



A Comparative Study of *in vivo* Plant and *in vitro* Callus Extracts of *Centratherum punctatum* Cass

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Authors' contributions

This work was carried out in collaboration among all authors. Author KMM performed the statistical analysis and wrote the protocol. Author JA designed the study and managed the literature search and wrote the first draft of the manuscript. Authors JA and SAS managed the analyses of the study. Authors GN and GSP performed the statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

The present study aims at comparative study between plant and callus extract, with respect to analysis of phytochemical constituents, antioxidant, antibacterial activity and cytotoxicity properties of *Centratherum punctatum* using aqueous medium and different solvents such as, methanol and ethyl acetate. *In vitro* studies in MS media supplemented with BAP 4.5 mg/L + Kn 4.0 mg/L has shown the high callus induction percentage of 92.33% with a maximum callus weight of 1.08 g. The phytochemical analysis of aqueous, methanol and ethyl acetate extract of *C. punctatum in vivo* plant and *in vitro* callus showed the presence of alkaloids, flavonoids, phenols and carbohydrates. The aqueous extract of both plant and callus showed the presence of tannins, proteins and steroids whereas the methanol extract showed the presence of tannins, amino acids and terpenoids. The ethyl acetate extract showed terpenoids and protein. FTIR analysis of plant and callus aqueous extract had a maximum characteristic band at 3399.87 cm⁻¹ and 3412.73 cm⁻¹ respectively indicating the presence of N-H stretching. GC-MS analysis revealed the presence of 11 different compounds in ethyl acetate extracts of plant and the callus extract revealed the presence of 15 different compounds which was absent in the plant extract. Plant extract exhibited maximum total phenol content than callus extract. The *in vitro* callus extract showed higher DPPH radical scavenging

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activity with lower inhibition percentage than *in vivo* plant extract. A maximum zone of inhibition was observed in methanol extract of *in vivo* plant and *in vitro* callus (15 mm and 14 mm respectively) against *Bacillus subtilis*. The ethyl acetate extract of *in vivo* plant and *in vitro* callus had a zone of 14 mm and 12 mm against *E. coli*. A maximum zone of inhibition (12 mm and 11 mm respectively) was observed in both methanol and ethyl acetate of *in vivo* plant and *in vitro* callus against *Staphylococcus aureus*. Antiproliferative analysis revealed that *in vivo* plant has inhibitory percentage of 23.6 whereas callus exhibited 28.5% against HeLa cells.

Keywords: *Centratherum punctatum*; phytochemical; GC-MS; FT-IR; DPPH; antibacterial activity; antiproliferative analysis.

1. INTRODUCTION

The medicinal plants have been utilized in the traditional framework of medicine. The social insurance framework dependent on plants goes back to vedic period and nature, instinct and in addition the gathered information over the span of times, has guided the human to find solutions to normal sicknesses from natural sources. The indigenous frameworks of medication to be specific ayurveda, siddha and unani have been in presence for a few hundred of years [1]. As indicated by World Health Organization restorative plants with different life supporting constituents would be the best beginning spot to get an assortment of disturbing, protected and novel medications. The medicinal values of plants lie in some chemical substances to produce a definite physiological action on the humans [2]. *Centratherum punctatum* is a medicinal plant belonging to the family Asteraceae. It commonly called as lark daisy, kesavardhini, kattushiragam, somraj and brazilian bachelor's button. It is a perennial bushy plant, growing up to 60 to 80 cm height of well branched stem [2]. It has been an extraordinary asset of medicinal values which are utilized for the treatment of several illnesses. It is rich in secondary metabolites and act as a health promoting compound in anti-inflammatory, antimicrobial, anti-allergic, anti-oxidant and cytotoxic anti-tumour. Thus it is also called as a traditional wound healer [2].

2. MATERIALS AND METHODS

2.1 Plant Material

Healthy *Centratherum punctatum* was purchased from botanical garden in Madhavaram, Chennai and authenticated by Dr. N. K. Uday Prakash, Department of biotechnology, Vels University, Chennai and deposited in the herbarium (MLHerb01/2019) at Marina labs. The plants were maintained in the garden.

2.2 Culture Technique

Murashige Skoog medium (MS medium) [3], supplemented with 3% sucrose and 0.8% agar and different growth hormones such as cytokinin 6-Benzyl amino purine (BAP); kinetin (Kn) for the initiation callus was used for the study. pH of the media was adjusted to 5.7 before autoclaving.

Table 1. Concentrations of growth regulators by different hormone

Treatments	Concentrations of growth regulators (mg/L)	
	BAP	Kn
T ₁	2.0	1.0
T ₂	2.5	1.5
T ₃	3.5	3
T ₄	4.0	3.5
T ₅	4.5	4
T ₆	3.0	1.5

2.3 Preparation and Inoculation of the Explant

The nodes (2-3 cm) were used as explants. It was washed with autoclaved distilled water for 3–4 times, for 10 minutes. This was followed by treatment with surfactant (Tween-20: 2–3 drops/100 ml water) for 5 minutes. Explants were then treated with 70% ethanol for 30 seconds and in the disinfectant (0.1% HgCl₂) for 10 minutes. This was again treated with 70% ethanol for 2 – 3 minutes and thrice with autoclaved distilled water. The explants were kept in a petriplate containing Whatmann filter paper to drain out the water [4]. The nodes were inoculated vertically on MS medium for culture initiation. The cultures for indirect regeneration were incubated at 25 ± 2°C under 16/8 hours light regime provided by cool white fluorescent lamp (60 μmol m⁻²s⁻¹).

