



E-ISSN: 2278-4136

P-ISSN: 2349-8234

JPP 2019; 8(1): 2219-2227

Received: 16-11-2018

Accepted: 18-12-2018

**Gitanjali Javir**

a) Ph.D Student, Department of Technology, Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra, India

b) Department of Biotechnology, Sinhgad College of Engineering, Affiliated to Savitribai Phule Pune University, Vadgaon (Bk.), Pune, Maharashtra, India

**Kalpna Joshi**

Professor and Head, Department of Biotechnology, Sinhgad College of Engineering, Affiliated to Savitribai Phule Pune University, Vadgaon (Bk.), Pune, Maharashtra, India

**Supada Rojatkar**

Former Scientist, CSIR-National Chemical Laboratory, Dr Homi Bhabha Rd, Pashan, Pune, Maharashtra, India

## Anticancer activity, phytochemical analysis of pet-ether extract by UPLC-ESI-QTOF/MS/MS and quantitative analysis of an active major constituent sesquiterpene lactone from *Cyathocline purpurea* [Buch-Ham ex D. Don.]

Gitanjali Javir, Kalpna Joshi and Supada Rojatkar

**Abstract**

**Background:** *Cyathocline purpurea* is known for its traditional therapeutic potential in Asian countries. However, limited reports are available on its anticancer activity and phytochemical analysis.

**Objective:** The aim of the present study was to investigate anticancer activity and phytochemical analysis of *C. purpurea* followed by isolation, identification, characterisation and quantification of an active constituent.

**Methods:** MTT assay was performed to check cytotoxicity of extract in a panel of human cancer cell lines along with non-cancerous human peripheral blood mononuclear cells (PBMCs). To elucidate phytochemicals responsible for anti-proliferative activity, we did phytochemicals analysis of the pet-ether extract by UPLC-ESI-QTOF/MS, MS/MS, and FTIR. Further isolation of an active constituent was carried out by repeated column chromatography coupled with thin layer chromatography. And their purity was assessed using UV-VIS, IR, <sup>1</sup>HNMR, <sup>13</sup>CNMR, DEPT, and mass spectra. Further, compound was quantified using HPLC.

**Result:** Pet-ether extract showed IC<sub>50</sub> values such as 73.99, 62.59 and 62.51 µg/ml against MDA-MB-231, MCF-7, and KB cell lines respectively. It is found to be more effective in NCI-H23 cell line with IC<sub>50</sub> value of 26.11 µg/ml whereas a lowest inhibition was seen in MDA-MB-453 cell line with IC<sub>50</sub> of 83.97 µg/ml. Several compounds belonging to Terpenes, Phenolic and aromatic, Fatty acids and amides, Steroids groups were identified. The yield of isolated active compound: 6α-hydroxy-4[14], 10[15]-guaianadien-8β,12-olide (SRCP1) was found to be 289.9246 µg/mg of pet-ether extract. It showed IC<sub>50</sub> value 9.98 µg/ml in NCI-H23 cell line. The SRCP1 showed better anti-migration potential than the standard drug actinomycin D in non-small cell lung cancer cells.

**Conclusion:** Pet-ether extract showed anticancer activity towards cancer cells associated phytochemicals were identified from *C. purpurea*. The active compound SRCP1 was isolated purified and characterised by spectroscopic analysis and assessed for anticancer activity.

**Keywords:** *C. purpurea*, anticancer, phytochemicals, UPLC-ESI-QTOF/MS, HPLC

**1. Introduction**

The *Asteraceae* family plants have been proven to be of medicinal value at a greater extent with lesser side effects [1]. One of the herb, *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze belonging to the same family, found in rice fields of the parts of northern and peninsular region of India, at an elevation of 1300m. Traditionally it is used to treat various diseases such as tuberculosis, malaria, rheumatism and conditions like bleeding, and inflammatory diseases. The chemical constituents reported from this plant are reported to have anti-oxidant [2] and anti-inflammatory [3] Very few phytochemicals such as lactones from an Australian origin reported to have anticancer activity [4]. Further, *Cyathocline purpurea* extracts are reported to have, anti-oxidant, anti-microbial, anti-fungal, anti-helminthic, anti-plaque, hypotensive, and insect repellent activities [5]. The phyto-constituent, 6α-hydroxy- 4 [14], 10 [15]-guaianadien-8β, 12-olide showed plant growth regulatory activity from Indian variety of *C. purpurea* [6].

Earlier phytochemical studies of *C. purpurea*, extracted with various solvents such as pet-ether, chloroform, ethyl acetate, and ethanol were performed to check the presence of functional groups using traditional biochemical tests [5]. However the anticancer activity, detailed phytochemical identification as well as quantification of an active constituent from *C. purpurea* has not been investigated to the best of our knowledge.

In the present study, we performed MTT assay on a panel of cancer cell lines followed by FTIR, UPLC-ESI-QTOF/MS of *C. purpurea* extract.

**Correspondence****Kalpna Joshi**

Professor and Head, Department of Biotechnology, Sinhgad College of Engineering, Affiliated to Savitribai Phule Pune University, Vadgaon (Bk.), Pune, Maharashtra, India

Identification of marker compounds was carried out by MS/MS. Further, isolation, identification, characterisation and evaluation of anticancer activity an active constituent were carried out on various cancer cell lines.

## 2. Materials and methods

### 2.1 Chemicals

Highest analytical grade organic solvents and chemicals (acetone, pet-ether (60-80 range), ethyl acetate, potassium dihydrogen phosphate, acetonitrile, methanol and ethanol) were purchased from SRL (Sisco Research Laboratories Pvt. Ltd.India).

### 2.2 Collection and authentication of plant material

*C. purpurea* used in present study was collected from rice fields of Pirangut area, near Pune, Maharashtra, India in March 2014. Authentication of plant was carried out at Botanical Survey of India, Pune and voucher specimen [BSI/WRC/Tech/2013/1094] was deposited with the institute.

### 2.3 Extraction and sample preparation

The aerial plant parts were shade dried at room temperature and then powdered using grinder. 100gms of fine powder were extracted using (500mlx3) pet-ether at room temperature by maceration. Extract was filtered through whatmann No.1 filter paper and solvents were removed using rotary evaporator under reduced pressure at 40°C to obtained 23.0g extract. Dried extract was dissolved in methanol and filtered with 0.22- $\mu$ m polytetrafluorethylene syringe filter (Millipore). The filtered extract was used for further analysis.

### 2.4 Cell culture and maintenance

NCI-H23 (human non-small cell lung cancer), MCF-7(HR positive breast cancer), MDA-MD-453(HER2 positive breast cancer), MDA-MB-231(Triple negative breast cancer), KB (Oral cancer) cell lines were obtained from national centre for cell sciences (NCCS), Pune. PBMCs (Human peripheral blood mononuclear cells) from whole blood of healthy volunteer were isolated by *Histopaque* density gradient method. NCI-H23 cells were cultured in RPMI-1640 medium. MDA-MB-453 and MDA-MB-231 cells were cultured in Leibovitz's medium (Gibco™). MCF-7, PBMCs and KB cells were cultured in DMEM (Gibco™) medium. All cell lines were grown at 37°C in supplementation with 10% Fetal Bovine Serum (FBS), and 1% antimycotic-antibiotic solution (Invitrogen). All the cells were maintained with 5% CO<sub>2</sub> except MDA-MB-453 cells.

