83 ± 0.66 mg $GAEg^{-1}$) while total flavonoids content was highest in acetone fraction (608.31 ± 0.98 mg CEg^{-1}). *Prosopis cineraria* posses highest Nitrogen (3125 ± 0.20 mg/100g) in acetone fraction followed by Potassium content (833 ± 0.32 mg/100g) in ethyl acetate fraction, Phosphorus content (675 ± 0.09 mg/100g) in aqueous fraction, Iron content (253.40 ± 0.02 mg/100g) in ethyl acetate fraction, Copper content (91.45 ± 0.02 mg/100g) in benzene fraction, Zinc content (21.94 ± 0.02 mg/100g) in methanol fraction and Manganese content (04.30 ± 0.01 mg/100g) in aqueous fraction. Acetone fraction was found to be maximum protein binding capacity at highest concentration, i.e. 8.30 ± 0.05%. The presence of active phytoconstituent with antioxidant properties may provide generous basis for the use of these plants in pharmaceutical industry.

Key words: *Prosopis cineraria* (L) Druce, phytochemicals, phenolics, flavonoids, mineral content.

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Introduction

All living systems require minerals and inorganic constituents for sustained normal life. Unlike other nutrients, minerals cannot synthesized by living organisms or animals by acquiring adequate amount of required elements from their survival in environment [1]. Beside simple phenolic acids, the greatest number of plant phenolic compounds belongs to the flavonoids which represent an important group of secondary metabolites. A variety of flavonoids and their glycosides occur in plant species. Among the secondary metabolites produced by the plants, a major class of phenolics which relates to tannins is defined as water soluble polymeric phenolics that precipitate in proteins [2]. Compounds containing one or more phenolic group with many phenolic hydroxyl groups are called polyphenolic compounds and these polyphenols are derived from the common building block skeleton viz., phenyl propanoid unit C_6-C_3 [3].

Prosopis cineraria (Linn.) Druce, sometimes known as *P. spicigera* L., is one of about forty-four species of flowering trees [4]. It is a native species of Western and South Asia's deserts, including Afghanistan, Iran, India, and Pakistan, and it was praised in early Sanskrit writing. *Prosopis* is a more popular species of Leguminosae family and Mimosaceae subfamily, which commonly famous for names, i.e., Kandi in Sindhu, Sumri in Gujarat, Khejri, Janti, Sangri, and Jandi in Rajasthan [5]. It is the state tree of Rajasthan and produces vegetable pods, fodder from the foliage, and fuel from the branches that have been cut. In abandoned places, camels, goats, and sheep are the principal consumers of khejri, which is the most imported feed species and provides nourishing and attractive green and dry fodder shown in Figure 1. It is also known as 'Wonder or Golden tree' and the 'King of Desert' [6]. Pods of *P. cineraria* are good source of vitamins A, C, iron, protein and other micro nutrients. Dried and boiled sangri are used as vegetable in human diet and is the most important constituent of popular dishes "Trikuta and Panchkuta" [7]. It is one of the most delicious and famous dishes of Rajasthani cuisine. Unripe pods of khejri fruit have high nutritional value and are being used in the preparation of various dishes as depicted in Figure 2. Sangri pods are also known for prevention of protein and mineral deficiency. Dried pods are locally known as "Kho -Kha". Since all of this plant's parts are extremely beneficial, this tree is known as Kalp-Taru [8]. Many pharmaceutical and nutraceutical products currently utilise the bioactive phytochemicals found in plants, particularly fruits, because they have been linked to a number of health advantages [9]. Plant fruits play a significant role in the human beings and animals health's by providing carbohydrates, fats, proteins, minerals and vitamins [10]. Therefore, the present study was aimed to screen the isolation of various chemical compounds, total phenols content (TPC), total flavonoid content (TFC), mineral content and Protein Binding efficiency form different fractions of pods of *P. cineraria.*

Figure 1. A complete plant of *Prosopis cineraria* (L.) Druce [25].

Figure 2. Sangri Pods (fruits) of *Prosopis cineraria* (L.) Druce[26].

Materials and Methods

Chemicals

All chemicals and solvents used in this research work were obtained from Himedia laboratories, CDH or SD fine chem. Limited Mumbai and theses were of analytical grade also.

