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Research Article

STUDIES ON THE PHYTOCHEMISTRY AND VALIDATION FOR ANTICANCER PROPERTIES OF *ECLIPTA PROSTRATE. Linn*

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ABSTRACT

Eclipta prostrate was sequentially extracted with hexane, chloroform and ethyl acetate three solvents separate the compounds on the basis of their polarity. The extracts were analyzed to delineate their chemical structure and properties using High performance Thin Layer Chromotography (HPTLC), Gas chromatography-Mass spectrometry (GC-MS) and Liquid Chromotography Mass Spectrometry (LC-MS) techniques. The separation of the various compounds in the crude extracts and phytochemical studies by elucidating their structures. HPTLC has revealed the presence of about 16 compounds in the hexane extract. Through GC-MS about 28 compounds have been characterized in the chloroform extract and about 32 compounds have been characterized in the Ethyl Acetate extract. Structural elucidation has been done on 11 compounds present in the Ethyl acetate extract with the help of LC-MS. The identified compounds were validated the anticancer property of the plant was tested with EAC tumor cell line using MTT assay for the inhibition of cell proliferation. Testing with all the three extracts indicate that the anticancer property is present in all the three extracts.

INTRODUCTION

Eclipta is thought to be native to Asia, but is widely naturalized around the world[1,2]. Eclipta germination is greater in warm, moist soils and as a result, it typically occurs in poorly drained fields, irrigated areas, or during periods of heavy rainfall[3]. The plant has several phytoconstituents and used in traditional medicinal research. *E. prostrate* is used in both sidha medicine as well as tribal practitioners as it has various medicinal properties. *E. prostrata* is used as a purgative, externally for skin diseases[4]. It is used for animal feed[5]. The ethnomedicinal herb is used for treatment of antiosteoporosis[6], anti inflammatory[7] antihyper lipemia [8, 9] gastrointestinal disorders, respiratory tract disorders, fever, hair loss and graying of hair [10] liver disorders [11], skin disorders [12], spleen enlargement [13]. *E. prostrate* shows excellent antibacterial properties [14]. Rahman[15] observed that the methanol extract of the whole plant of *E.prostrata* and one of its isolated compounds reveals potent antidiabetic activity.

There are various instruments involved in quantifiyin and qualitating the sample extract. High performance Thin Layer Chromotography (HPTLC) has used for evaluation of such botanical materials of therapeutic value. HPTLC quantify the phytoconstituents, expected to be present in a medicinal plant *E. prostrata* proved to be effective in reducing CSH of dental plaque bacteria which would in turn affect their ability to adhere to tooth enamel[16]. HPTLC is used to ensure the quality as well safety of the herbal drugs present in a plant [17]. The phytochemical analysis of the *E.prostrate* has been analyzed through Gas chromatography-Mass spectrometry (GC-MS) and its medicinal properties are also studied[18]. The ethyl acetate extract of *E. prostrata* possesses a potential antioxidant capacity, which is used to prevent oxidative stress-induced neurodegeneration in Parkinson's disease[19]. Liquid Chromotography Mass Spectrometry (LC-MS) can be used as the qualitative and quantitative methods could also be reliable tools for quality control analyses [20] MTT assay in particularly its simplicity and effectiveness, to assess the anti-inflammatory and anti-cancer activities at preliminary levels [21]. Therefore, this present study has taken for the detailed structural details of the biological compounds of the plant *E.prostrate* by HPTLC, GC-MS and LC-MS and validation of the anticancer property of the extracts prepared with solvents of different polarity.

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MATERIALS AND METHODS

Plant collection

E.prostrate was collected from the herbal garden of AMET deemed to be University, Kanathur, Chennai, Tamil Nadu, India. The collected leaves were brought to the department laboratory, washed, shade dried and ground to a fine powder of 100g.

Extraction

The extraction was done by Soxhlet apparatus with 25gm of powder. The Soxhlet apparatus was filled with 50 ml of hexane and the extraction was carried out at 400 c for about 6 hrs. After 5 hours extract was collected and condensed to a solvent free extract. The plant residue after the extraction with hexane was again extracted with chloroform, a moderately polar solvent as explained for hexane extraction. After 6 hours of extraction the extract was collected and condensed to a solvent free extract. The same was repeated with a high polar solvent namely ethyl acetate.

HPTLC

Standardization of qubercitin in E. prostrata by HPTLC

Preparation of standard solution: About 1 mg of qubercitin standard was taken in a eppendorf tube (1mL). 1 ml of methanol was added to it and mixed by vortexing mixture to dissolve the sample completely.

Preparation of sample solution

About 101.9mg of the extract was dissolved in 5ml of hexane, 103.2 mg of extract was dissolved in 10ml of chloroform and 103.2mg of extract was dissolved in 5ml of ethyl acetate of *E. prostrata* was taken in three separate 1mL of eppendorf tubes.

Chromotographic conditions

HPTLC analysis was performed using isocratic technique. Mobile phase was optimized with toluene and methanol in a ratio of 9:1 v/v. The temperature was maintained at 25°C and mobile phase was developed in a twin trough glass chamber. The standard stock solution was applied consequently in the range of 2-10 µL with 2 µL gradual increments. Hexane, chloroform and ethyl acetate extract of *E. prostrata* was applied at the concentration 101.9mg/mL, 103.2 mg and 103.2mg mg/ml respectively. The tracks on HPTLC plate were used for standardization, including standard and sample solution, respectively in a band form.