2.4 Preparation of the Extract

The *in vivo* grown plant was collected and shade dried for 2 to 3 weeks. The dried plant materials

was then ground into a coarse powder in a mechanical blender and stored in an air tight container. 25 g of plant powder was subjected to extraction using 250 ml of distilled water (boiled at 100°C for 30 minutes). 25 g of plant powder was subjected separately for extraction in Soxhlet with 250 ml of methanol and ethyl acetate. After the completion of extraction, the extracts were transferred to rotary evaporator to evaporate the solvent and concentrating the extract [5]. The *in vitro* grown callus was dried under room temperature for 3 days. The dried callus was macerated using mortar and pestle. 10 grams of coarsely macerated callus was subjected separately for the extraction using 100 ml distilled water and solvent such as methanol and ethyl acetate [6].

2.5 Total Ash Content

Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators. About 2.0 g of powdered *C. punctatum* was weighed accurately and evenly distributed among the crucible. The content was shared with a heater and then burnt in a muffle furnace for 2 hours at 600 ± 10°C. The crucible was allowed to cool in desiccators. The desiccated, cool basin was then weighed with the ash [7].

$$\% \text{ Total ash} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100$$

2.6 Phytochemical Analysis

The phytochemical tests were carried out for the *Centratherum punctatum* plant and callus extracts using standard procedures.

2.6.1 Test for alkaloids

Dragendroff's test: To 250 µl of the extract, 1 ml of con. HCl and few drops of Dragendroff's reagent was added. Formation of red or orange precipitate indicated the presence of alkaloids [7].

Test for flavonoids: To 500 µl of 2N NaOH 300 µl of the extract was added. An intense yellow colour indicates the presence of flavonoids in the sample [8].

Test for phenols: To 250 µl of the extract, 250 µl of 2% Ferric chloride solution was added. Appearance of black indicates the presence of phenol in the sample [9].

Test for tannins: To 250 µl of the sample was added to 500 µl of distilled water and filtered few

drops of ferric chloride was added to the filtrate. Formation of bluish green or black precipitate indicates the presence of tannins in the sample [10].

Test for steroids: To 250 µl of extract, 250 µl of chloroform and 250 µl of conc. sulphuric acid was added. Appearance of red colour indicates the presence of steroids [11].

Test for terpenoids: 250 µl of the extract was mixed with 250 µl of chloroform followed by the addition of 250 µl of conc. sulphuric acid. A reddish brown interface layer formed indicates the presence of terpenoids [7].

Test for quinines: To 250 µl of the extract, 250 µl of conc. sulphuric acid was added. Appearance of red colour indicates the presence of quinines [11].

Test for proteins: To 250 µl of the extract, 7 drops of millon's reagent was added. Formation of white precipitate that turns red on gentle heating indicates the presence of proteins [7].

Test for amino acids: To 1 ml of the extract, 1 ml of Ninhydrin reagent were added. Appearance of purple colour indicates the presence of amino acids [7].

2.6.2 Test for carbohydrates

Molisch test: Few drops of molish reagent and conc. sulphuric acid were added to the extract, formation of reddish violet ring was observed at the junction of two layers indicates the presence of carbohydrates [11].

Test for saponins: To 250 µl of crude extract, 250 µl of water was added. The appearance of stable foam indicates the presence of saponins in the sample [11].

2.7 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The aqueous extract of *in vivo* plant and *in vitro* callus were subjected to FTIR analysis. The Characteristic peak values were recorded to find out the respective functional groups [7].

2.8 Gas Chromatography – Mass Spectrometry analysis (GC-MS)

The ethyl acetate extract of *in vivo* plant and *in vitro* callus of *Centratherum punctatum* were

subjected to GC – MS analysis at VIT University, Vellore. Identification of specific components was done by comparing mass spectra and the retention times with the data provided with the NIST library and confirmed [7]. The peak area percentages of the chromatography represent the abundance of the compounds in the extract. The peak area percentage was calculated by the formula:

$$\text{Peak area percentage (\% of component)} = \frac{\text{area under peak}}{\text{total area}} \times 100$$

2.9 Total Phenolic Content

Folin Ciocalteu method was followed for determining the total phenol content in the plant and callus extract of *C. punctatum* of aqueous, methanol and ethyl acetate. Distilled water (500 µl) and Folin Ciocalteu reagent (100 µl) was added to 1000 µl (1 mg/ml) of the extract and incubated for 6 minutes at room temperature. The final volume was made up to 5 ml with water, after addition of 1.25 ml of 7% sodium carbonate. The absorbance was measured at 760 nm using UV – Vis spectrophotometer after an incubation period of 90 minutes. Same method was repeated for other extracts. The total phenol content was expressed as mg Tannic acid equivalents (TAE) per dry weight of the flower using a standard plot of Tannic acid [12].

2.10 DPPH Radical Scavenging Activity

Aqueous, methanol and ethyl acetate extract of *in vivo* plant and *in vitro* callus of *C. punctatum* of various concentrations of 200, 400, 600, 800, 1000 µl (1 mg/ml) was taken in a test tube and made up to 1 ml using methanol. 1 ml of 0.1 mM DPPH was added to all the test tubes and kept in the dark for 30 minutes at room temperature. The absorbance of the solution was read at 517 nm in UV visible spectrophotometry. The % inhibition and IC₅₀ values were calculated with DPPH as the control and Ascorbic acid as the standard. The concentration of µg of dry weight of material per ml of solvent (µg/ml) that inhibits the formation of DPPH radicals by IC₅₀ value [13].