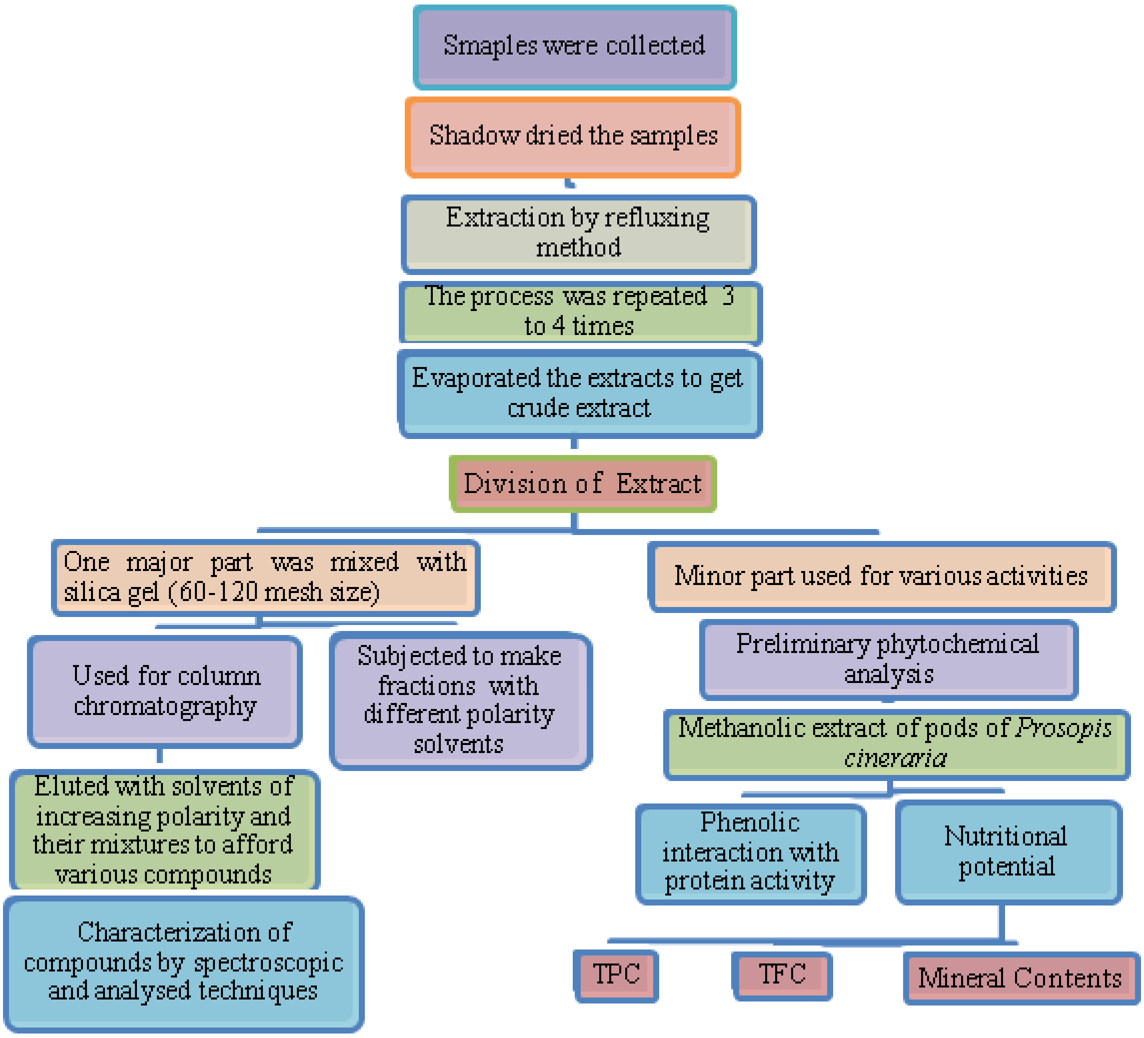
Collection of plant material

Prosopis cineraria pods sample were procured in the month of July to Oct from campus of CCS HAU, Hisar and sidewise area of Haryana state in India. Samples of the collected pods were water washed, followed by shadow drying. For study purposes, the dried samples were appropriately divided into little pieces and stored in an airtight box.

Preparation of extracts/ fractions

The above-mentioned chopped samples were used for the extraction, which was done using the refluxing method for eight hours with methanol. Crude extract was produced by repeating the procedure three to four times, collecting the extractive, and evaporating it using a rotary evaporator. With the help of column chromatography, around half of the crude extract was combined with silica gel with a mesh size (60–120 mesh size). Hexane, benzene, ethyl acetate, methanol, and combinations of these solvents with various degrees of polarity were used to elute the column. The leftover extract was separated using solvents of various polarities, including water, hexane, benzene, chloroform, and ethyl acetate. The resultant fractions were then evaporated to yield crude bulk, which was then kept in a refrigerator and employed in further experiments.

Flowchart: Showing extraction, fractionation and isolation of *Prosopis cineraria* (L.) Druce pods



Preliminary phytocochemicals screening

The fresh prepared methanol extract of fruits of *P. cineraria* was used for quantitative analysis of phytochemicals by using standard parameters and this helps in the identification of various classes of bioactive chemical compounds.

Column chromatography

Methanol extract of plant parts was combined with silica gel with a mesh size of 60 to 120 for column chromatography. Hexane silica gel slurry was placed within a glass column with a 1000 mm x 40 mm dimension. A little amount of the fruit's methanol extract was added to the column and eluted using hexane, benzene, ethyl acetate, methanol, and their mixes as solvents with increasing polarity. Thin layer chromatography plates were used to monitor each eluted sample that was collected. From this column chromatography, four distinct chemical compounds with the letters I to IV were obtained.

Estimation of Total Phenol Content (TPC)

The Folin-Ciocalteau method [15] was used to determine the phenolic content of the plant component extract, and gallic acid (2.5-100 µg/ml) was utilized as a reference standard for constructing the calibration curve. The final volume was built up to 10 ml using distilled water after the fractions (1 mg/ml) were combined with 1 ml of saturated sodium carbonate (20%) and 1 ml of (1N) Folin-Ciocalteau reagent. The reaction mixture was left in the dark for 20 minutes to allow for colour development, and after that, a UV-V spectrophotometer set to a wavelength of 725 nm was used to measure the absorbance. By applying a regression equation that was derived from a standard curve and expressed as mean ±standard deviation or mg GAE/ g (Galic acid equivalent), of dry extract, the total amount of phenolics was determined.

Estimation of total flavonoids content (TFC)

By employing catechin as a reference for the calibration curve and aluminium chloride colorimetric assay [16] to measure the total flavonoid content of plant extract. 100 ml of distilled water was used to dissolve 10 mg of catechin, which was then diluted to create different concentrations (2.5, 5, 10, 20, 40, 60, 80, and 100 µg/ml). A 10 ml volumetric flask was used to combine one ml of each plant extract and catechin with 4 ml of distilled water, along with 0.3 ml of sodium nitrate (5%) and 0.3 ml of aluminium chloride (10%). After waiting for five minutes, 2 ml of sodium hydroxide (1M) were added, and distilled water was used to make the remaining volume to the required level. Following that, the UV-V spectrophotometer measured the absorbance in comparison to a blank. Using a linear regression equation derived from the calibration curve, the total flavonoids were estimated as mean ±standard deviation and expressed as mg CE/g (Catechin equivalent) of dry extract.

Estimation of Mineral Content

Atomic absorption spectrometer measurements were used to assess the concentrations of minerals including Fe, Cu, Zn, and Mn. By taking 0.5g of each fraction and adding 20 ml of a diacid mixture (HNO_3: $HClO_4$: 4:1), the samples were digested by wet oxidation and left overnight. The following day, samples were digested on a hot plate, and the final volume was diluted with distilled water to a level of 25 ml. The readings were recorded using AAS and a blank surface. The following formula was used to determine the mineral content, which was then represented as mg per 100g of the extract.

Mineral content = (Abs_{Sample}- Abs_{Blank}) × Dilution factor

Following the same process as previously, the NPK mineral content in the plant sample was digested; however, instead of using the 20 ml HNO_3: $HClO_4$ - 4:1 diacid mixture, 10 ml of H_2SO_4 - 4:1 diacid mixture was utilised. Utilizing Nesseler's reagent, colorimetry was used to quantify the amount of nitrogen [24]. Using the ammonium sulphate standard curve, the nitrogen concentration was determined and represented in mg/100g [17]. Vanadomolybdo-phosphoric yellow colour analysis was used to assess the phosphorus concentration as well [18]. The potassium level in the acid digest plant sample was evaluated using a flame photometer by measuring absorbance against a control, and the phosphorus concentration was calculated using a standard plot of phosphate solution. Using the standard curve of potassium chloride, the potassium concentration (mg/100g) was estimated.