Applied 2µl of standard &10µl of test solutions was spotted on a precoated silica gel 60 F254 HPTLC plate (E.Merck) of uniform thickness 0.2mm using Linomat5 sample applicator. Developed the plate in the solvent system to a distance of 8cm. Scanned the plate densitometrically at 254nm using TLC Scanner 3. Observed the plate under UV light at 254nm using Camag Reprostar3.

After development, plates were air dried. The dry plate was treated with sulfuric acid-anisaldehyde spraying reagent. The plate was kept at 110°C for 5 min in hot air oven and evaluation was carried out in 366 and 540 nm. Colored bands were observed at 540 nm. The spot capacity can be increased by developing the plate with two different solvents, using two-dimensional chromatography. The procedure begins with development of sample loaded a plate with solvent first. After removing it, the plate is rotated 90° and developed other solvent.

LC-MS

LC-MS is a powerful technique that has very high sensitivity and selectivity and so is useful in many applications. Its application is oriented towards the separation, general detection and potential identification of chemicals of particular masses in the presence of other chemicals (i.e., in complex mixtures), e.g., natural products from natural-product extracts, and pure substances from mixtures of chemical intermediates. Preparative LC-MS systems can be used for rapid mass-directed purification of specific substances from such mixtures. The results showed a good linear correlation, precision, accuracy, and repeatability that could be used for contents determination

GC-MS

The methanol extract of *E. prostrata* was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i autosampler and a GC-MS equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in the electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adapted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Anticancer activity

The cytotoxic effect of *E. prostrate* was done by MTT assay, though the phytoconstitutuents of the isolated compounds by GC-MS possess anticancer properties. Growth of EAC cells in the presence of different extracts of Ethyl acetate was determined by MTT (3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) assay. This assay was performed as described in the modified method of Mosmann (1983). Cells were seeded in 96 well microctitre plate at 2 × 103 cells per well and were exposed to 0, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, 1.953125 and 0.9765625 µg/ml of each extract for 48 h. MTT was prepared at a concentration of 5 mg/ml in sterile phosphate buffered saline system (PBS). A 20 µl aliquot of the stock solution of MTT was added to each well. After 3h of incubation at 37oC, 150 µL of DMSO was added to each well in order to dissolve the formazan crystals. Optical absorbency was measured at 570nm using a 96 well Spectramax

Microplate Reader (Biotek plate reader, USA). The results obtained were calculated and presented as a percentage of control values.

RESULTS AND DISCUSION

HPTLC

HPTLC has been carried out for all the extracts such as hexane, chloroform and ethyl acetate and results on given in Fig no 1. A 3D graphic display of the HPTLC data is presented in Fig 2. The peak heights are given in table-1. This spectrum of HPTLC for the variability extracts such as hexane, chloroform and ethyl acetate are compared with that of Quercetin (standard) in Fig no (3,3a.3b & 3c). The details of the standard and other extracts given in table: 1. The resolution front of the standard is 0.53 to 0.61. The maximum peak height was 334.9, in the HPTLC and further indicates that 3 distinct compounds present in large quantities. The chloroform extract contains 7 compounds and the peak detail is given in Fig: 3b. The spectrum of ethyl acetate extract shows 5 distinct compounds, whereas the chloroform and ethyl acetate shows one compound with considerably higher concentration.

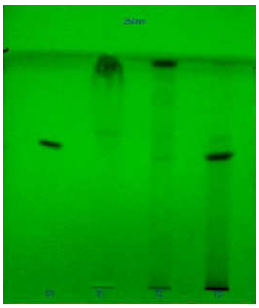


Fig 1 HPTLC profile of *E.prostrata* photo documentation under UV at 254nm

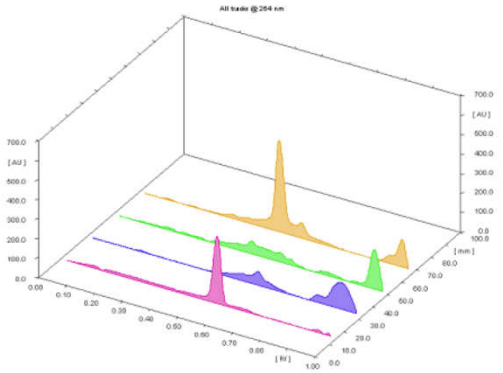


Fig 2 The 3D properties of the extract obtained by HPTLC for the hexane, chloroform and ethyl acetate extracts at 254nm.