2.11 Antibacterial Activity

Two Gram positive organisms *Bacillus subtilis*, *Staphylococcus aureus* and one Gram negative organism *Escherichia coli* were used to determine the antibacterial activity. All the test

organisms were maintained on nutrient agar slants in the laboratory. The antibacterial activities of the aqueous, methanol and ethyl acetate extract of *C. punctatum in vivo* plants and *in vitro* callus were determined by agar well diffusion method. The culture suspension was adjusted by comparing with 0.5 Mc Farland turbidity standard. The plates were swabbed with 100 µl of the test organisms. A sterile cork borer was used to make wells of 5 mm diameters. 5% DMSO served as the negative control and concentration of 100 µl (1 mg/ml) extract were added into the respective wells. The plates were then incubated at 37°C overnight. The resulting zones of inhibition were measured using a ruler calibrated in millimeters. The average of the triplicate reading were taken to be the zone of inhibition of the bacterial isolates [14].

2.12 Antiproliferative Analysis

Sub culture of cell line: Concentration of the cell suspension was adjusted by adding 100 µl of a 5 x 10⁵ cells (HeLa cells)/ml solution to each well and incubated for 24 - 48 hours in CO₂ incubator.

Drug treatment: 10 µl of drug dissolved in DMSO was added to each well. It was placed on a shaking table at 150 rpm for 5 minutes, to thoroughly mix the samples into the media. It was then incubated at 37°C, 5% CO₂ for 12 - 24 hours.

MTT assay: 2 ml of MTT solution was added per 96 well plate at 5 mg/ml in DMSO/PBS. 20 µl of MTT solution was added to each well. It was placed on a shaking table at 150 rpm for 5 minutes and incubated at 37°C, 5% CO₂ for 4 hours for MTT metabolization. Formazan (MTT metabolic product) was resuspend in 200 µl of DMSO and placed on a shaking table at 150 rpm for 5 minutes. Optical density was read at 560 nm and subtract background at 670 nm, optical density should be directly correlated with cell quality. The cell density and percentage cell viability was calculated using the following formula.

$$\text{Cell density} = \text{OD Sample} - \text{OD blank}$$

The average cell density of triplets:

$$\text{Percentage of Cell viability (\%)} = \frac{\text{OD sample}}{\text{OD Control}} \times 100$$

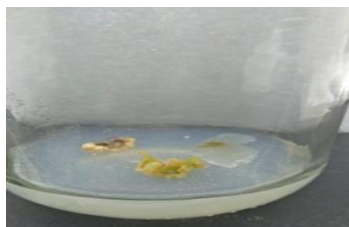
3. RESULTS AND DISCUSSION

3.1 Callus Induction Percentage and Weight

MS medium supplemented with BAP and Kn in (T_5 MS + 4.5 mg/L BAP + 4 mg/L Kn) showed the highest callus induction percentage (92.33%) and callus weight 1.08 g (Fig. 1A) followed by (T_6 3.0 mg/L BAP + 1.5 mg/L Kn) showing the highest callus induction percentage 87.54% and callus weight 0.82 g (Table 2, Fig. 1B).



(A)



(B)

Fig. 1(A). Callus induction from node explants of *Centratherum punctatum* on MS medium supplemented with T_{10} - 4.5 mg/L BAP and 4 mg/L Kn. **(B)** Callus induction from node explants of *C. punctatum* on MS medium supplemented with T_{11} - 3.0 mg/L BAP and 1.5 mg/L Kn

Similarly, Shirin et al. [15] reported *in vitro* multiple shoot proliferation method for *Tridax procumbens* L. using nodal explants. MS medium was supplemented with different concentration and combination of plant growth regulators such as BAP, Kn, NAA, IBA and GA_3 . In nodal segment the highest culture response 95% was observed. Archana et al. [16] reported the micropropagation of *Eclipta alba* (L.) Hassk using nodal segment where multiple shoot were obtained by culturing the explants on MS media supplemented with various concentration of BAP and Kn and in combination with IAA and NAA. The highest culture response was 90% of shoot induction.

3.2 Total Ash Content

C. punctatum is a medicinal plant used by many of the herbal practitioners to treat many diseases. The amount of ash content provides a measure of total amount of mineral matter in a plant. In the present study, it is noted *in vivo* plant contain maximum of 8% total ash content. Similarly, Chitra and Brindha [2] reported the *C. punctatum* possess 10.2% of total ash. Sivasubramanian and Brindha [17] reported that *C. punctatum* possess 6.8% of total ash. Measuring ash content is important because mineral matter may be the cause of pharmacological effect.

3.3 Phytochemical Analysis

The aqueous, methanol and ethyl acetate extract of *Centratherum punctatum* *in vivo* plant and *in vitro* callus showed the presence of alkaloids, flavonoids, phenols and carbohydrates. Presence of tannins was observed on aqueous and methanol extract of both plant and callus extract. Steroids was present in aqueous extract of both plant and callus. The presence of terpenoids was noted in methanol and ethyl acetate extract from both plant and callus. The presence of protein was observed in aqueous and ethyl acetate extract from plant and callus. The presence of amino acid were seen in methanol extract of (from) both and callus (Table 3).

Similarly, [2] reported the presence of alkaloids, phenols, quinines, flavones, sterols and protein.