Estimation of protein binding efficiency/ protein phenol interaction

Tannic acid (50 µg/ml) was used as a standard to assess the spectrophotometric method's ability to measure the protein binding efficiency of fruit extracts/fractions. 0.25g of each fraction was dissolved in 5 ml of methanol, and 2, 4, 6, 8 and 10 µg/ml of these solutions were put as spots on Whatmann No. 1 filter paper. The entire experiment was performed three times. After allowing the spots to dry, BSA (Bovine Albumin Serium) was sprayed on them until the paper was totally moist. The unbound BSA was removed from the filter paper by vigorously washing it with acetate buffer three times after 30 minutes. Three times, Ponceau S dye was applied to the paper, and the strained strips were then rinsed in 0.2% acetic acid (v/v) until the colour of the strips stopped. The strained area was chopped into small pieces and maintained in separate test tubes after the strained strips and blank were air dried. The contents of the test tubes were eluted with 3 ml of NaOH (0.1N), added 0.3 ml of acetic acid (10%), and centrifuged afterward. After centrifugation, the colour of the liquid supernatant was assessed using 525 nm wavelengths. The linear regression equation, which was derived from the tannic acid standard curve and calculated as mean ±standard deviation (S.D.), and represented as µg/ml tannic acid of dry extract [19], was used to quantify the quantity of protein binding efficiency.

Statistical data analysis

The experiment was performed three times, and the findings were calculated and expressed as mean ±standard deviation (SD). Microsoft Excel 2007 was used to conduct all measurements for one- and two-way ANOVA with a mean ($p<0.05$) in the OPSTATE.

Result and Discussion

Preliminary phytochemical screening

The methanol extract of pods of *P. cineraria* was screened for the detection of various phytochemical components by using standard phytochemical procedures. The extracts were tested for saponins, tannins, carbohydrates, cardiac glycosides, alkaloids, flavonoids, terpenoids and fat and fixed oil, phytosterols, proteins, amino acids and anthraquinone glycosides are presented in Table1 [20].

Table 1.Preliminary phytochemical parameters for test on pods methanol extract of *P. cineraria*

Phytochemical parameters	Name of the test	Results
Saponins	Frothing test	+
Tannins	Ferric chloride test	+
Carbohydrates	Fehling's test, Tollen's reagent test	+
Cardiac glycosides	Keller-Killiani test	+
Anthraquinone glycosides	Hydroxyanthraquinine test	-
Alkaloids	Hager's test	+
Flavonoids	Alkaline reagent test	+
Terpenoids	Salkowski test	+
Phytosterols	Liebermann- Burchard's test	-
Protein	Biuret test	-
Amino acids	Millon's test	-
Fats and fixed oil	Copper sulphate test	+

The presence is shown by the + sign, whereas the absence is denoted by the - sign.

Compounds isolation and structure elucidation of pods of *P. cineraria*:

Compound I (1-Heptatricontanol): After being eluted with pure hexane and recrystallized from benzene, the white needle-shaped substance (27 mg) with a melting point of 89-90^{0}C (Lit. mp. 90^{0}C) [11] was produced (1:1). After development with iodine, the R_f value in benzene: hexane (1:1) was discovered to be 0.31. The compound's paraffinic alcohol composition was shown by the absorption peak at 3398 cm^{-1}, which also indicated the existence of a hydroxyl group. Its GC-MS with a molecular mass of 536 led to the derivation of the chemical formula $C_{37}H_{76}O$.^{1}H NMR (δ, DMSO-d_6): 0.86 (t, J= 4.0Hz, 3H, 1×CH_3), 1.24 (br, 68H, 34×CH_2), 2.52(m, J=8.0Hz, 2H, 1×CH_2), 3.31(t, 2H, 1×CH_2-OH). IR (KBr, $V_{max,}cm^{-1}$): 3398, 2900, 2849, 1062, 723. GC-MS (m/z, % intensity): 478(0.5), 325(5), 269(3), 151(100), 135(72), 81(50).

The triplet peak at 0.86 with coupling constant J= 4.0 Hz integrating three protons of the methyl group at terminal position was visible in the ^{1}H NMR spectra of DMSO-d_6. A total of thirty four methylene groups were allocated to a broad peak of sixty eight protons at 1.24. A multiplet of two methylene protons at 2.52 linked to the hydroxyl group in the -position. There is also a triplet of two methylene protons at 3.31 connected to the hydroxyl group. All of the spectral information stated above was in agreement with 1-Heptatricontanol published data [11], which was a report of the compound's isolation and characterization from *P. cineraria* pods.

Compound II (3, 8, 8-trimethoxy-3-piperidyl-2, 2'binaphthalene-1, 1', 4, 4'-tetrone): The compound II was obtained on elution with benzene: hexane (1:3) as a solid (50mg), melting point 214-217ºC. It was recrystallized from ethyl acetate. Absorption peaks at 1670cm^{-1} indicated the presence of keto group. The molecular formula $C_{28}H_{25}NO_7$ with molecular mass 487 was deduced from its GC-MS [12]. ^{1}H NMR (δ, DMSO-d_6): 3.98(t, 6H, 2× OC**H**$_3$), 4.21(t, 3H, 1× OC**H**$_3$), 7.32(d, 1H, 7, 7' H), 7.71(d, 1H, 5, 5'H), 7.86(t, 1H, 6, 6'H). IR (KBr, $V_{max,}$ cm^{-1}): 1670, 1650, 1639 and 1467. GC-MS (m/z, % intensity): 394(2.5), 341(3.5), 279(11), 207(16), 149(100), 113(12).

^{1}H NMR spectra of compound II in DMSO-d_6 exhibited Two methoxy groups might be the cause of a triplet at 3.98 integrating six protons. One methoxy group was hinted at in a second triplet with three protons integrating at 4.21. At 7.32, a doublet appeared, signifying one proton that belonged to the methine group. Additionally, the methine group may be responsible for a doublet at 7.71 integrating one proton. At 7.86, a triplet incorporating a proton hinted at the methine group. The following structure of compound II fits our data for the molecule perfectly. 3, 8, 8-trimethoxy-3-piperidyl-2, 2' bimaphthalene-1, 1', 4, 4'-tetrone [12] is one possible name for it.