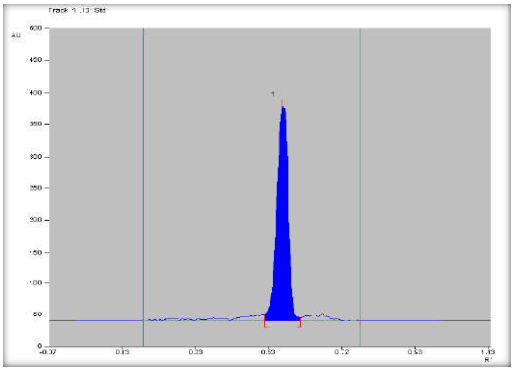


Fig 3 Standard sample of quercitin

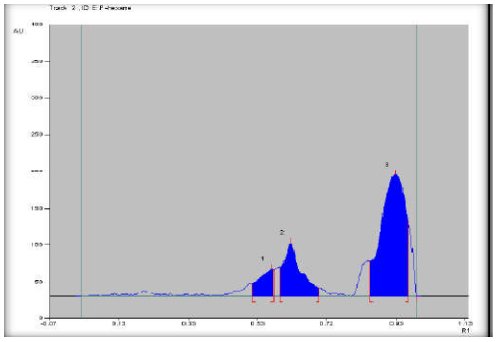


Fig 3a Hexane extract of *E.prostrata*

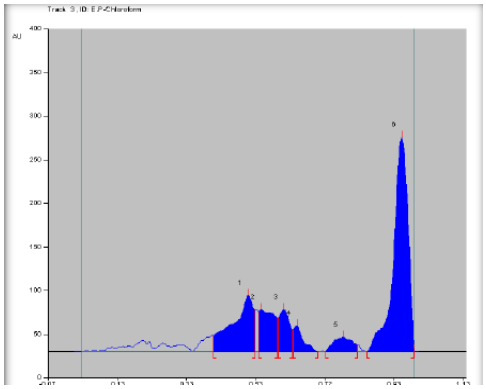


Fig 3b Chloroform extract of *E.prostrata*

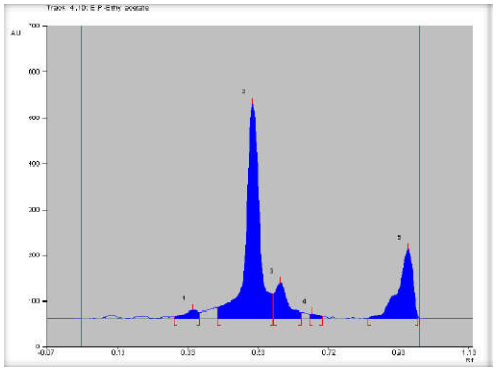


Fig 3c Ethyl acetate extract of *E.prostrata*

Fig 3 List of compounds analyzed by HPTLC spectrum of variable extracts

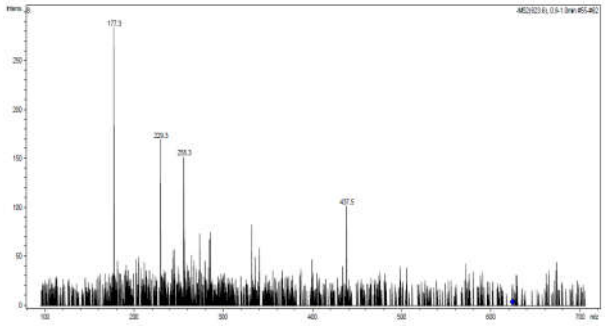
Table 1 Analytical results of extract (Hexane, chloroform, ethyl acetate) with a standard of Quercetin

Peak	Start Rf	Start height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.52	5.1	0.57	334.9	100.00	0.61	0.4	7269.3	100.00
					Sample-1 Hexane				
1	0.51	17.9	0.57	37.4	12.73	0.57	36.3	1204.4	9.90
2	0.59	40.7	0.62	72.4	24.61	0.70	11.4	2845.4	23.39
3	0.80	0.1	0.84	41.3	14.3	0.85	39.3	936.9	7.70
4	0.85	39.3	0.92	143.0	48.63	0.96	74.5	7178.6	59.01
					Sample-2 Chloroform				
1	0.40	18.3	0.50	64.2	14.85	0.52	47.9	2921.6	22.68
2	0.53	46.0	0.54	48.4	11.19	0.59	38.4	1628.2	12.64
3	0.59	38.6	0.61	48.0	11.09	0.63	25.0	1105.8	8.58
4	0.63	25.1	0.65	29.4	6.80	0.70	0.1	638.9	4.96
5	0.73	0.3	0.78	16.5	3.82	0.82	8.1	684.5	5.31
6	0.85	0.1	0.89	13.4	3.09	0.89	12.5	245.1	1.90
7	0.89	12.6	0.95	212.6	49.16	0.97	43.9	5658.3	43.92
					Sample-3 Ethyl acetate				
1	0.29	4.4	0.34	20.5	2.82	0.36	16.0	650.5	3.02

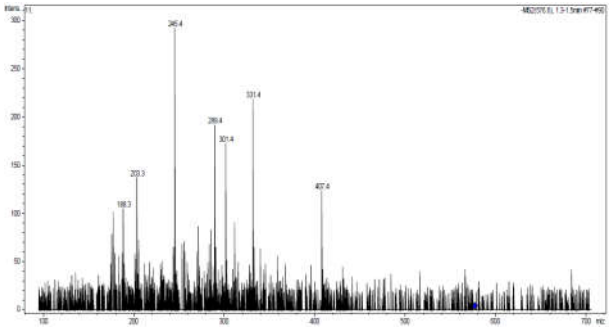
2	0.41	26.3	0.51	471.5	64.77	0.57	55.4	14155.0	65.65
3	0.57	55.8	0.59	79.2	10.87	0.65	16.2	2270.8	10.53
4	0.67	12.6	0.68	13.1	1.80	0.71	5.9	255.4	1.18
5	0.87	5.2	0.95	143.6	19.73	0.98	4.9	4230.1	19.62