### 2.5 MTT assay

Cell cytotoxicity was determined using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) conversion assay. Briefly, cells ( $1 \times 10^5$  cells/ml) were seeded in 96 well plate and incubated at 37°C in a humidified incubator with and without 5% CO<sub>2</sub> for overnight for adherence. Those were treated with various concentrations of pet-ether extract such as 1, 5, 10, 20, 40, 80 and 100 $\mu$ g/ml. After 24 h treatment, 20 $\mu$ L of 5 mg/ml MTT was added to each well and incubated for additional 4 h. The formazan crystals in each well were dissolved by adding 100  $\mu$ L of dimethyl sulfoxide (DMSO).The amount of purple formazan produced relative to number of viable cells was determined by measuring the absorbance at 570 nm using Dynex ELISA plate reader. IC<sub>50</sub> values were calculated using standard equation obtained from Microsoft office excel worksheet.

### 2.6 Phytochemical analysis using UV-VIS

The purity of the isolated compound was confirmed by TLC using 7:3 (pet-ether: ethyl acetate) as mobile phase. For UV-Vis spectroscopy obtained whole plant extracts was serially diluted to obtain a working solution/ extract of 1mg/ml concentration. The working solution of extract was filtered through syringe filter and scanned in the range of 200 to 800 nm by using UV-Visible spectrophotometer (Shimadzu UV 1800) at room temperature. Obtained data was processed using Origin 8 software.

### 2.7 Phytochemical analysis using FTIR

The dried pet-ether extract was loaded in ATR- FTIR spectroscopy (Shimadzu IRAffinity-1S 00466 Serial No.A221354), at frequency regions of 4000–500 cm<sup>-1</sup> by coding 30 scans and at resolution of 4 cm<sup>-1</sup>. All spectra were subtracted against a background of air spectra. After every scan, a new reference of air background spectra was taken. The ATR plate was carefully cleaned by scrubbing with acetone 70% twice followed by drying with soft tissue before being filled in with the next sample, making it possible to dry the ATR plate. Obtained data was processed through IR solution software.

### 2.8 UPLC-ESI-QTOF/MS analysis

Pet-ether extract was analysed using Agilent 6540 QTOF MS equipped with an electrospray ionisation (ESI) source. The chromatographic separation analysis was carried out with an Agilent Binary LC 1260 with Agilent Zorbax Eclipse plus C18 column (2.1x50mm, 1.8 $\mu$ m). The column was maintained at 40°C. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of acetonitrile. The gradient elution was as follows: 0min-5% B; 18min-95% B; 27min-95% B; 27.1min-5% B; 30min-5% B; 30min-5% B and 5 min post-acquisition time with flow rate of 0.3ml/min. The samples were maintained at 4°C during analysis and the injection volume was 1 $\mu$ L. For injection wash methanol was used. The mass spectrometer was operated in negative and positive ionisation modes and data were acquired over the mass range of m/z 60 to 1600 with a sampling rate of 2 Hz. In the ionisation mode, the capillary was set at 3500 V, the nebuliser gas was set at 35psig, and the dry gas was set at 8 L/min at 350°C, sheath gas temperature were set at 300°C. Nozzle voltage was set at 0V, fragmentor at 150 and skimmer1 at 45. The centroid data type was acquired from the instrument and Mass hunter Workstation software v.B.05.01 was used for analysis.

### 2.9 Targeted identification of compounds by MS/MS

Agilent Binary LC 1260 with Triple Quad LC-MS mass spectrometer was used (Agilent Technologies, CA, US). Agilent Zorbax Eclipse Plus C18, 2.1X50mm 1.8 $\mu$ m columns were used to achieve an optimal separation of all selected compounds and to obtain good peak shapes. The flow rate was 0.3 ml/minute with a mobile phase of (A) water (0.1% formic acid) and (B) Acetonitrile. Samples were acquired for 0-5 min with isocratic 70% B. Electrospray ionisation (ESI) was used in the positive mode (ESI<sup>+</sup>) and in the negative mode (ESI<sup>-</sup>). 5  $\mu$ L was used for the injection volume and the Column temperature was maintained at 40°C. The Agilent 6540 QTOF MS system comprised a degasser, binary pump, cooled auto-sampler, column oven, and 6540 mass spectrometer. The gas temperature was 325°C and flow was 5 l/minute; sheath gas temperature was 300°C with flow of

5 L/minute and nebuliser pressure of 30 psi in negative ion mode and 25 psi in positive ion mode. Capillary voltage was 2500°C and 2000°C for positive and negative polarity, respectively. Fragmentor, 170 and Skimmer1 were set at 45 in both the ionic modes. Nozzle voltage, 0 were kept in negative ion mode whereas it was kept 1000 in positive ion mode. Mass range m/z 100-800 for positive mode while 100-700 for negative ion mode. MS scan speed were 2Hz and MS/MS scan speed were 3Hz. The centroid data type was acquired by using Mass hunter workstation software v.B.05.01. Identification was achieved based on product ions compared with literature and databases.

### 2.10 Isolation of SRCP1 from pet-ether extract

The pet-ether extract showed potential anticancer activity. Hence, we have investigated the extract for further purification and carried out its bio-evaluation. The pet-ether extract 20.0 g was further extracted using 100% pet-ether (8.63g), 5.0% acetone (5.23g), 10% acetone (3.38g) and 100% acetone (1.28g). The fraction was collected separately and concentrated under vacuum at 40°C. Similar fractions used for TLC showed maximum yield of interested compound in 5.0% acetone fraction. The fraction (5.0g) was subjected to silica gel column chromatography with mobile phase of pet-ether and ethyl acetate (7:3 V/V) followed by LH-20 sephadex gel column chromatography. The pure compound SRCP1 (Peak 7) showed the RF value of 0.6 on the TLC plate (Pet-ether: Ethyl acetate; 7:3). The pure compound (0.2g) was isolated from the 5.0% acetone fraction.

### 2.11 Characterization of SRCP1 from pet-ether extract

The chemical structure and characterisation of SRCP1 has been established by UV-VIS, <sup>1</sup>H-NMR, <sup>13</sup>CN-MR, DEPT, IR and Mass spectra.