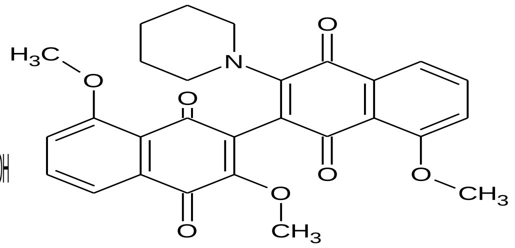
Compound III (β-sitosterol): After elution with ethyl acetate:benzene (1:1) and recrystallization from pure ethyl acetate solvent, a white crystalline material with a melting point of 135-136 ^{0}C (Lit. mp. 136-137^{0}C) [13] and the R_f value of 0.65 was generated (30 mg). The reaction of Liebermann and Burchard turning green indicates the presence of steroids. The presence of a hydroxyl group in the molecule was shown by a 3405 cm^{-1} absorption peak. Based on the molecular mass of 414, the GC-MS analysis confirmed the formula to be $C_{29}H_{50}O$.^{1}H NMR (δ, DMSO-d_6): 0.69(t, J=8.0Hz, 3H, 1×C_{28}-C**H**$_3$), 0.81(d, J=8.0Hz, 6H, 1×C_{26}-C**H**$_3$, and 1×C_{27}- C**H**$_3$), 0.85(t, J=8.0Hz, 3H,C_{29}-C**H**$_3$), 0.93(d, J=7.0Hz, 3H, C_{21}-CH_3), 1.20(s,3H, C_{19}-C**H**$_3$), 2.17-2.38(m, 29H, 11×-C**H**$_2$ and 7×-C**H**), 4.74-4.82(m,1H, C_3-C**H**-OH), 5.32(br, J=4.0Hz, 1H, =C**H**). IR (KBr, $V_{max,}$ cm^{-1}): 3405, 2931, 2849, 1649, 1456, 1365, 1019 and 799. GC-MS (m/z, % intensity): 353.9(95), 339.9(15), 280.1(70), 220.2(35), 146.4(25), 72.7(100).

^{1}HNMR spectra in DMSO-d_6 exhibited a three-proton triplet at 0.69 with a coupling constant of 8.0 Hz and a methyl group positioned at C28. A six-proton doublet at 0.8, J = 8.0 Hz, integrating two methyl groups at C_{26} and C_{27}. The presence of one methyl group at C_{29} position was again suggested by a triplet of three protons at 0.85 with J value 8.0 Hz. At 0.93, a methyl group with a centre at C_{21} was represented by three protons with a coupling constant of (J=7.0 Hz). A methyl group with a centre at C_{19} may be the cause of a singlet of three protons at 1.2. Seven methines and eleven methylene groups were indicated by a multiplet of twenty nine protons with a range of 2.17 to 2.38. Once more, a multiplet with a 4.75-4.82 range integrates one proton at the hydroxyl groups -position. A broad signal with a J value of 4.0 Hz that can be assigned to an olefin proton at 5.32 of one proton. The identity of compound III as -sitosterol was proved by a full agreement between the data of compound III and the literature data of -sitosterol [13].

Compound IV (Catechin): This compound, which has a melting point of 213-214 ºC (Lit. mp. 212-214 ºC), was produced after being eluted with benzene and ethyl acetate (1:1) and recrystallized from the methanol solvent [14]. The existence of the hydroxyl group was revealed by the absorption peaks at 3558 and 3350 cm^{-1}. From LC-MS, the molecular mass and formula (290, $C_{15}H_{14}O_6$) were determined. ^{1}H NMR (δ, $CDCl_3$): 2.71(d, 2H, J=8.4Hz, C $_{4a}$-**H**), 2.88(dd, 1H, J=6.0Hz, C_{4b}-**H**), 5.17(d, 1H, J=7.9Hz, C_2-**H**), 5.29(m, 1H, C_3-**H**), 6.69(d, 1H, J=2.3Hz,C_6-**H**), 6.75(d, 1H, J=2.1Hz, C_8-**H**), 7.19(dd, 1H, J=8.4Hz and 2.4Hz, $C_{5'}$-**H**),7.26(m, 2H, $C_{2'}$,$C_{6'}$-**H**). IR (KBr, $V_{max,}$ cm^{-1}): 3538, 3350, 2919, 2360, 1610, 1445, 1228, 1048, 939,756, 602. LC-MS (m/z, % intensity: 290(100), 278(30), 272(13), 205(7)

^{1}H NMR spectra of this compound in $CDCl_3$ showed two protons in a doublet at 2.71 with coupling constant 8.4 Hz in the C_{4a} position. Once more, the C_{4b} location displayed a doublet at 2.88 for one proton with a J value of 6.0 Hz. A second doublet with a J value of 7.9Hz is assigned at position C_2 and is at 5.17 for one proton. A proton multiplet integrating at 5.29 on the C_3 location. Appearance of a doublet with one proton integrated at positions C_6 and C_8, at 6.69 and 6.75 Hz, respectively. A doublet of doublet with a central frequency of 7.19Hz, coupling constant J=8.4Hz, and a proton assigned at $C_{5'}$ represented by 2.4Hz. Due to their positions at $C_{2'}$ and $C_{6'}$, a second multiplet of two protons emerged at 7.26. The spectral data analysis perfectly matches the catechin literature data [21]. According to a review of the literature, this is a catechin report from a *Prosopis cineraria* pod.

I



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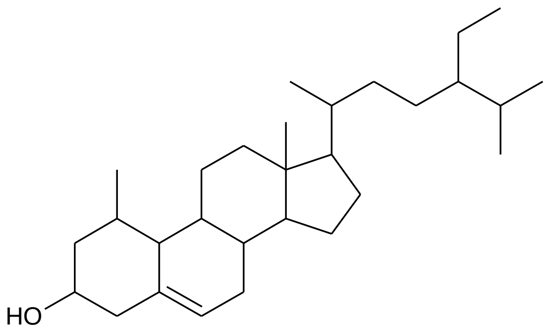
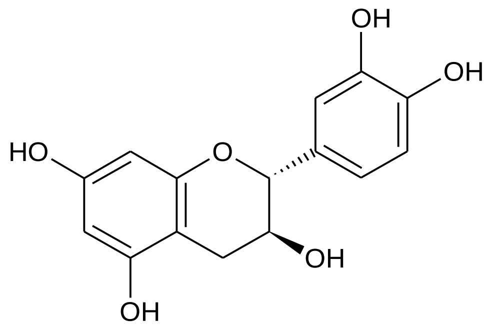
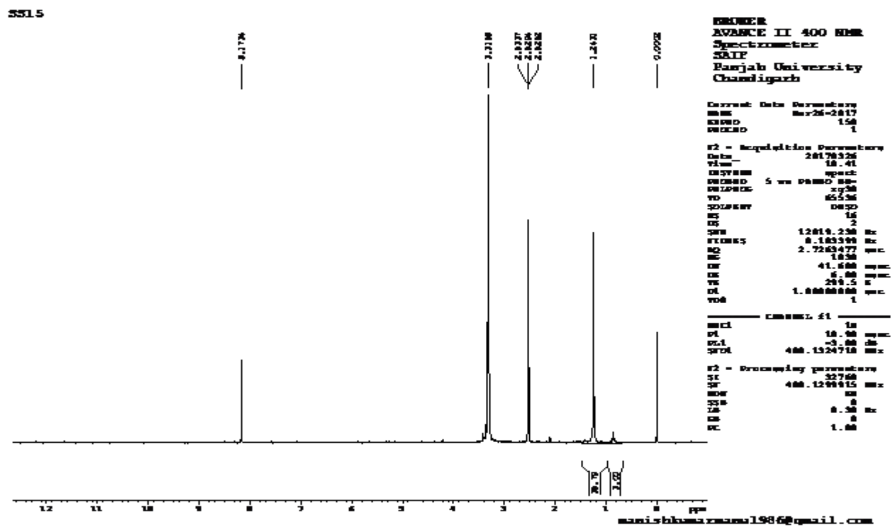