LC-MS

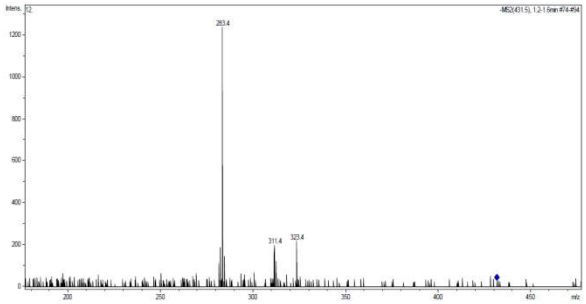
The following structural compounds have been identified in the LC-MS analysis is given in Fig 4. The identified compounds are Isorhamnetin-3- Galactoiside-6" Rhamnoside, Procyanidin, Vitexin, 3-Caffeoylquinic acid, Delphinidin-3-O-beta-glucopyranoside, Kaempferol-3-Glucuronide, 1,5-diCaffeoylquinic acid, Eriodictyol-7-O-glucoside, 3,7-Dihydroxy-3',4'-dimethoxyflavone, Quercetin-3,4'-O-di-beta-glucopyranoside , Baicalin.



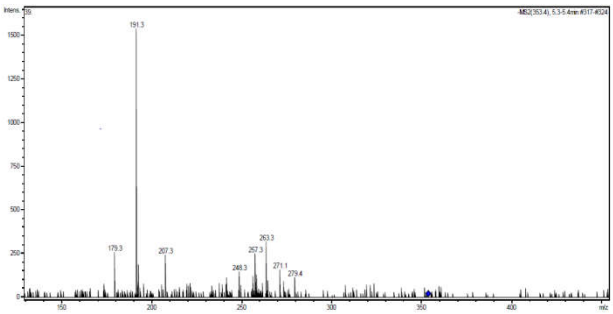
Isorhamnetin-3- Galactoiside-6" Rhamnoside