3.4 Fourier Transform Infrared Spectrophotometer (FT-IR)

Aqueous plant extract of *Centratherum punctatum* exhibited a maximum characteristic band at 3399.87 cm^{-1} and lowest at 538.61 cm^{-1} indicating the presence of N-H stretching and C-I stretching deformation respectively (Fig. 2). The aqueous callus extract exhibited a maximum characteristic band at 3412.73 cm^{-1} and lowest at 557.73 cm^{-1} indicating the presence of N-H stretching and C-Br stretching deformation respectively (Fig. 3). Similarly, Naveen and Neelakantan [17] reported the FTIR spectra functional groups with a peak value of alkanes at 2927.8 , amides at 1564.3 , aromatic at 1426.2 , aliphatic amines at 1093.6 and alkyl halide at 466.3 for *C. punctatum*.

Table 2. Effect of various concentrations of BAP and Kn on MS medium for callus initiation of *Centratherum punctatum* from nodal explants

Treatments	Concentrations of growth regulators (mg/L)		No.of days for callus induction	Callus induction percentage (%)	Callus weight (g)
	BAP	Kn			
T ₁	2.0	1.0	4	63.66 %	0.78
T ₂	2.5	1.5	4	70 %	0.81
T ₃	3.5	3	4	80 %	0.62
T ₄	4.0	3.5	3	60 %	0.71
T ₅	4.5	4	3	92.33 %	1.08
T ₆	3.0	1.5	4	87.54 %	0.82

Table 3. Phytochemical screening of aqueous, methanol and ethyl acetate *in vivo* plant and *in vitro* callus extract of *Centratherum punctatum*

S. no.	Phytochemicals test	Aqueous		Methanol		Ethyl acetate	
		Plant	Callus	Plant	Callus	Plant	Callus
1.	Alkaloids	+	+	+	+	+	+
2.	Flavonoids	+	+	+	+	+	+
3.	Phenols	+	+	+	+	+	+
4.	Tannins	+	+	+	+	-	-
5.	Steroids	+	+	-	-	-	-
6.	Terpenoids	-	-	-	-	+	+
7.	Quinines	+	+	-	-	-	-
8.	Proteins	+	+	-	-	+	+
9.	Amino acids	-	-	+	+	-	-
10.	Carbohydrates	+	+	+	+	+	+
11.	Saponins	-	-	-	-	-	-

(+ indicates positive ; - indicates negative)

3.5 Gas Chromatography – Mass Spectrometry Studies

In the present study, ethyl acetate extract of *in vivo* plant *C. punctatum* have revealed the presence of many compounds. Ethyl acetate extracts showed the presence of 1-Octadecyne, 3,7,11,15-Tetramethyl-2-Hexadecan-1-OL, 1,1'-bicyclopentyl, 2-hexadecyl, 1-Hexyl-2-Nitrocyclohexane, Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl) Methyl ester, CIS-, Dotriacontane, 2-octadecyl-propane-1,3- DIOL, 2,4,4-Trimethyl-3-Hydroxymethyl-5A-(3-Methylbut-2-Enyl)-cyclohexene, 3-O-Acetyl-6-Methoxycycloartenol, 2-isopropyl-5-Methylcyclohexyl 3-(1-(4-chlorophenyl)-3-Oxobutyl)-C, 17-pentatricontene (Fig. 4). The ethyl acetate extract of *in vitro* callus *C. punctatum* have revealed the presence of many compounds which were absent in the *in vivo* plant extract. *C. punctatum* ethyl acetate callus extracts showed the presence of 7-Dehydrocholesteryl isocaproate, 9,19-

cycloergost-24(28)-EN-3-OL, 4,14-Dimethyl-, acetate, (3.BETA., 4.ALPHA., 5.ALPHA.), 1,3,3-Trimethyl-2-hydroxymethyl-3,3-Dimethyl-4-(3-Methylbut-2-Enyl)-cyclohexene, 1,2-pentanediol, 5-(6-bromodecahydro-2-hydroxy-2,5,5A,8A-tetramethyl-1-Naphthalenyl)-3-Methylene Tetradecane, 1-chloro-, 2,6,6-Trimethyl-Bicyclo (3.1.1) Hept-3-ylamine, Cis -9,10-Epoxyoctadecan-1-OL, Dodecanal, Hexadecane, 1,16-Dichloro-, 3H-cyclodeca [B] furan-2-one, 4,9-Dihydroxy-6-Methyl-3,10-Dimethylene, 1-Eicosanol, Heptadecanoic acid, heptadecyl ester (Fig. 5). The GC-MS profile can be used as biochemical markers in the pharmaceutical industries. Few of the compounds in the present study has been shown to have anticancer and antioxidant activity in literature. Similarly, Sivasubramanian and Brindha [17] reported the presence of 30 different compounds along with anti-cancerous compounds such as engenol, saphulenol, viridifloral, hexadecanoic acid, phthalic acid, bis (7-methyloctyl) ester, Eicosane and squalene in the GC-MS analysis.