III



IV

Figure 3. Isolation of chemical compounds (I-IV)



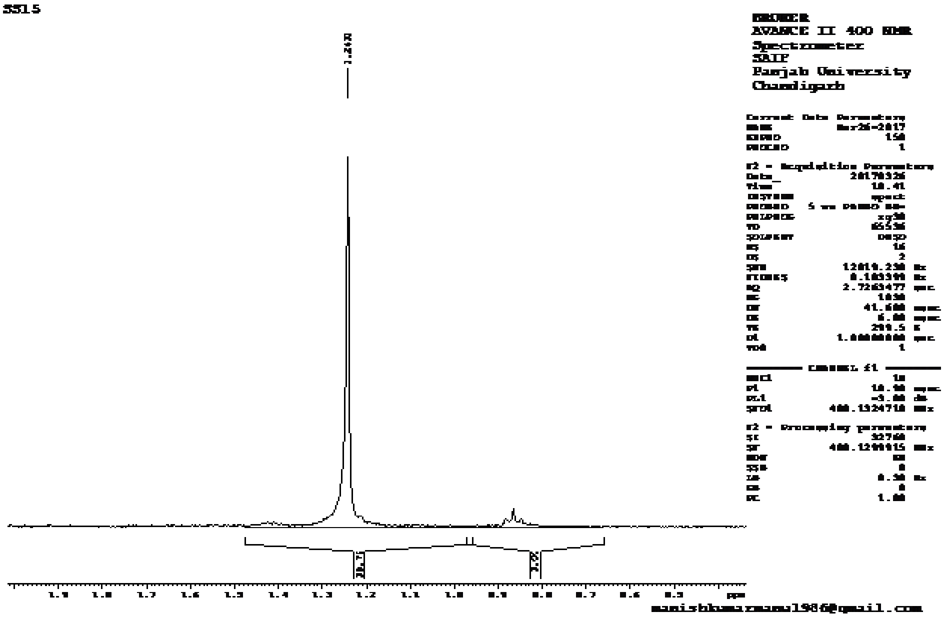


Figure 4. [1]H NMR Spectra of compound 1.

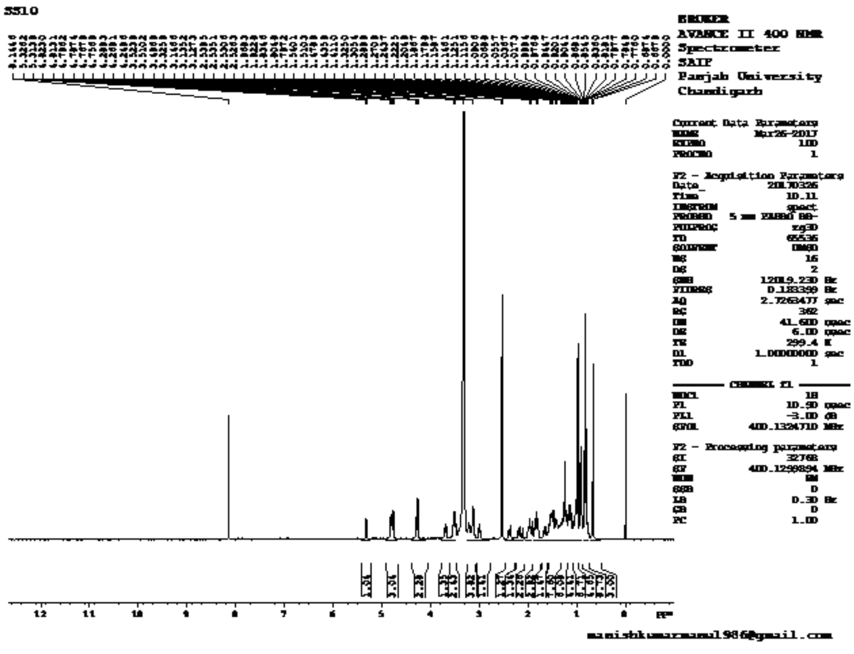
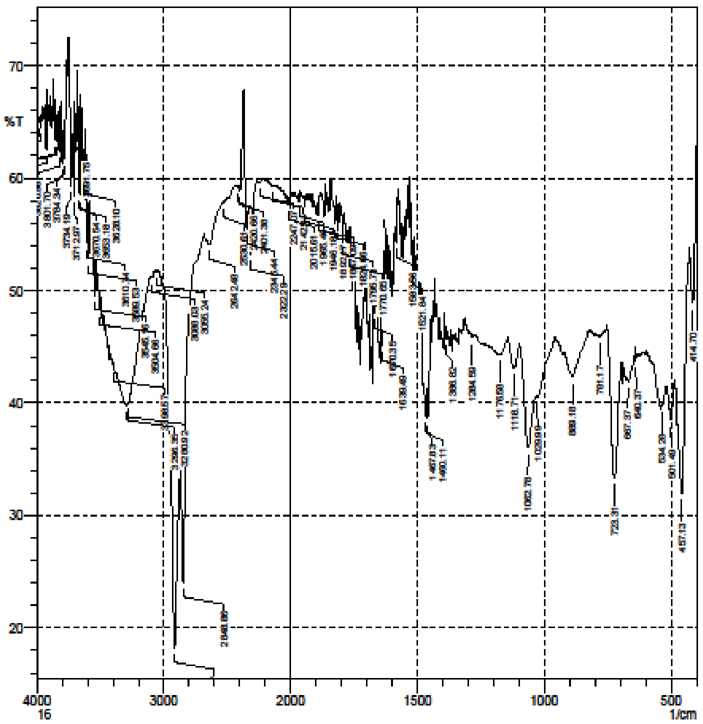


Figure 5. [1]H NMR spectra of compound 3.