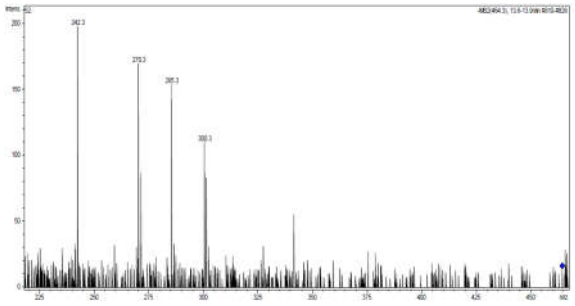
Procyanidin



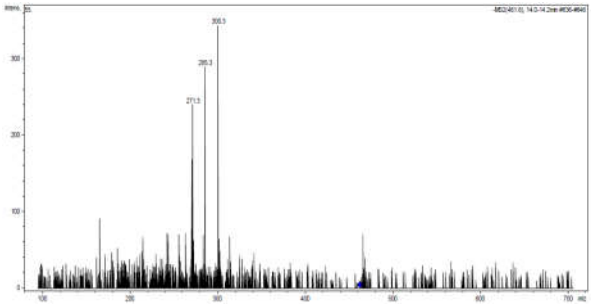
Vitexin



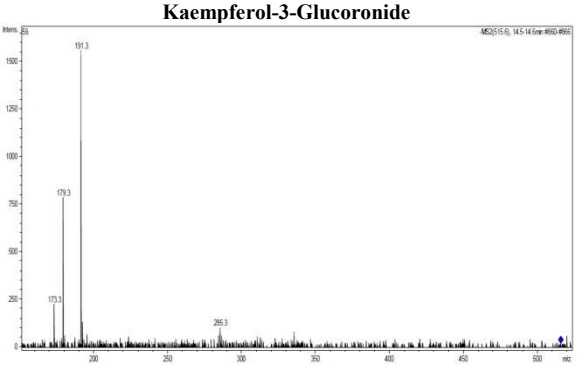
3-Caffeoylquinic acid



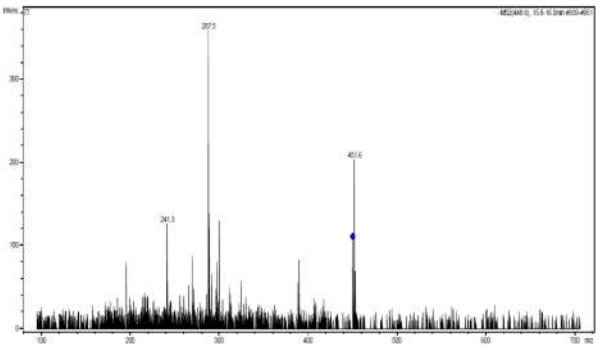
Delphinidin-3-0 - beta- glucopyranoside



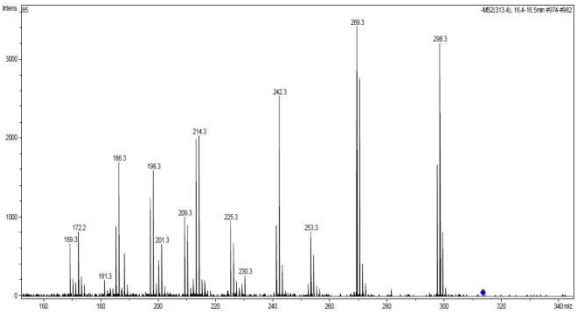
Kaempferol-3-Glucoronide



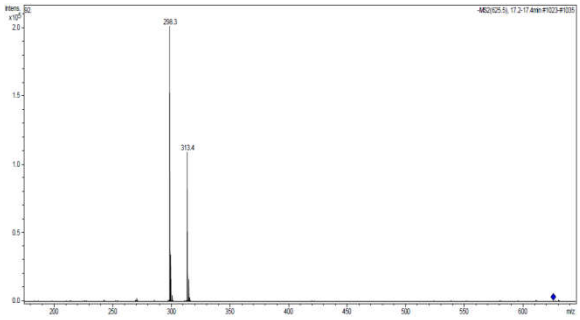
1-5-dicaffeoylquinic acid



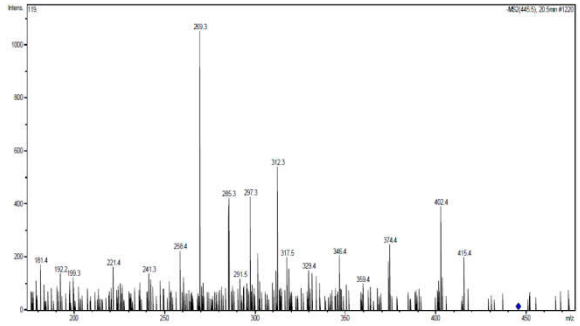
Eriodictyol-7-0- glucoside



3-7- dihydroxy3'4-dimethoxyflavone



Quercetin-3,4'-O-di-beta-glucopyranoside



Baicalin

Fig 4 Spectrum details of compounds obtained by LC-MS extracted with Ethyl acetate

GC-MS

GC-MS studies indicate that about 27 compounds are present in the extract. The molecular formula and the molecular weight of each compound are given in table 3. The spectrum of GC-MS of the chloroform extract of *E. prostrate* is presented in Fig:5. The predominant compounds were found to be 3,7,11,15-Tetramethyl-2- (table 2), some of the compounds present in large quantities are 2-Hexanol, (R)-, 3,7,11,15-Tetramethyl-2-, n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- and Stearic acid (table 2).

GC-MS studies indicate that about 32 compounds are present in the ethyl acetate extract. The molecular formula and the molecular weight of each compound are given in table 2. The spectrum of GC-MS of Ethyl acetate extract of *E. prostrate* is presented in Fig: 6. The predominant is found to be n-Hexadecanoic acid (table 3), some of the compounds present in large quantities are n-Hexadecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-, 1-Octadecene, n-Propyl acetate.

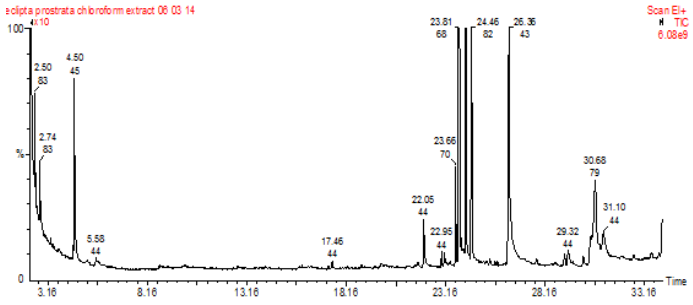


Fig 5 GC-MS chromatogram analysis of chloroform of *E.prostrata*

Table 2 Compounds identified in the chloroform extract of *E. prostrate* in the

S.No	Name of the compound	Formula	MW	Retention Time	Peak area
1.	3-Penten-2-ol	$C_5H_{10}O$	86	2.74	0.96
2.	2-Hexanol, (R)-	$C_6H_{14}O$	102	4.50	4.95
3.	Octan-2-one, 3,6-dimethyl-	$C_{10}H_{20}O$	156	5.12	0.15
4.	Bicyclo[2.1.1]hex-2-ene, 2-ethenyl-	C_8H_{10}	106	5.58	0.7666
5.	Decane, 3,6-dimethyl-	$C_{12}H_{26}$	170	17.46	0.2468
6.	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	18.93	0.1386
7.	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180	19.61	0.1005
8.	Dodecanoic acid	$C_{12}H_{24}O_2$	200	19.95	0.3213
9.	Undecane, 3,6-dimethyl-	$C_{13}H_{28}$	184	21.62	0.0899
10.	(Z)6-Pentadecen-1-ol	$C_{15}H_{30}O$	226	21.72	0.1531
11.	cis-9-Hexadecenal	$C_{16}H_{30}O$	238	21.79	0.1526
12.	1-Nonadecanol	$C_{19}H_{40}O$	284	22.05	2.073
13.	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228	22.95	0.2903
14.	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	23.10	0.5010
15.	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280	23.66	1.816
16.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	23.81	48.944
17	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	23.95	0.0374
18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	24.46	16.577
19	Pentadecanal	$C_{15}H_{30}O$	226	25.38	0.1812
20	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	26.36	7.483
21	Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-4a,7- dimethyl-1-methylene-, [4aS-(4aà,4aá,7á,10aá)]-	$C_{19}H_{28}$	256	27.74	0.2355
22	1,2-Octadecanediol	$C_{18}H_{38}O_2$	286	29.13	0.4290
23	Phytol	$C_{20}H_{40}O$	296	29.32	0.9546
24	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264	30.48	0.4719
25	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	30.68	4.232
26	Stearic acid	$C_{18}H_{36}O_2$	284	31.10	5.176
27	Palmitoyl chloride	$C_{16}H_{31}ClO$	274	34.07	2.557

GC-MS analysis (Sample injected: 1.4µL)