### 2.12 Quantitative analysis of SRCP1 by HPLC

Extract was analyzed by Agilent 1100 series quaternary HPLC system, equipped with auto sampler. Chromatographic separation was carried out using isocratic mode on a C<sub>18</sub>, with a mobile phase consisting of potassium dihydrogen phosphate (pH-3): acetonitrile (30:70 v/v). Flow rate was used as 1 ml/min, and the eluate was scanned at 207 nm at 25°C. All the injections were run in triplicate, and the injection volume was 20 µL. The run time was 10 min, and the total peak areas were used to quantify the studied components. For construction of calibration curves, accurately measured aliquots within the range of 31.2-500 µg/ml of purified SRCP1 were used in triplicate for each concentration. The obtained peak areas were used to construct a calibration curve of SRCP1 and based on the regression equation the amount of SRCP1 present in pet-ether extract were determined.

### 2.13 Anticancer activity of SRCP1

The isolated SRCP1 were dissolved in DMSO at concentration of 100 mM, filtered and stored at -80°C for further use. The concentrations 2ng-20000ng/ml was used for MTT assay. The MTT assay was performed as described in earlier method sub point 2.5.

### 2.14 Wound scratch assay

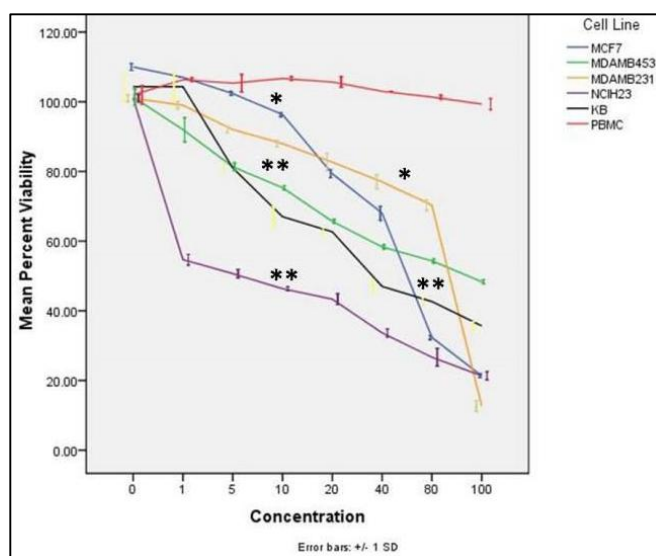
NCI-H23 cells were allowed to grow at confluency more than

90 %. The scratches were prepared using 200µl tip. The cells were washes with 1X PBS twice and drugs were added. Images were taken at 0 h and 24 hr after drug treatment.

## 3. Results and Discussion

### 3.1 Anticancer activity of pet-ether extract

Cells were treated with 1-100µg/ml of pet-ether extract for 24 h with a panel of cancer cell line and non-cancerous PBMCs as showed in Fig. 1. Pet-ether extract is found to be more effective on NCI-H23 cell line with IC<sub>50</sub> value of 26.11µg/ml whereas a lowest inhibition was seen in MDA-MB-453 cell line with IC<sub>50</sub> of 83.97µg/ml. This could be due to the abundant presence of terpenoids, fatty acids in pet-ether extract. Pet-ether extract showed IC<sub>50</sub> values such as 73.99, 62.59 and 62.51µg/ml against MDA-MB-231, MCF-7, and KB cell lines respectively. Interestingly, extract did not showed cytotoxicity towards human PBMCs within selected concentration range indicating the selective cytotoxicity towards cancer cells.

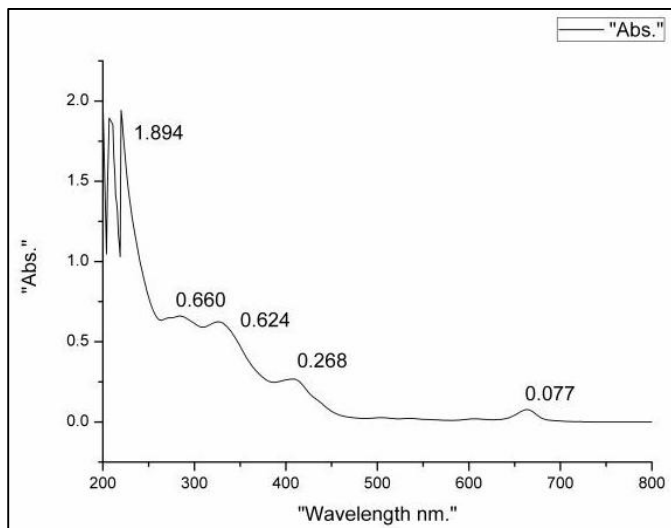


**Fig 1:** Percent viability of MCF-7, MDA-MB-453, MDA-MB-231, KB, NCI-H23 and PBMCs after treatment of *Cyathocline purpurea* pet-ether extract in the concentration range of 1-100µg/mL.

Experiments were carried out at n=6 and the data were represented as Mean± SD. Significance value were calculated by One way ANOVA followed by Tukey's multiple comparison test.

### 3.2 Phytochemical analysis using UV-VIS

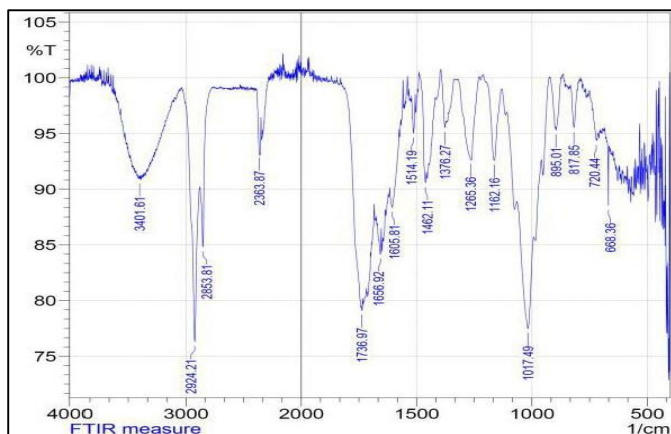
The quantitative spectrophotometric study of extract was carried out by UV-VIS spectrophotometer was screened between 200 to 800 nm. The UV-VIS spectrophotometric profile showed the peaks between 200–700 nm for pet-ether extract. The number of peaks and its absorption maxima ( $\lambda_{max}$ ) has showed correlation with polarity of the solvent. The extracts prepared have maximum peaks in UV and visible wavelength and these peaks are at in the range of 200-400 nm. For pet-ether extract the peaks shown at 204, 218, 259, 270, 340, 410 and 660 nm with absorbance of 1.913, 1.894, 0.640, 0.624, 0.268 and 0.077 indicating  $\lambda_{max}$  at 207 nm Fig. 2. Extracts shows absorbance peak within the range of 200-210 nm which shows the presence of sesquiterpene lactones and similar  $\lambda_{max}$  is also seen in HPLC analysis.