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Dr. D. Kumar)

SHIMADZU

	Peak	Intensity	Corr. Int	Base (H)	Base (L)	Area	Corr. Are
1	414.7	48.8802	9.4946	426.27	399.26	7.3753	1.263
2	457.13	31.9475	16.1969	487.99	428.2	23.5969	4.4146
3	501.49	38.4748	3.1368	518.85	489.92	11.5468	0.5246
4	534.28	39.7791	0.2379	536.21	522.71	5.307	0.0391
5	640.37	43.7577	0.9519	644.22	634.58	3.4272	0.0423
6	667.37	41.9782	0.4086	671.23	653.87	6.4377	0.0385
7	723.31	33.3791	11.8641	752.24	696.3	22.9694	3.6859
8	781.17	45.8812	0.4479	792.74	754.17	12.9292	0.1096
9	889.18	42.3166	2.7625	921.97	819.75	36.2655	1.2503
10	1029.99	40.5295	0.5354	1035.77	958.62	28.2321	0.1926
11	1062.78	36.0296	6.5453	1097.5	1037.7	24.1352	2.1042
12	1118.71	43.167	2.3247	1143.79	1099.43	15.7565	0.592
13	1176.58	44.3364	1.1734	1219.01	1145.72	25.5583	0.4647
14	1284.59	45.8124	0.3667	1292.31	1278.81	4.5504	0.0198
15	1386.82	45.7336	1.0226	1392.61	1381.03	3.8825	0.051
16	1460.11	38.6334	3.121	1462.04	1446.61	5.5886	0.1765
17	1467.83	38.8388	2.4061	1473.62	1463.97	3.8159	0.1065
18	1521.84	52.0467	4.2717	1525.69	1516.05	2.635	0.1898
19	1583.56	54.2427	2.1397	1585.49	1575.84	2.3618	0.0674
20	1639.49	45.0925	5.2472	1643.35	1629.85	4.0476	0.2733
21	1670.35	48.0081	2.0018	1674.21	1664.57	3.0039	0.0947
22	1770.65	53.4328	2.1	1776.44	1766.8	2.5707	0.1072
23	1795.73	54.534	2.779	1799.59	1788.01	2.9032	0.091
24	1824.66	56.0248	1.9525	1830.45	1818.87	2.8261	0.0916
25	1867.09	56.5964	2.1988	1882.52	1865.17	4.0959	0.0841
26	1892.17	56.7119	1.048	1896.03	1884.45	2.764	0.0365
27	1946.18	57.4404	1.2546	1961.61	1938.46	5.4234	0.0708
28	1965.46	57.9642	0.6579	1967.39	1963.53	0.9032	0.0095
29	2015.61	57.6329	0.387	2017.54	1998.25	4.5266	0.0078
30	2142.91	59.3197	0.1248	2181.49	2135.2	10.4493	0.0275
31	2247.07	59.6688	0.021	2249	2218.14	6.8777	0.0006
32	2322.29	53.2159	4.5395	2331.94	2266.36	15.9495	0.7336
33	2345.44	55.4725	5.0693	2372.44	2333.87	8.987	1.019
34	2401.38	58.9441	2.5559	2411.02	2374.37	7.6634	0.4571
35	2420.66	59.2442	0.0647	2439.95	2412.95	6.1298	0.0061
36	2530.61	57.8186	0.0352	2532.54	2441.88	21.1101	0.0823
37	2642.48	54.0952	1.6954	2684.91	2534.46	38.1363	0.7552
38	2848.86	24.0322	12.3553	2870.08	2686.84	63.8046	3.9445
39	2916.37	18.2552	21.1959	3005.1	2872.01	65.2776	14.3186
40	3055.24	51.7517	0.0914	3061.03	3051.39	2.7561	0.0046
41	3088.03	51.1731	0.162	3095.75	3084.18	3.3596	0.0077

Figure 6. IR spectra of compound 1.

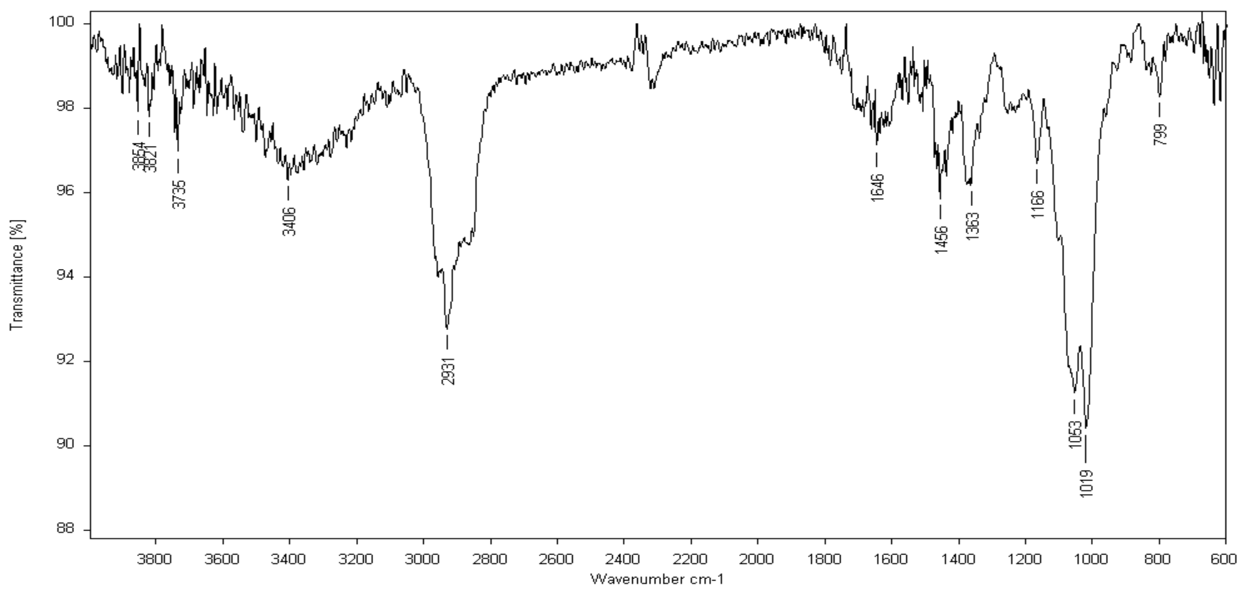

Figure 7. IR spectra of compound 3.