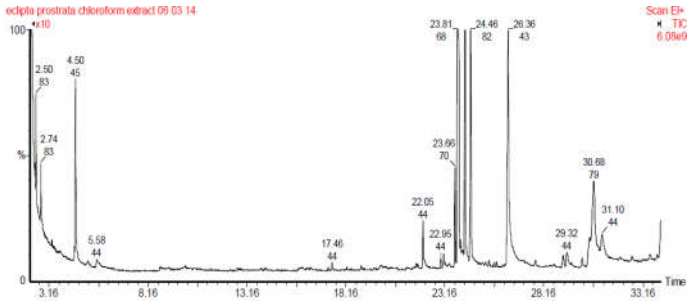


Fig 6 GC-MS chromatogram analysis of Ethyl acetate of *E.prostrata*

Table 3 Compounds identified in the ethyl acetate extract of *E. prostrate* in the GC-MS analysis (Sample injected: 1.4µL)

S.No	Name of the compounds	Formula	MW	Retention Time	Peak Area	% Peak area
1	n-Propyl acetate	$C_5H_{10}O_2$	102	2.87	26509642	18.5328
2	Cyclopropane, (2-methylenebutyl)-	C_8H_{14}	110	7.41	103127	0.0721
3	Glycerin	$C_3H_8O_3$	92	8.81	1224024	0.8557
4	1-Butanol, 2-methyl-, acetate	$C_7H_{14}O_2$	130	10.72	83672	0.0585
5	Cyclohexanone, 2-acetyl-	$C_8H_{12}O_2$	140	12.41	107174	0.0749
6	1H-Pyrrole-2,5-dione, 3-ethenyl-4-methyl-	$C_7H_7NO_2$	137	12.78	888476	0.6211
7	3-Hexadecene, (Z)-	$C_{16}H_{32}$	224	14.71	584648	0.4087

8	5-Methyl-5-octen-1-ol	$^{C}9^{H}18^{O}$	142	14.99	259642	0.1815
9	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	$^{C}13^{H}18^{O}2$	206	15.06	673397	0.4708
10	10-(1-Methylallyl)tricyclo[6.3.1.0(2,7)]dodeca-2(7),3,5-trien-10-ol	$^{C}16^{H}20^{O}$	228	15.54	137172	0.0959
11	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	$^{C}13^{H}18^{O}$	190	16.45	360112	0.2518
12	D-Glucopyranose, 4,6-O-hexylidene-	$^{C}12^{H}22^{O}6$	262	17.06	805716	0.5633
13	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$^{C}11^{H}16^{O}2$	180	17.70	700901	0.4900
14	E-15-Heptadecenal	$^{C}17^{H}32^{O}$	252	17.88	4850170	3.3907
15	Eicosane	$^{C}20^{H}42$	282	17.97	151926	0.1062
16	3,5-Heptanedione, 2,2,6,6-tetramethyl-	$^{C}11^{H}20^{O}2$	184	18.44	431641	0.3018
17	(Z)6-Pentadecen-1-ol	$^{C}15^{H}30^{O}$	226	19.53	390729	0.2732
18	Hexadecanal	$^{C}16^{H}32^{O}$	240	19.81	2627077	1.8366
19	1,7-Dodecadiene	$^{C}12^{H}22$	166	20.39	748824	0.5235
20	1-Octadecene	$^{C}18^{H}36$	252	20.73	13243587	9.2585
21	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$^{C}20^{H}40$	280	21.21	268824	0.1879
22	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$^{C}20^{H}40^{O}$	296	21.33	24200322	16.9184
23	2-Pentadecanone,	$^{C}18^{H}36^{O}$	268	21.48	3282448	2.2947

Anticancer activity

The lowest polarity solvent used is the Hexane. The concentrations used are from 1µg/ml to 1000µg/ml phytochemical of *E. prostrata*. The anticancer activity of the active principle was tested with the growth of EAC (Ehrlich's ascites carcinoma (EAC-) tumor cell lines employing MTT assay. The MTT assay is a colorimetric assay for assessing cell viability. NADH-dependent cellular oxidoreductase the Hexane extract (30µg/ml and 65% inhibition). The IC50 concentration of the chloroform extract is 3972.2 µg/ml dose which is in no way better than the IC50 concentration of the Hexane extract (402.6). This inhibitory effect could be attributed to only a consortium of phytochemicals present in the plant that could be extracted with chloroform. These compounds are moderately polar in nature and this anticancer property of this extract could be attributed to a consortium of active principles listed in table-2.

The Hexane a low polar solvent and then chloroform a moderate polar solvent the residue was used to extract with Ethyl acetate a high polar solvent. Thus, this extract will contain only high polar chemical present in the plant sample. The Ethyl acetate extract of *E.prostrate* was studied with the help of EAC tumor cell line using MTT assay. The results are shown in Fig no: 7 An observable inhibition of the of the tumor cell proliferation was recorded in ~15µg/ml chloroform extract and a maximum of >75% inhibition was recorded in 1000µg/ml dose. These results are more or less similar to that of the chloroform extract. The correlation coefficient between the dose of Ethyl acetate extract and the %inhibition was found to be R2=0.986 which indicates that the relationship between the dose and the inhibition is almost perfectly positive. A comparison of the commencement of the inhibition and the maximum inhibition ~15µg/ml and >75% inhibition was recorded in 1000µg/ml dose is higher than that of the Hexane extract (30µg/ml and 65% inhibition) but similar to that of the chloroform extract. The IC50 concentration of the chloroform extract is ~206 µg/ml dose which is much lower than that of the Hexane (403 µg/ml and that of the Chloroform (397 µg/ml) which indicates that the active principles present in the Ethyl acetate extract, which are of high polar nature are more effective as anticancer activity. This inhibitory effect could be attributed to only a consortium of phytochemicals present in the plant that could be extracted with Ethyl acetate. These compounds are highly polar in nature and this anticancer property of this extract could be attributed to a consortium compound given in table 3.