**Fig 2:** UV-VIS analysis of pet ether extracts of *Cyathocline purpurea* by UV-VIS spectrophotometer.

### 3.3 Phytochemical analysis of pet-ether extracts using FTIR

Identification of the functional groups and chemical bonds (based on peak values) in pet-ether extracts of *C. purpurea* was done using ATR-FTIR spectroscopy Fig 3. Pet-ether extract showed maximum peak values as shown in Table 1. The presence of peak within a range of  $3200\text{--}3570\text{ cm}^{-1}$  and  $1565\text{--}1800\text{ cm}^{-1}$  are because of O-H and C=O stretch which indicates the presence of phenolics. They have also shown presence of alkaloids for peak range at  $1240\text{--}1380$  (C-N stretch). Saponins were confirmed on the basis of obtained peaks at  $1600\text{--}1750\text{ cm}^{-1}$  (C=O stretch),  $1125\text{--}1250\text{ cm}^{-1}$  (C-O stretch) which show accordance with Moses *et al* [43]. Quinones were present in extracts of *C. purpurea* which were confirmed due to the peaks obtained in between  $1125\text{--}1250\text{ cm}^{-1}$  with C-O stretch. The esters peak for C=O stretch at  $1680\text{--}1800\text{ cm}^{-1}$  and C-O stretch at  $1125\text{--}1250\text{ cm}^{-1}$  implying presence of terpenoids and steroids. Flavonoids were confirmed with O-H stretch at  $3200\text{--}3570\text{ cm}^{-1}$  and C=O stretch at  $1600\text{--}1750\text{ cm}^{-1}$ . Terpenes were ascertained with C-H stretch at  $2815\text{--}3000\text{ cm}^{-1}$ , and =C-H bend at the  $1450\text{--}1510\text{ cm}^{-1}$  and showed agreement with Moses *et al.* [43]. We recorded such peaks for all extracts of leaf, stem and root. Presence of C=N stretch at  $2150\text{--}2390\text{ cm}^{-1}$  verified presence of cyanogenic glycosides [43]. Cardiac glycosides were documented by presence of C=O stretch at  $1700\text{--}1750\text{ cm}^{-1}$  and C-O stretch at  $1200\text{--}1280\text{ cm}^{-1}$  and showed correspondence with the findings of Moses *et al.*, 2013 [7].



**Fig 3:** FTIR analysis of pet ether extract of *Cyathocline purpurea*.

### 3.4 Phytochemical analysis using UPLC-ESI-QTOF/MS

#### 3.4.1 Screening of phyto-constituents from *C. purpurea* acetone extracts using various databases

UPLC-ESI-QTOF/MS data analysis using Masshunter and Metlin database search revealed the presence of various primary, secondary metabolites and intermediate compounds. The identified secondary metabolites belong to terpenoids, flavonoids, steroids, quinones, fatty acids, fatty amides and phenols. List of phytochemicals in pet-ether extract along with molecular formula, molecular mass and m/z showed in Table 2. The database such as Pub Chem, Chem Spider and LIPID MAPS Lipidomics Gateway were used to confirm the class of compound as per their m/z value and tentative molecular formula obtained from UPLC-ESI-QTOF/MS.

#### 3.4.2 Phytochemicals in pet-ether extracts of *C. purpurea* depending on their classification

Duartin (-) a flavonoids were observed in pet-ether extract. The detailed retention time, m/z ion, mass and molecular formulae of duartin (-) is listed Table 2. Our findings are in line with Bihani G *et al.*, 2014 [8]. Flavonoids were found to be absent in pet-ether extract of *C. purpurea* when analysis were carried out by traditional test such as  $\text{FeCl}_3$  test, Alkaline reagent test and Shinoda test; indicating the UPLC-ESI-QTOF/MS is more sensitive technique for determination of a single flavonoid.

A total of 9 terpenoids were identified by UPLC-ESI-QTOF/MS method, indicating abundance in the pet-ether extracts. Detailed terpenoids were listed in Table 2. Although we are unable to find sesquiterpene lactones such as santamarine, 9-beta-acetoxycostunolide and 9-beta-acetoxyparthenolide which were reported in Australian variety of *C. purpurea* [4]. Iso-safrole which was present in pet-ether extract is used in fragrance industry but its isoform safrole is not safe to use for human consumption as it causes hepatic toxicity [9].

Quinones are class of organic compound formally derived from aromatic compounds by conversion of an even number of  $-\text{CH}=\text{}$  groups into  $-\text{C}(=\text{O})-$  groups with any necessary rearrangement of double bonds, resulting in a fully conjugated cyclic dione structure. UPLC-QTOF-MS showed presence of three quinones: embelin, idebenone metabolite (QS-10), and lactone of PGF-MUM in *C. purpurea* extracts. Embelin was observed in positive as well as negative ionization mode hence we propose embelin to be considered as marker compound. Embelin isolated from other plant source is reported to have anticancer properties [10]. We are the first to report the presence of Quinones from *C. purpurea*.

Steroids are biologically active organic compounds with four rings arranged in a specific molecular configuration. The selected plant also contains steroids such as lithocholate 3-O-glucuronide which is used as a cholestatic agent [11].

Almost 14 fatty acids were predominantly present in pet-ether extract. They are major source of energy for animal metabolism. One of them, butyric acid is a pharmacologically active compound which functions as an agonist of hydroxycarboxylic acid receptor 2. It has immunomodulatory [12], anti-diabetic [13], anti-microbial [14, 15] and anti-carcinogen activities [12]. The volatile esters of valeric acid tend to have pleasant odours and are used in perfumes and cosmetics. Earlier Clavirin I used as anti-infectious agent to treat infections of lung ear, skin and bladder. Clavirins reported for cytotoxic and antitumor activity [16].

Fatty acid amides are important signaling molecules in the mammalian nervous system, binding to many drug receptors

and demonstrating control over sleep, locomotion, angiogenesis, and many other processes [17]. In present study we found two fatty amides. N-(2-hydroxyethyl) icosanamide is used as biomarker for Alzheimer's disease [18] whereas anandamide used in an ayurvedic preparations preparation such as hashish and marijuana [19]. Increased brain's anandamide level helps to treat anxiety and depression [20]. It is present in both the ionic mode of pet-ether extract, hence considered as marker compound.

Total of eight phenolic and aromatic acids are found in pet-ether extract. Phenolics and aromatic acid possess numerous pharmacological activities such as valeryl salicylate act as a cyclooxygenase-1 (COX-1) inhibitor [21], nanoxylinol as a spermicidal [22], haloperidol as an antipsychotic medication [23] etc. Further, terephthalic acid identified which is used in clothing and plastic bottle industry.

Taken together, phytoconstituents identified from *C. purpurea* pet-ether extract possess medicinal and industrial benefits.