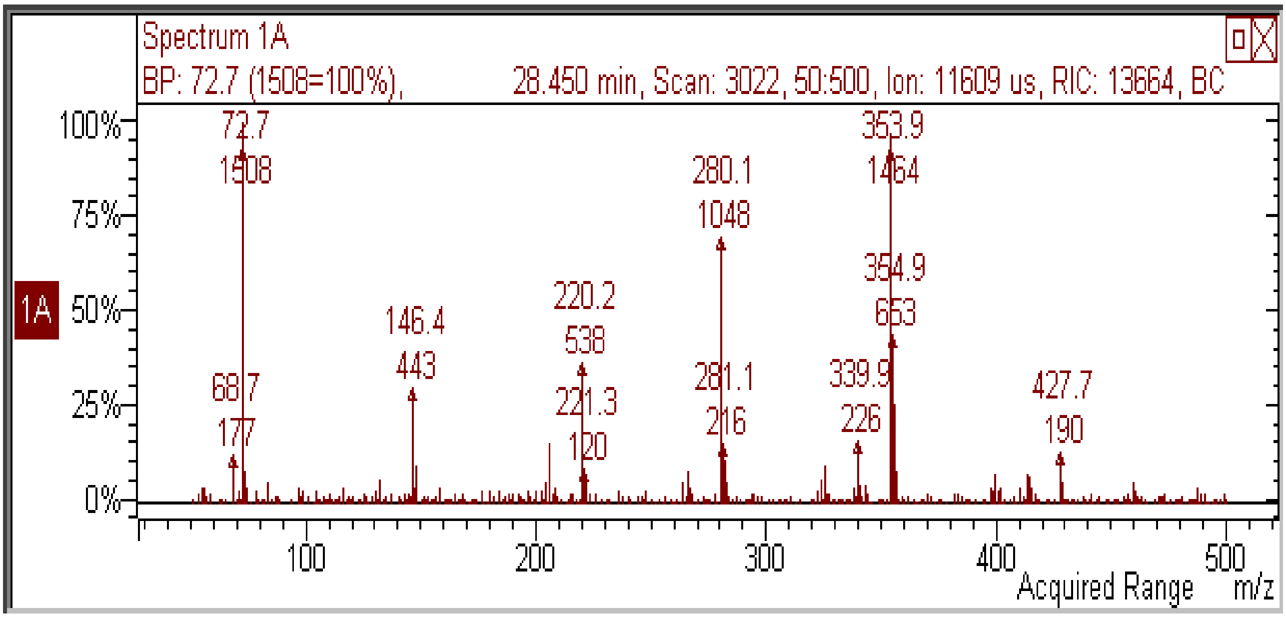

Figure 8. GC-MS of compound 3.

Total Phenolics Content

The carbon-based aromatic chemicals known as phenols, which are found in plants, are essential to maintaining human health. It was capable of a variety of biological processes. As a result, the total phenolics content of the various *P. cineraria* pod fractions was determined (Table 2). The TPCs of different pod fractions from *P. cineraria* are computed using the linear regression equation derived from the standard gallic acid plot and are represented in terms of gallic acid equivalent (GAE). Overall result showed that the total phenolics content varied from 42.29 ± 0.04 mg GAEg^{-1} of dry extract of hexane fraction to 844.83 ± 0.66 mg GAEg^{-1} of methanol fraction in pods of *P. cineraria.* The maximum phenol content was found in methanol fraction of pods of *P. cineraria* [22].

Total Flavonoids Content (TFC) of the extracts of *Prosopis cineraria.*

The most significant class of polyphenols in the human diet, flavonoids are typically found in plants. In order to express the total flavonoids content of the various Prosopis cineraria pod fractions in terms of catechin equivalent (CE). The following linear regression equation, which was derived from the catechin standard plot, was used to compute the TFCs. The present data also reported in table 2 revealed that hexane fraction contained minimum amount of flavonoids content, i.e., 118.92 ± 0.58 mg CEg^{-1} and acetone fraction of pod contained maximum amount of flavonoids content, i.e., 608.30 ± 0.98 mg CEg^{-1} [20].

Table 2. Total phenol content (TPC) and Total flavonoid content (TFC) from pods extract/ fractions

Sr. No.	Extract/ fractions	TPC(mg GAE/g)	TFC(mg CE/g)
1.	Hexane	42.29 ± 0.04	181.92 ± 0.58
2.	Benzene	171.76 ± 0.82	290.13 ± 0.40
3.	Chloroform	144.65 ± 0.79	260.74 ± 0.70
4.	Ethyl acetate	181.28 ± 1.43	535.04 ± 0.40
5.	Acetone	176.52 ± 1.64	608.31 ± 0.98
6.	Methanol	844.83 ± 0.66	190.13 ± 0.40
7.	Water	122.25 ± 0.84	172.05 ± 0.43

All the values are mean ± S.D.

Gallic acid equivalent in milligrammes per gramme (mg GAE/g)

Catechin Equivalent in Milligrams per Gram (mg CE/g)

Mineral contents isolated from various extracts of pods of *P. cineraria.*

Prosopis cineraria (L.) Druce is one of the chief indigenous tree of the plains and the most important source of medicinal and nutritional areas which play vital role in many healing benefits and feeding processes. Therefore, from its valuable properties, have to decide the estimation of mineral contents from various fractions of *P. cineraria*. The results revealed that *Prosopis cineraria* possessed the highest Nitrogen (N) content, followed by Potassium (K), Phosphorus (P), Iron (Fe), Copper (Cu), Zinc (Zn), and Manganese (Mn) content[17, 23].

The potassium content of *P. cineraria* pods ranged from 833 0.32 mg/100g in the ethyl acetate fraction to 241 0.03 mg/100g in the aqueous fraction (Table 3). The lowest Cu and Zn values (03.40 0.04 mg/100g and 02.75 0.90 mg/100g, respectively) were found in the acetone fraction of *P. cineraria* pods. The iron concentration in the pods of this plant ranged from 48.75 0.01 mg/100g in the acetone fraction to 253.40 0.02 mg/100g in the ethyl acetate fraction. Manganese was found to be present in extremely small amounts in *P. cineraria* pods. Between 0.05 and 0.01 mg/100 g (benzene percentage) and 4.30 and 0.01 mg/100 g. (water fraction). Compared to other components of this plant, the sangri pods have the highest concentration of nitrogen. In the hexane fraction, it ranged from 2525 0.09 mg/100g to 3125 0.02 mg/100g. The amount of phosphorus in the pod fractions in khejri pods was observed to vary between highest (675 0.09 mg/100g) in the aqueous fraction and minimum (312 0.90 mg/100g) in the ethyl acetate fraction. The mineral content of *P. cineraria* pod fractions showed that nitrogen (N) was followed by phosphorus (P), potassium (K), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) in that sequence (Mn) [22].