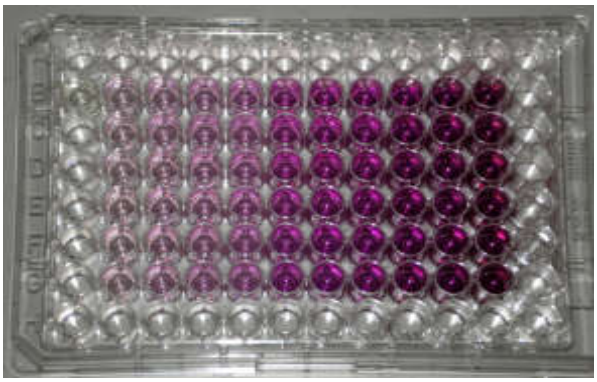


Fig 7 MTT assay plate

3972 = IC50 = 402.6 µg/mL

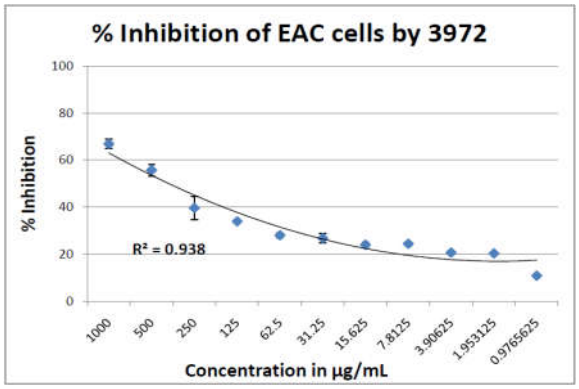


Fig 7 Hexane extract

3973=IC50=397.2µg/mL

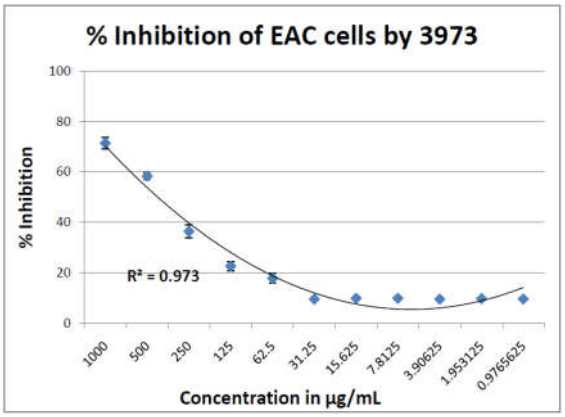


Fig 7a Chloroform extract

3974 = IC50 = 205.8 µg/mL

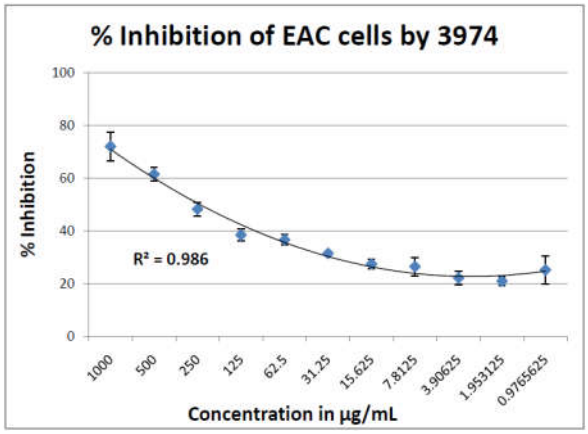


Fig 7b Ethyl acetate extract

Fig 7 Construction effect of *E. prosrate* (Hexane, Chloroform and Ethyl acetate) on the proliferation of EAC tumour cells analyzed with MTT assay

CONCLUSION

E.prostrate was extracted with hexane, chloroform and ethyl acetate. The chloroform and ethyl acetate extracts were shown 87 compounds through GC-MS and LC-MS. Among these 87 compounds, four compounds possess anticancer properties. Through GC-MS, about 28 compounds have been characterized in the chloroform extract and about 32 from the ethyl acetate extract. The anticancer activity was tested with EAC tumor cell line using MTT assay for the inhibition of cell proliferation. The IC50 information (402, 397 and 205µl/ml respectively in hexane, chloroform and ethyl acetate respectively) indicates the high polar compounds extracted with ethyl acetate has higher anticancer activity than the compounds extracted with the other two solvents. Hence, the ethnomedicinal herb *E.prostrate* which has promising value on high medicinal values can be further taken for anticancer and pharmacological research.

Conflict of interest:
There is no conflict of interest.