**Table 1:** Phytochemical analyses of pet-ether extract of *Cyathocline purpurea* by FTIR.

No	Peak-Reference range(cm <sup>-1</sup> )	Corr. Area (Acetone extract)	Functional group assignment
1	668.36	0.05	C-Br stretch
2	720.44	0.028	C-Cl stretch
3	817.85	0.238	=C-H bend
4	895.01	0.079	P-O-C
5	1017.49	1.875	C-O Stretch
6	1162.16	0.827	C-O Stretch
7	1265.36	0.047	C-N stretch
8	1376.27	0.06	O-H bend
9	1462.11	0.032	C-O stretch
10	1514.19	0.071	C-O stretch
11	1605.81	0.422	C=O Stretch
12	1656.92	0.06	C=O Stretch
13	1736.97	0.017	C=O Stretch
14	2363.87	-0.003	C=N stretch
15	2853.81	0.872	C-H stretch
16	2924.21	3.789	C-H stretch
17	3401.61	0.003	O-H stretch

**Table 2:** Identification of chemical constituents of *Cyathocline purpurea* pet ether extract by UPLC-QTOF-MS.

Label	Proposed Identity	Obs. RT	Ionisation mode	Product Ions (m/z)	Obs. Mass	Difference (DB. ppm)	MFG Formula	Tgt Formula
<b>Terpenes</b>								
Cpd 69: Ophiobolin A	Ophiobolin A	10.28	Positive	400.28	400.26	0.95	C <sub>25</sub> H <sub>36</sub> O <sub>4</sub>	C <sub>25</sub> H <sub>36</sub> O <sub>4</sub>
Cpd 92: Oleandolide	Oleandolide	11.54	Positive	386.25	386.23	1.23	C <sub>20</sub> H <sub>34</sub> O <sub>7</sub>	C <sub>20</sub> H <sub>34</sub> O <sub>7</sub>
Cpd 102: Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate	Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate	12.06	Positive	311.18	310.17	0.04	C <sub>17</sub> H <sub>26</sub> O <sub>5</sub>	C <sub>17</sub> H <sub>26</sub> O <sub>5</sub>
Cpd 113: 6b,11b,16a,17a,21-Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	6b,11b,16a,17a,21-Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	12.83	Positive	415.21	432.21	0.66	C <sub>24</sub> H <sub>32</sub> O <sub>7</sub>	C <sub>24</sub> H <sub>32</sub> O <sub>7</sub>
Cpd 123: 11-Hydroxyiridodial glucoside pentaacetate	11-Hydroxyiridodial glucoside pentaacetate	13.23	Positive	539.21	556.21	3.66	C <sub>26</sub> H <sub>36</sub> O <sub>13</sub>	C <sub>26</sub> H <sub>36</sub> O <sub>13</sub>
Cpd 151: Isosafrole	Isosafrole	14.66	Positive	145.06	162.06	-0.55	C <sub>10</sub> H <sub>10</sub> O <sub>2</sub>	C <sub>10</sub> H <sub>10</sub> O <sub>2</sub>
Cpd 33: N-(3-oxo-hexanoyl)-homoserine lactone	N-(3-oxo-hexanoyl)-homoserine lactone	12.69	Negative	194.08	213.09	2.73	C <sub>10</sub> H <sub>15</sub> N O <sub>4</sub>	C <sub>10</sub> H <sub>15</sub> N O <sub>4</sub>
cpd 43: Punctaporin B	Punctaporin B	14.51	Negative	233.15	252.17	-3.57	C <sub>15</sub> H <sub>24</sub> O <sub>3</sub>	C <sub>15</sub> H <sub>24</sub> O <sub>3</sub>
6 $\alpha$ -hydroxy-4 [14], 10 [15]-guainadien-8 $\alpha$ , 12-olide	6 $\alpha$ -hydroxy-4 [14], 10 [15]-guainadien-8 $\alpha$ , 12-olide	11.19	Positive	247	246.12	3.00	C <sub>15</sub> H <sub>18</sub> O <sub>3</sub>	C <sub>15</sub> H <sub>18</sub> O <sub>3</sub>
<b>Flavonoids</b>								
Cpd 130: Durtin (-)	Durtin (-)	13.4	Positive	337.1	332.12	-0.82	C <sub>18</sub> H <sub>20</sub> O <sub>6</sub>	C <sub>18</sub> H <sub>20</sub> O <sub>6</sub>
<b>Quinones</b>								
Cpd 162: Embelin	Embelin	14.84	Positive	294.2	294.18	-0.11	C <sub>17</sub> H <sub>26</sub> O <sub>4</sub>	C <sub>17</sub> H <sub>26</sub> O <sub>4</sub>
Cpd 174: Idebenone Metabolite (QS-10)	Idebenone Metabolite (QS-10)	15.45	Positive	357.12	352.18	-0.29	C <sub>19</sub> H <sub>28</sub> O <sub>6</sub>	C <sub>19</sub> H <sub>28</sub> O <sub>6</sub>
Cpd 179: Lactone of PGF-MUM	Lactone of PGF-MUM	15.71	Positive	279.15	296.16	2.26	C <sub>16</sub> H <sub>24</sub> O <sub>5</sub>	C <sub>16</sub> H <sub>24</sub> O <sub>5</sub>
Cpd 32: Embelin	Embelin	12.65	Negative	293.17	294.18	0.26	C <sub>17</sub> H <sub>26</sub> O <sub>4</sub>	C <sub>17</sub> H <sub>26</sub> O <sub>4</sub>
<b>Steroids</b>								
Cpd 186: Lithocholate 3-O-glucuronide	Lithocholate 3-O-glucuronide	15.82	Positive	591.29	552.32	1.63	C <sub>30</sub> H <sub>48</sub> O <sub>9</sub>	C <sub>30</sub> H <sub>48</sub> O <sub>9</sub>
<b>Fatty acids</b>								
Cpd 83: C16 Sphinganine	Sphinganine	10.88	Positive	274.27	273.26	0.37	C <sub>16</sub> H <sub>35</sub> N O <sub>2</sub>	C <sub>16</sub> H <sub>35</sub> N O <sub>2</sub>
Cpd 205: 2-Hydroxy-3-(4-methoxyethylphenoxy)-propanoic acid	2-Hydroxy-3-(4-methoxyethylphenoxy)-propanoic acid	16.48	Positive	233.09	240.09	1.66	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>
Cpd 224: 12-oxo-14,18-dihydroxy-	12-oxo-14,18-dihydroxy-9Z,13E,15Z-	17.29	Positive	329.17	324.19	1.88	C <sub>18</sub> H <sub>28</sub> O <sub>5</sub>	C <sub>18</sub> H <sub>28</sub> O <sub>5</sub>