Table 3 Mineral composition (mg/100g) from various pods fractions of *P. cineraria*

Sr. No.	Extract/ Fractions	Iron (Fe)	Copper (Cu)	Zinc (Zn)	Manganese (Mn)	Nitrogen (N)	Phosphorus (P)	Potassium (K)
1.	**Hexane**	71.65±0.08	29.15±0.09	08.64±0.99	00.10±0.09	2525±0.09	525±0.03	181±0.02
2.	**Benzene**	145.80±0.09	91.45±0.02	06.77±0.78	00.05±0.05	ND	587±0.02	203±0.09
3.	**Chloroform**	183.85±0.01	29.95±0.09	08.77±0.88	00.30±0.11	2260±0.10	362±0.01	174±0.01
4.	**Ethyl acetate**	253.40±0.02	61.55±0.09	11.50±0.54	00.20±0.02	ND	312±0.90	833±0.32
5.	**Acetone**	48.75±0.01	03.04±0.04	02.75±0.90	00.35±0.04	3125±0.20	362±0.07	745±0.09
6.	**Methanol**	166.75±0.01	06.25±0.03	21.94±0.02	00.40±0.03	ND	450±0.02	143±0.21
7.	**Water**	61.20±0.06	05.00±0.01	20.51±0.01	04.30±0.01	ND	675±0.09	241±0.03

All the values are mean ± S.D.

ND – Not determined

Protein binding capacity / protein phenol interaction of pods of *P. cineraria*

Mean percent of binding efficiency of pods fractions are shown in table 4 with their different concentration rates. Acetone fraction was found to be maximum activity at highest concentration i.e. 8.30 ± 0.05%. Methanol and hexane fractions exhibited very good activity values with 5.62 ± 0.04% and 4.07 ± 0.04% at highest concentrations. Very less activity was shown by ethyl acetate and benzene fractions (2.03 ± 0.03% and 2.05 ± 0.05%) at 10 μg/ml test concentration. Comparative protein binding capacity of various fractions of pod were found in increasing order, i.e., acetone > methanol > hexane > chloroform > water > benzene > ethyl acetate. There is step wise increasing value of protein phenol interaction showed it's nutritional and digestibility power of pods of *P. cineraria* going on increase respectively [19].

Table 4. Protein binding capacity/ protein phenol interaction (%) of pods fractions

Sr. No.	Fractions	Concentrations (µg/ml)					
		00	02	04	06	08	10
1.	Hexane	2.36±0.03	2.72±0.06	2.94±0.05	3.54±0.02	3.94±0.06	4.07±0.04
2.	Benzene	1.15±0.03	1.38±0.06	1.60±0.04	1.75±0.04	1.83±0.05	2.05±0.05
3.	Chloroform	1.42±0.02	1.62±0.04	1.86±0.03	2.27±0.03	2.76±0.03	2.97±0.02
4.	Ethyl acetate	1.30±0.03	1.52±0.03	1.73±0.05	1.83±0.05	1.96±0.04	2.03±0.03
5.	Acetone	3.84±0.06	4.63±0.03	5.05±0.07	5.62±0.04	6.40±0.05	8.30±0.05
6.	Methanol	2.82±0.05	3.17±0.07	3.84±0.05	4.18±0.06	4.93±0.03	5.62±0.04
7.	Water	1.22±0.04	1.59±0.03	1.83±0.03	2.39±0.03	2.48±0.03	2.75±0.03

All values are mean ± standard deviation (S.D)

µg/ml means microgram per milliliters

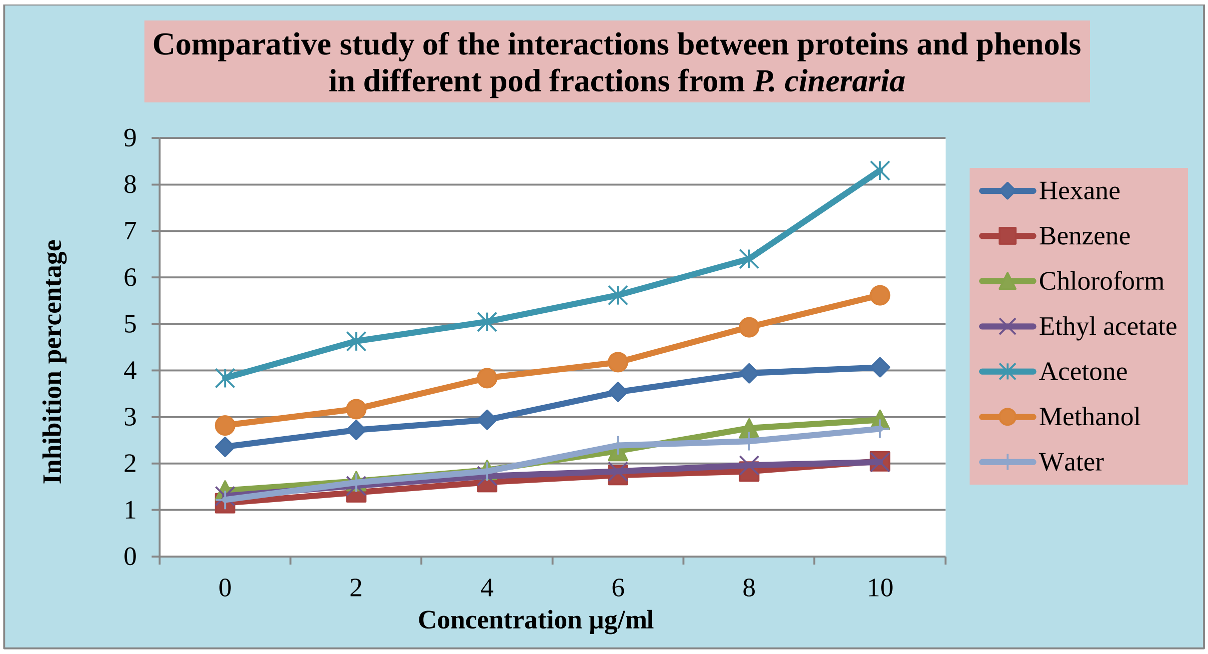

Figure 9. Interaction of phenols with *P. cineraria* pod protein.

Conclusion

According to the study, *P. cineraria* contains a healthy level of total phenols and flavonoids. the significance of plants as sources of medicinal lead compounds and as a means of treating various ailments. It serves as a great source of micronutrients as well. The highest effective total phenolic and total flavonoid content was identified in the methanol and acetone fractions of the pod, demonstrating its efficacy as a strong source of natural antioxidant. It is abundantly obvious from all of this plant's components that P. cineraria has a number of medicinal characteristics and can be used to cure a variety of conditions without causing any negative side effects. To discover the specific elements that make up antioxidative systems and develop applications for food and other products, more research is necessary.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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