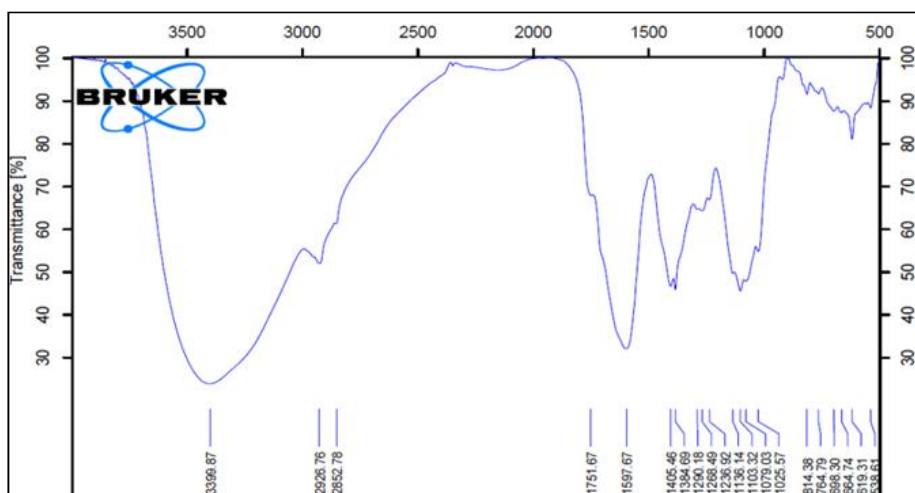


Fig. 2. FTIR spectrum of aqueous *in vivo* plant extract of *Centratherum punctatum*

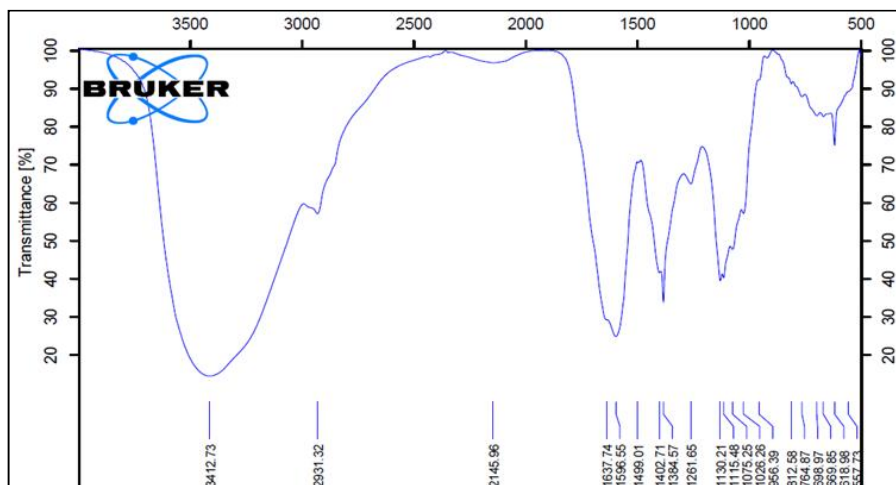


Fig. 3. FTIR spectrum of aqueous *in vitro* callus extract of *Centratherum punctatum*