9Z,13E,15Z-octadecatrienoic acid	octadecatrienoic acid							
Cpd 153: 2,3-Dinor-6,15-diketo-13,14-dihydro-PGF1a	2,3-Dinor-6,15-diketo-13, 14-dihydro-PGF1a	14.66	Positive	231.16	338.17	1.16	C <sub>18</sub> H <sub>26</sub> O <sub>6</sub>	C <sub>18</sub> H <sub>26</sub> O <sub>6</sub>
Cpd 185: clavirin I	clavirin I	15.82	Positive	313.14	290.15	2.8	C <sub>17</sub> H <sub>22</sub> O <sub>4</sub>	C <sub>17</sub> H <sub>22</sub> O <sub>4</sub>
Cpd 80: (+)-6-methyl caprylic acid	(+)-6-methyl caprylic acid	10.81	Positive	158.15	158.13	-0.21	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>
Cpd 62: cis-5-Tetradecenoylcarnitine	cis-5-Tetradecenoylcarnitine	9.79	Positive	370.29	370.29	1.34	C <sub>21</sub> H <sub>40</sub> N O <sub>4</sub>	C <sub>21</sub> H <sub>40</sub> N O <sub>4</sub>
Cpd 26: methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate	methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate	12.04	Negative	309.17	310.17	-3.00	C <sub>17</sub> H <sub>26</sub> O <sub>5</sub>	C <sub>17</sub> H <sub>26</sub> O <sub>5</sub>
Cpd 92: 4,8-dimethyl-hexadecanoic acid	4,8-dimethyl-hexadecanoic acid	20.95	Negative	283.26	284.27	-3.70	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
Cpd 7: 4-methyl-dodecanedioic acid	4-methyl-dodecanedioic acid	9.71	Negative	225.14	244.16	0.41	C <sub>13</sub> H <sub>24</sub> O <sub>4</sub>	C <sub>13</sub> H <sub>24</sub> O <sub>4</sub>
Cpd 16: 18-hydroxy-9S,10R-dihydroxy-stearic acid	18-hydroxy-9S,10R-dihydroxy-stearic acid	11.69	Negative	331.25	332.25	-0.399	C <sub>18</sub> H <sub>36</sub> O <sub>5</sub>	C <sub>18</sub> H <sub>36</sub> O <sub>5</sub>
Cpd 78: 3-ethyl-3-methyl-tridecanoic acid	3-ethyl-3-methyl-tridecanoic acid	19.37	Negative	255.23	256.24	-1.61	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
Cpd 34: 10,11-epoxy-3,7,11-trimethyl-2E,6E-tridecadienoic acid	10,11-epoxy-3,7,11-trimethyl-2E,6E-tridecadienoic acid	12.83	Negative	311.18	266.18	-3.48	C <sub>16</sub> H <sub>26</sub> O <sub>3</sub>	C <sub>16</sub> H <sub>26</sub> O <sub>3</sub>
<b>Fatty amides</b>								
Cpd 232: N-(2-hydroxyethyl)icosanamide	N-(2-hydroxyethyl)icosanamide	17.87	Positive	338.34	355.34	1.72	C <sub>22</sub> H <sub>45</sub> N O <sub>2</sub>	C <sub>22</sub> H <sub>45</sub> N O <sub>2</sub>
Cpd 200: Anandamide (20:5, n-3)	Anandamide (20:5, n-3)	16.16	Positive	328.26	345.26	0.78	C <sub>22</sub> H <sub>35</sub> N O <sub>2</sub>	C <sub>22</sub> H <sub>35</sub> N O <sub>2</sub>
<b>Phenolics and aromatic acids</b>								
Cpd 190: Nanoxynol	Nanoxynol	15.97	Positive	634.45	616.41	1.40	C <sub>33</sub> H <sub>60</sub> O <sub>10</sub>	C <sub>33</sub> H <sub>60</sub> O <sub>10</sub>
Cpd 132: Atrolactic acid	Atrolactic acid	13.41	Positive	149.06	166.06	-2.79	C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>	C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>
Cpd 161: 2-Isopropyl-3-methoxycinnamic acid	2-Isopropyl-3-Methoxycinnamic Acid	14.84	Positive	221.11	220.10	0.62	C <sub>13</sub> H <sub>16</sub> O <sub>3</sub>	C <sub>13</sub> H <sub>16</sub> O <sub>3</sub>
Cpd 207: Terephthalic acid	Terephthalic acid	16.49	Positive	149.02	166.02	0.07	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>
Cpd 182: valeryl salicylate	Valeryl Salicylate	15.74	Positive	205.08	222.08	1.92	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>
Cpd 56: p-Hydroxymexiletine	p-Hydroxymexiletine	9.21	Positive	178.12	195.12	-1.41	C <sub>11</sub> H <sub>17</sub> N O <sub>2</sub>	C <sub>11</sub> H <sub>17</sub> N O <sub>2</sub>
Cpd 51: a-[1-(diethylamino)ethyl]-p-hydroxy-Benzyl alcohol	a-[1-(diethylamino)ethyl]-p-hydroxy-Benzyl alcohol	15.79	Negative	250.14	223.15	-3.96	C <sub>13</sub> H <sub>21</sub> N O <sub>2</sub>	C <sub>13</sub> H <sub>21</sub> N O <sub>2</sub>
Cpd 85: Haloperidol	Haloperidol	19.38	Negative	374.13	375.13	1.86	C <sub>21</sub> H <sub>23</sub> Cl F N O <sub>2</sub>	C <sub>21</sub> H <sub>23</sub> Cl F N O <sub>2</sub>

### 3.5 Identification of marker compounds by UPLC-ESI-QTOF/MS/MS

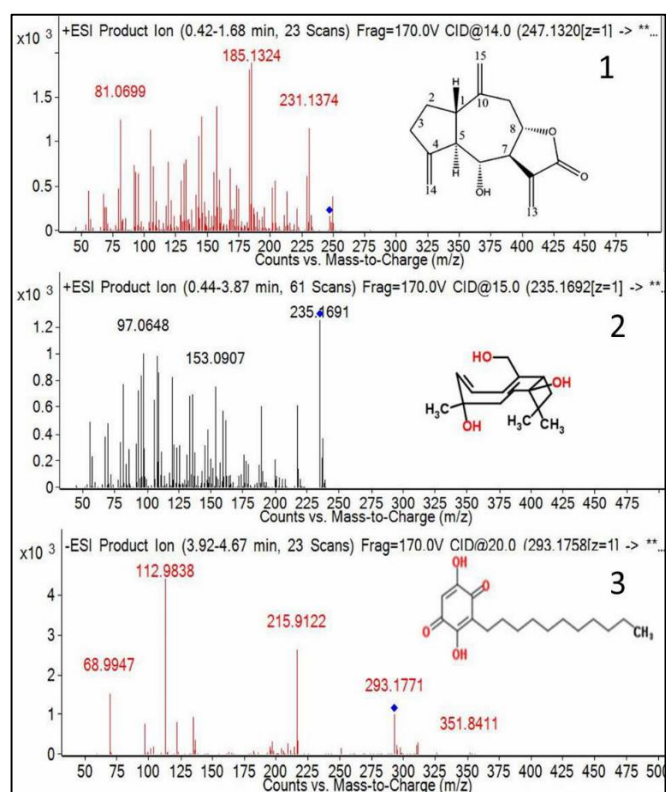
The MS/MS fragmentation pattern was helpful in identifying the accurate skeleton of the compound. Those are belonging to existing formula were tentatively identified without reference compound by the aspects such as comparison to clear extracted chromatogram, determining accurate mass within error of +/- 5ppm, judging reasonable fragmentation pathway corresponding to structure, that was referring to the reported literature and comparing with known and similar compounds to validate correctness. Peaks were positively confirmed by comparison with retention time and fragmentation pattern with standard or with reported literature are enlisted in Fig. 4.