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References

1. Holm, L. G., J. V. Poncho, J. P. Herberger, and D. L. Plunknett. 1979. *A Geographical Atlas of World Weeds*. New York: John Wiley and Sons.
2. USDA National Resources Conservation Service. 2014. "The Plants Database." Greensboro, NC: National Plant Data Team. http://plants.usda.gov.
3. Prostko, E. P. 2012. "Eclipta Identification and Control in Peanut." Circular 869. Athens, GA: University of Georgia Coop. Extn.
4. Neal MC, 1965. In: Gardens of Hawaii. Bernice Pauahi Bishop Museum Special Publication No. 50. Honolulu, Hawaii, USA: Bishop Museum.
5. Moody K; Elliot PC, 1984. The use of rice-field weeds for animal feed. In: Report of the Crop-livestock Systems Research, Monitoring Tour, Philippines and Thailand. Los Ba±os, Laguna, Philippines: International Rice Research Institute, 52-64.
6. LeeM.K, N. R. Ha, H. Yang, S. H. Sung, and Y. C. Kim, "Stimulatory constituents of Eclipta prostrata on mouse osteoblast differentiation," Phytotherapy Research, vol. 23, no. 1, pp. 129-131, 2009.
7. Tewtrakul, S. Subhadhirasakul, P. Tansakul, S. Cheenpracha, and C. Karalai, Antiinflammatory constituents from E.prostrata using RAW264.7 macrophage cells, Phytotherapy Research, vol. 25, no. 9, pp. 1313-1316, 2011.
8. Kim D I, S.-H. Lee, J.-H. Choi, H. S. Lillehoj, M.-H. Yu, and G.-S. Lee, "The butanol fraction of Eclipta prostrata (Linn) effectively reduces serum lipid levels and improves antioxidant activities in CD rats," Nutrition Research, vol. 28, no. 8, pp. 550-554, 2008.
9. Kumari C S, S. Govindasamy, and E. Sukumar, "Lipid lowering activity of Eclipta prostrata in experimental hyperlipidemia," Journal of Ethnopharmacology, vol. 105, no. 3, pp. 332–335, 2006.
10. Roy R K, M. Thakur, and V. K. Dixit, "Hair growth promoting activity of *Eclipta alba* in male albino rats," *Archives of Dermatological Research*, vol. 300, no. 7, pp. 357–364, 2008
11. Wagner H, B. Geyer, Y. Kiso, H. Hikino, and G. S. Rao, "Coumestans as the main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulacea*," *Planta Medica*, vol. 5, pp. 370-374, 1986.
12. Chan C F, W.-Y. Huang, H.-Y. Guo, and B. R. Wang, "Potent antioxidative and UVB protective effect of water extract of *Eclipta prostrata* L," *The Scientific World Journal*, vol. 2014,
13. Rownak Jahan, Abdullah Al-Nahain, Snehali Majumder, and Mohammed Rahmatullah., Ethnopharmacological Significance of *Eclipta alba* (L.) Hassk. (Asteraceae)., International Scholarly Research Notices Volume 2014, Article ID 385969, 22 pages http://dx.doi.org/10.1155/2014/385969.
14. Khan AV and Khan AA, Ehnomedicianl uses of E.prostrata. Linn., Indian Journal of traditional Knowledge. Vol 7(2), April, 2008,pp- 316- 320.
15. Nithya R. John, Viraj C. Gala, Ashok M. Bhagwat And Chhaya S. Sawant., HPTLC analysis of eclipta prostrata and psidium guajava extracts and their effect on cell-surface hydrophobicity of a consortium of dental plaque isolates. *Int J Pharm Pharm Sci*, Vol 5, Suppl 3, 935-940.
16. Rahman M.S, M. Z. Rahman, B. Begum, R. Chowdhury, Sk. Nazrul Islam & Mohammad A. Rashid. Antidiabetic principle from E.prostrata. Lat. *Am. J. Pharm*. 30 (8): 1656-60 (2011)
17. Bhattacharjee A, Chakraborty A, Dutta D, Mukhopadhyay G (2017) Standardization of Eclipta Alba by HPTLC, HPLC and AAS. Pharm Anal Acta 8: 541. doi:10.4172/2153-2435.1000541.
18. Wyson WJ, Deventhiran M, Saravanan P, Anand D and Rajarajan S : Phytochemical Analysis of Leaf Extract of *Eclipta Prostrata* (L.) by GC- MS Method. Int J Pharm Sci Res 2016; 7(1): 272-78.doi: 10.13040/IJPSR.0975-8232.7(1).272-78.
19. Yamaratee Jaisin, Piyanee Ratanachamnong, Supaluk Prachayasittikul, Ramida Watanapokasind, Chitraporn Kuanpradit. Protective effects of ethyl acetate extract of

Eclipta prostrata against 6-hydroxydopamine-induced neurotoxicity in SH-SY5Y cells. Science Asia 42 (2016): 259-265. doi: 10.2306/scienceasia1513-1874.2016.42.259.

20. Lifeng Han, Erwei Liu, Agyemang Kojo, Jing Zhao, Wei Li, Yi Zhang, Tao Wang, and Xiumei Gao1, Qualitative and Quantitative Analysis of Eclipta prostrata L. by LC/MS. *The Scientific World Journal*. Volume 2015, Article ID 980890, 15 pages http://dx.doi.org/10.1155/2015/980890.
21. Ashutosh Bahuguna, Imran Khan, Vivek K. Bajpai and Sun Chul Kang. MTT assay to evaluate the cytotoxic potential of a drug. *Bangladesh J Pharmacol*. 2017; 12:115-118. DOI: 10.3329/bjp.v12i2.30892

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