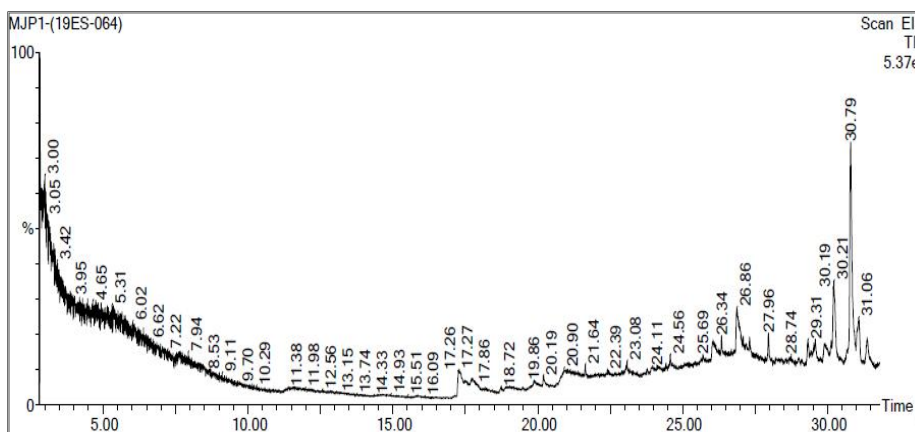


Fig. 4. Chromatogram of ethyl acetate extract of *in vivo* plant

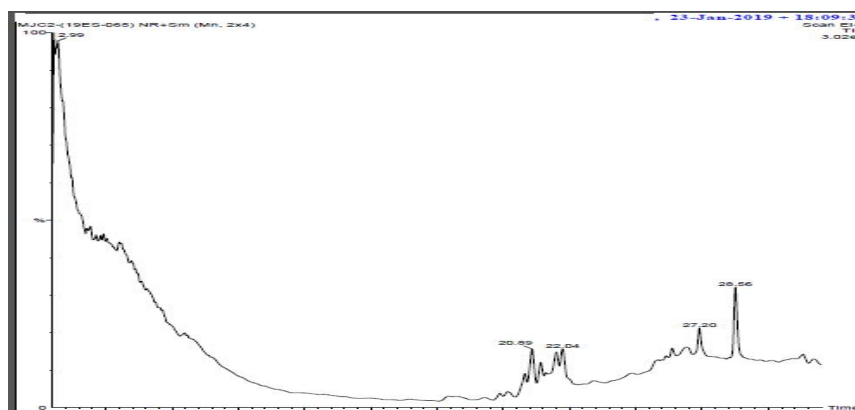


Fig. 5. Chromatogram of ethyl acetate extract of *in vitro* callus

3.6 Total Phenol Content

The total phenolic content for *C. punctatum* (1000 µg/ml) was calculated from the standard graph of tannic acid with the standard curve equation, $Y=17.68x - 0.017$, $R^2 = 0.973$. The total phenolic content in aqueous extract of *in vivo* plant and *in vitro* callus was 79.46 mgTAE/g dry weight and 69.4 mgTAE/g dry weight respectively. Methanol extract of plant was 75.79 mgTAE/g dry weight and in callus was 64.25 mgTAE/g dry weight. Total phenolic content in ethyl acetate extract of plant was 73.3 mgTAE/g dry weight and callus was 58.03 mgTAE/g dry weight (Fig. 7) currently, plant materials rich in phenolics are used in the food industry because they decrease the oxidative degradation of lipids and maintain the quality and nutritional value of food. We determined that the total phenolic content was slightly more in the aqueous extract compared to the methanol and ethyl acetate extract.

3.7 DPPH Free Radical Scavenging Assay

In the present study, the DPPH radical scavenging activity was found to be efficient in all the three extract such as aqueous, methanol and ethyl acetate extract of both *in vivo* plant and *in vitro* callus of *C. punctatum*. The lowest inhibition percentage indicates maximum scavenging activity. The aqueous extract of both plant and callus showed the lowest inhibition of 63.16% with IC_{50} value 3.412 µg/ml and 65.10% with IC_{50} value 3.257 µg/ml respectively. The methanol extract of both plant and callus showed the lowest inhibition of 60.01% with IC_{50} value 3.935 µg/ml and 60.46% with IC_{50} 3.3774 µg/ml respectively. The ethyl acetate extract of both plant and callus showed the highest inhibition of 61.90% with IC_{50} value 3.677 µg/ml and 63.68%

with IC_{50} value 3.463 µg/ml respectively. The *in vitro* callus extract shows maximum scavenging activity as it has lower inhibition percentage when compared to the *in vivo* plant extract (Fig. 9). Similarly, Sivasubramanian and Brindha [17] reported antioxidant activity of ethanol extract of aerial plant of *C. punctatum*. The extract also showed significant antioxidant activity comparable with that of standard ascorbic acid in the DPPH free radical scavenging assay.

3.8 Antibacterial Activity

The aqueous extract (1 mg/ml) of *C. punctatum in vivo* plant and *in vitro* callus was effective against all the three bacteria. The *in vivo* plant extract and *in vitro* callus extract had zone inhibition of 14 mm and 13 mm against *Bacillus subtilis* respectively. The *in vivo* plant extract had a zone of inhibition of 15 mm against *E. coli*. The *in vivo* plant extract and *in vitro* callus extract had a zone of inhibition 11 mm and 9 mm against *Staphylococcus aureus* respectively. The methanol extract (1 mg/ml) of *C. punctatum in vivo* plant extract and *in vitro* callus extract had a zone of inhibition of 15 mm and 14 mm against *Bacillus subtilis* respectively. The *in vivo* plant extract and *in vitro* callus extract had a zone of inhibition of 14 mm and 11 mm against *E. coli* respectively. The *in vivo* plant extract and *in vitro* callus extract had a zone of inhibition of 12 mm and 11 mm against *Staphylococcus aureus* respectively. The ethyl acetate extract (1 mg/ml) of *C. punctatum vivo* plant and *in vitro* callus extract had a zone of inhibition of 18 mm and 7 mm against *Bacillus subtilis* respectively. The *in vivo* plant and *in vitro* callus extract had zone of inhibition of 14 mm and 12 mm against *E. coli* respectively. The *in vivo* plant and *in vitro* callus extract had zone of inhibition of 12 mm and 11 mm against *Staphylococcus aureus* respectively (Fig. 10). Similarly, Shirin et al. [18] reported that