Peak 1 ( $t_R = 1.05$ ), showed  $m/z = 247.13$  determined as  $[M-15]^+$  leading to  $m/z 231.13$  in positive ESI mode. This peak showed molecular formula (C<sub>15</sub>H<sub>18</sub>O<sub>3</sub>) Fig.4 [1]. This skeleton is related with the guaianolide group of sesquiterpene lactones. This compound was reported previously from *C. purpurea* but isolated from ethyl acetate fraction of methanolic extract [3]. The MS/MS fragment from the present study was confirmed chemical structure of the peak 1 and it was identified as 6 $\alpha$ -hydroxy-4<sup>[14]</sup>, 10<sup>[15]</sup>-guaianadien-8 $\beta$ , 12-olide. The compound has been reported to have anti-inflammatory activity via COX-2 inhibition.

Peak 2 ( $t_R = 2.16$ ),  $m/z = 235.1695$ ,  $[M-18]^+$  found in ESI positive mode with molecular formula (C<sub>15</sub>H<sub>24</sub>O<sub>3</sub>) Fig.4 [2]. The mass,  $m/z$  ion and molecular formula were compared with previous report by [24] and [25]. The peak 2 was identified as Punctaporin B. It was found to be present in *Cucumber Carolina* and *Vernonia cinerea* (L) Less.

Peak 3 ( $t_R = 4.29$ ) with  $m/z = 293.17$ ,  $[M-1]^+$  was observed in negative ESI mode with molecular formula (C<sub>17</sub>H<sub>26</sub>O<sub>4</sub>). The MS/MS fragmentation pattern showed  $m/z$  of 351.84, 293.17

Fig 4 [3]. These ions were compared with studies done by [10] and [26]. The peak 3 was identified as Embelin. It is reported from *Embelia ribes* previously and posse's anti-cancer activity against MCF-7 cell line with IC<sub>50</sub> value 80 $\mu$ g/ml [10].



**Fig 4:** MS/MS spectra, proposed fragmentation pattern and structure of compound [1, 2] positive ion mode and [3] negative ion mode from *Cyathocline purpurea*.

### 3.6 Isolation and spectroscopic properties of SRCP1 from *C. purpurea* extract

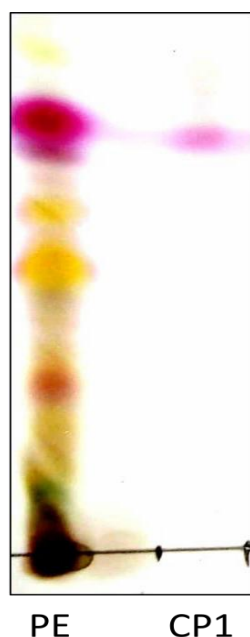
The isolation of the pure compound SRCP1 was carried out by repeated column chromatography with different solvent systems and coupled with Prep. TLC by using Pet-ether-Ethyl acetate (7:3) solvent system to obtained 200mg a pure compound (SRCP1). The details of physical and spectroscopic data of isolated compound are as follows:

Isolated compound obtained as a white crystalline solid, melting point: 116-118°C. IR  $\lambda$  max  $\text{cm}^{-1}$ : 3499(OH), 2948(aliphatic), 1750(C=O), 1646(un-saturation) and 1278(C-O, stretching). Molecular Formula:  $\text{C}_{15}\text{H}_{18}\text{O}_3$ , Molecular wt: m/z (rel.int.): 246.  $^1\text{H-NMR}$  (500 MHz), in  $\text{CDCl}_3$ :  $\delta$  (ppm) 2.35 (1Hm, H-1), 1.74 – 1.89 (2H m, H-2), 2.32-2.44 (2H m, H-3), 2.27(1H, m H-5), 3.70 (1 H t, J=9.2 Hz, H-6), 2.89 (1H m, H-7), 4.42 (1H, m, H-8), 3.22(1H, br. d, J = 16.4 Hz, H-9 $\alpha$ ), 2.66(1H dd, J = 160 Hz, 12.0 Hz 9  $\beta$ ), 6.33 (dd, 2H, J= 2.4, 2.8 Hz, H-13), 5.00(1H s, H-14a), 5.05(1H, s, H-14b), 5.10(1H, s, H-15a), 5.16(1H, s, H-15b).  $^{13}\text{C-NMR}$  (100MHz), in  $\text{CDCl}_3$ :  $\delta$  (ppm) 45.15(d, C-1), 29.44(t, C-2), 33.39(t, C-3), 152.43(s, C-4), 57.63(d, C-5), 74.36(d, C-6), 53.12(d, C-7), 76.64(d, C-8), 39.51(t, C-9), 144.02(s, C-10), 137.61(s, C-11), 170.15(s, C-12), 124.36(t, C-13), 108.85(t, C-14) and 111.23 (t, C-15), and ESIM-MS: (Rel.int; m/z): 247.5(M+1)<sup>+</sup>.

The IR,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , DEPT and mass spectra of SRCP1 were showed in Supplementary file. The structure and stereochemistry of isolated compound was established as 6 $\alpha$ -hydroxy-4<sup>[14]</sup>, 10<sup>[15]</sup>-guainadien-8 $\beta$ , 12-olide with the comparison of its physical and spectroscopic data reported in the literature <sup>[3]</sup>.

#### 3.6.1 Characterization of SRCP1 by TLC

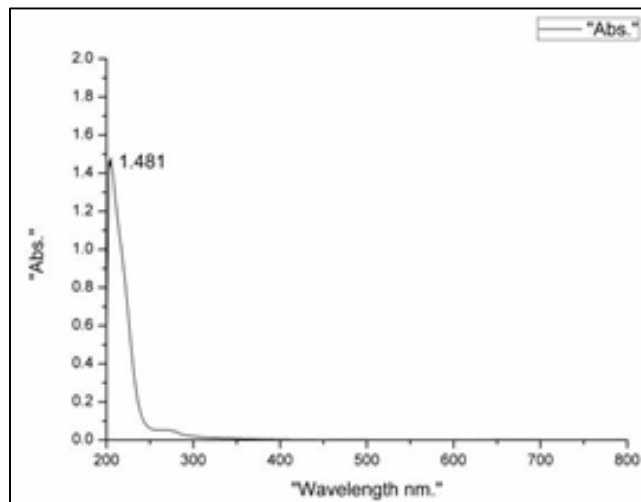
The thin layer chromatography of pet-ether extract and SRCP1 were carried out using pet-ether: ethyl acetate (7:3) mobile phase. For development of TLC the sample of 2 $\mu\text{l}$  were loaded and allowed to run at least 3/4<sup>th</sup> of TLC plate. Then plate was dried and developed using visualization solution ethanol: sulphuric acid (9:1) followed by incubation at 90°C for 30sec. The resultant sample PE represents pet-ether extract whereas CP1 represents SRCP1 and were showed in Fig. 5.