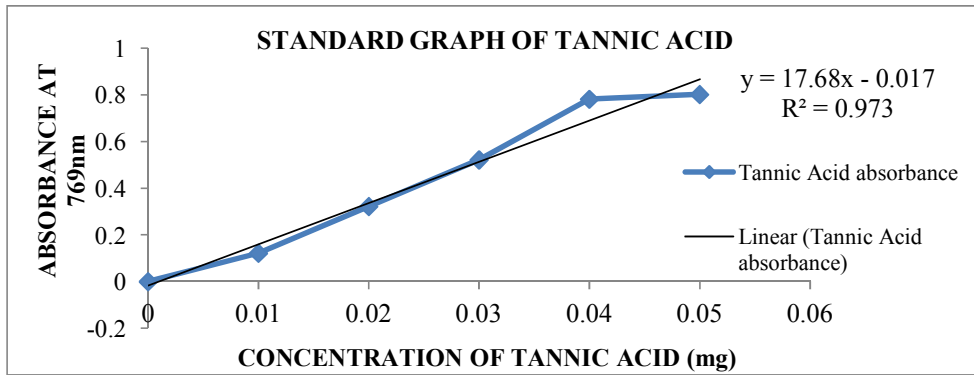


Fig. 6. Total phenolic content standard graph

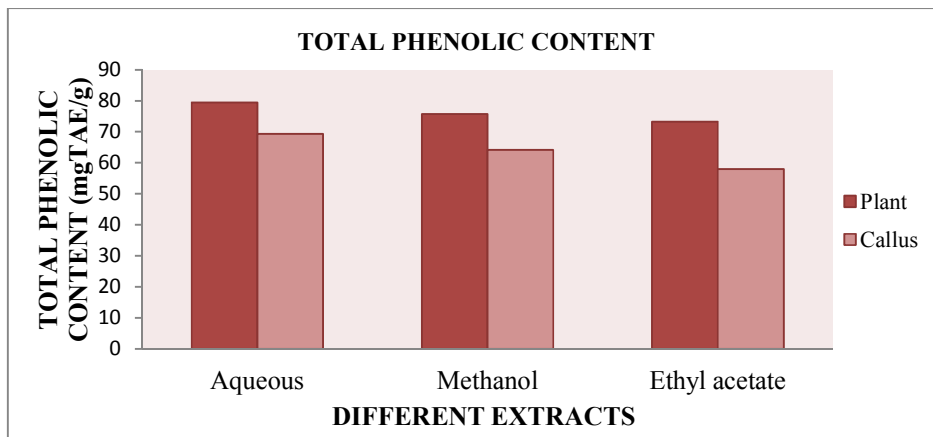


Fig. 7. Total phenolic content of *Centratherum punctatum*

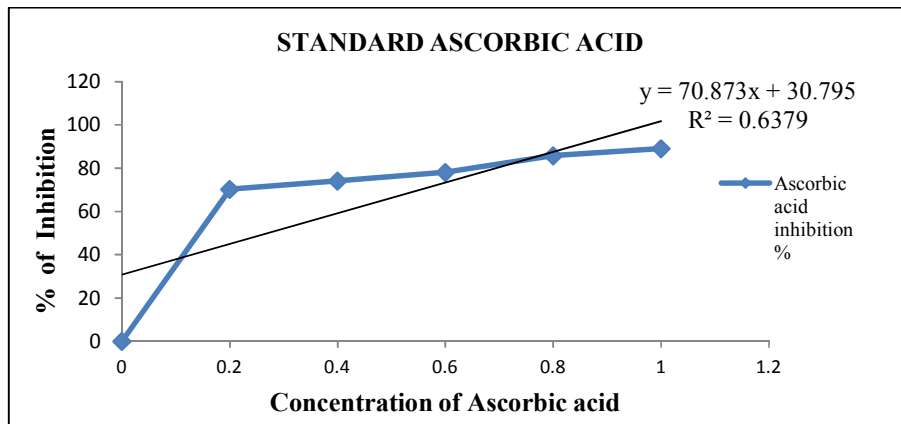


Fig. 8. DPPH free radical scavenging assay standard graph

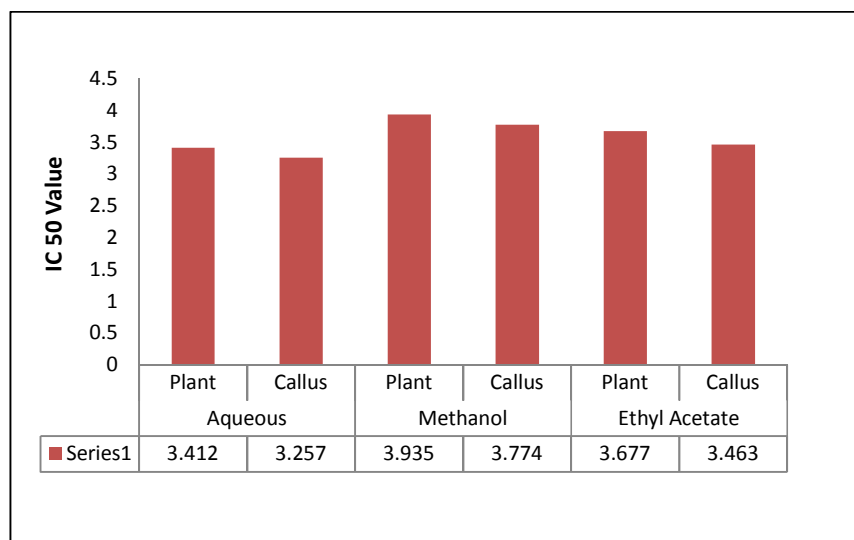


Fig. 9. DPPH free radical scavenging assay IC 50 value of *Centratherum punctatum* plant and callus of all the extracts

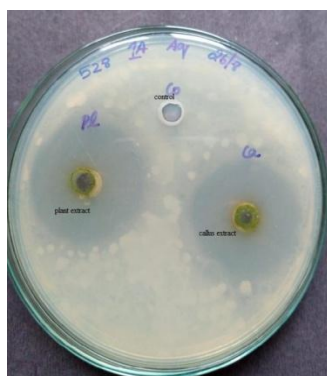
leaf extract of *C. punctatum* showed inhibitory activity against four out of five pathogenic bacteria including the multidrug resistant *Acinetobacter baumannii* and *Staphylococcus aureus*. Chitra and Brindha [19] reported antibacterial potential of *C. punctatum* against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli* and *proteus*. The maximum activity was observed in aqueous extract when compared with the ethanol extract.

3.9 Antiproliferative Analysis

Aqueous extracts of *in vivo* plant and callus of *C. punctatum* was studied for inhibitory property on

HeLa cell lines by MTT assay. This study revealed that *in vivo* plant has inhibitory percentage of 23.6% whereas callus has 28.5% as inhibitory percentage against HeLa cells (Fig. 11). Thus it shows *in vitro* callus has more potential to inhibit HeLa cells when compare to plant. Similarly, Sivasubramanian and Brindha [17] reported Cytotoxic effect of Ethanol extract of *Centratherum punctatum* against Ehrlich Ascites Carcinoma cell lines by incubating with different concentrations exhibited the presence of anticancer fractions such as Eugenol, Spathulenol, Viridiflorol, Hexadecanoic acid, Phthalic acid, bis(7-methyloctyl) ester, Eicosane and Squalene.

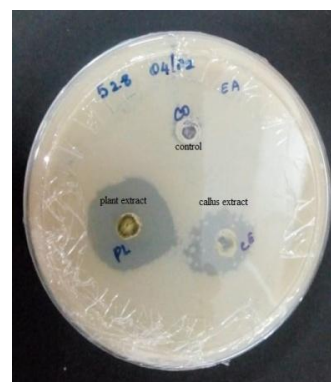
1. *Bacillus subtilis*



Aqueous

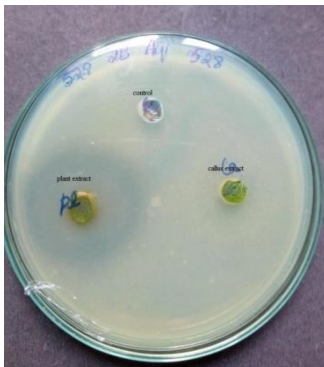


Methanol

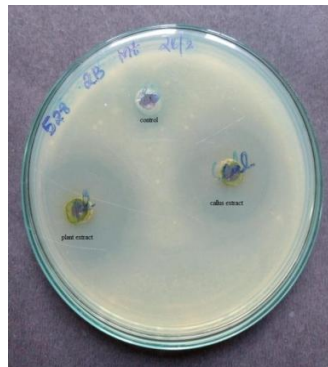


Ethyl acetate

2. E. coli



Aqueous

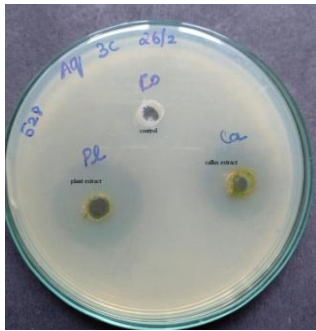


Methanol

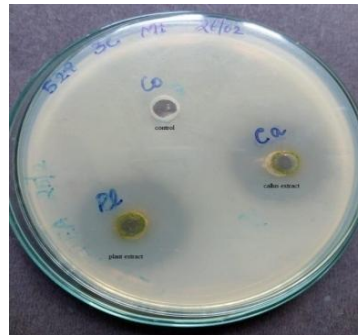


Ethyl acetate

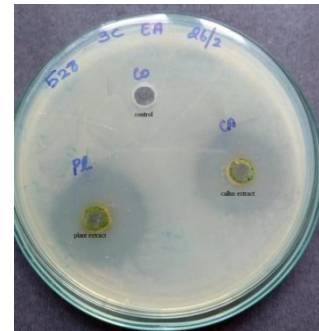
3. Staphylococcus aureus



Aqueous



Methanol



Ethyl acetate

Fig. 10. Antibacterial activity of *in vivo* plant and *in vitro* callus extract of aqueous, methanol and ethyl acetate of *Centratherum punctatum* against

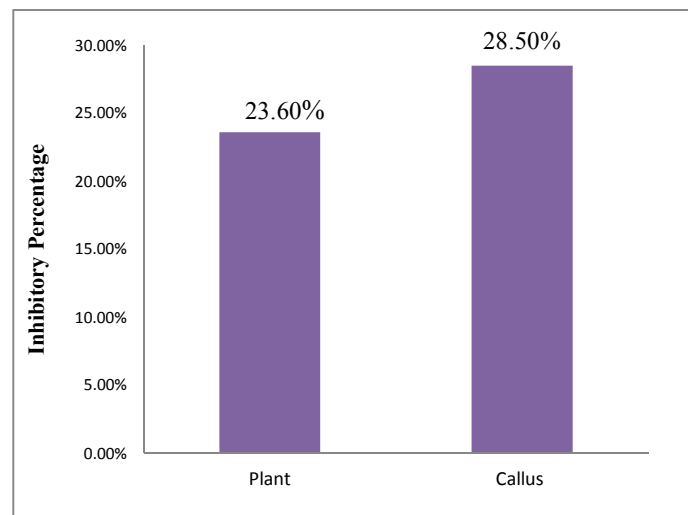


Fig. 11. Antiproliferative analysis of *Centratherum punctatum* plant and callus against HeLa cells

4. CONCLUSION

Centratherum punctatum has high medicinal properties and has become endangered and is at extinction risk. The regenerated plants can be useful for constant supply of raw materials for secondary metabolite extraction. Maximum content of total ash was found. The preliminary phytochemical screening of aqueous, methanol and ethyl acetate extract of both *in vivo* plant and *in vitro* callus of *C. punctatum* revealed the presence of alkaloids, flavonoids, phenols and carbohydrates. FT-IR analysis revealed the presence of some functional constituents in the extract. GC-MS analysis of ethyl acetate extract of both plant and callus revealed the presence of 11 and 15 compounds respectively. Both *in vivo* plant and *in vitro* grown callus of aqueous, methanol and ethyl acetate extracts possess potent antioxidant activity. *C. punctatum* of aqueous, methanol and ethyl acetate extract of both *in vivo* plant and *in vitro* callus was more active against the test organism. Anticancer analysis showed a considerable amount of inhibitory percentage against HeLa cells. Thus, the present study revealed the presence of various bioactive compounds indicating that this plant and callus extract compounds can be used as therapeutic drugs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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