**Fig 5:** Thin layer chromatographic analysis of pet ether extracts of *Cyathocline purpurea* (PE) and isolated compound SRCP1 (CP1)

#### 3.6.2 Characterization of SRCP1 by UV-VIS spectrophotometer

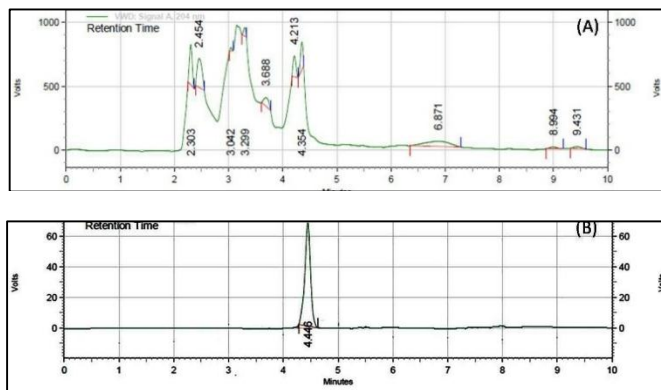
The obtained results of UV-VIS spectrophotometric analysis confirm the presence of single peak indicating the purity of compound. The isolated compound SRCP1 showed  $\lambda_{\text{max}}$  at 204 nm with absorbance of 1.481 as showed in Fig. 6.



**Fig 6:** UV-VIS analysis of isolated compound SRCP1 by UV-VIS spectrophotometer.

#### 3.7 Quantitative analysis of SRCP1 by HPLC

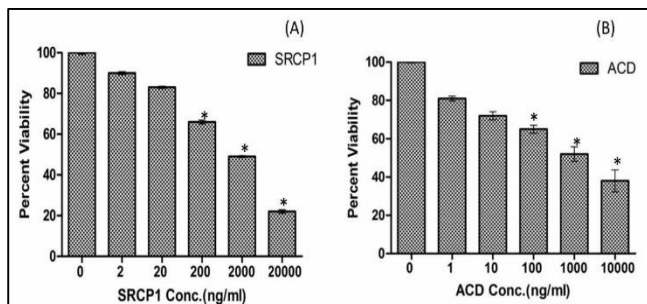
HPLC analysis was performed to quantify SRCP1 from pet-ether extract of *C. purpurea*. Maximum SRCP1 concentration was recorded in pet-ether extract 289.9246  $\mu\text{g}/\text{mg}$  with RF value 4.3 min. The resultant HPLC percent area of pet-ether extract Fig.7 (A) and purified SRCP1 Fig. 7 (B) were shown in Fig. 7.



**Fig 7:** HPLC analyses of *Cyathocline purpurea* pet ether extract (A) and purified compound SRCP1 (B).

#### 3.8 Anticancer activity of SRCP1

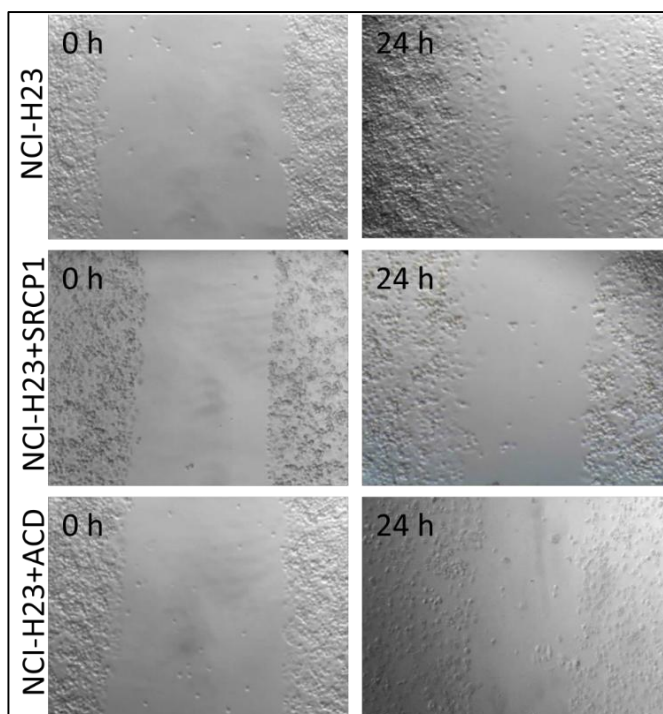
The MTT assay was performed onto the NCI-H23 cell line because it has shown lowest  $\text{IC}_{50}$  value when treated with pet-ether extract. The concentrations ranging from 2ng-20000ng were used in triplicate. The SRCP1 shows dose dependant reduction in proliferation i.e represented as percent viability as shown in Fig. 8 (A). The standard drug also showed reduction in percent viability as shown in Fig. 8 (B). The resultant  $\text{IC}_{50}$  value was calculated after 24hr of drug treatment and it is 9.98  $\mu\text{g}/\text{ml}$ , which was less as compared to the extract. But, the standard drug actinomycin D shows  $\text{IC}_{50}$  value 8.5  $\mu\text{M}$ . Although, the purified compound SRCP1 found to be more active as compared to the pet-ether extract in the present study.



**Fig 8:** Percent viability of NCI-H23 cells after 24 h treatment with purified compound-SRPC1 (A) And standard drug-actinomycin-D-ACD (B). The data were represented as Mean $\pm$  SD and analysed by paired t test. (p value for SRCP1-0.04\*, for ACD-0.01\* when compared with control)

### 3.9 Anti-migration potential of SRCP1

SRCP1 shows less wound closure as compared to the cells treated with medium only Fig. 9. Further actinomycin D (ACD) showed more wound closure as compared to SRCP1, indicating SRCP1 has an anti-migration potential.



**Fig 9:** Wound scratch assay of NCI-H23 cells with treatment of medium only (1st panel), treatment of SRCP1 (9.8 $\mu$ g/ml) (2nd panel) and with treatment of ACD (100nm) for 24 hr.

### 4. Conclusion

Our data indicate the abundance of fatty acids followed by terpenoids, phenolics and aromatic acids, quinines, fatty amides etc. But, flavonoids and steroids are found in very less amount in *C. purpurea* extracts. We have identified three marker compounds such as punctaporin B, 6 $\alpha$ -hydroxy-4<sup>[14]</sup>, 10<sup>[15]</sup>-guainadien-8 $\beta$ , 12-olide and embelin, they can be used for the identification of *C. purpurea* plant. Further, the active constituent isolated and characterised by TLC, UV-VIS, <sup>1</sup>HNMR, <sup>13</sup>CNMR IR and mass spectra. The quantitation of purified compound 6 $\alpha$ -hydroxy-4<sup>[14]</sup>, 10<sup>[15]</sup>, guainadien-8 $\beta$ , 12-olide was carried out using HPLC. The anticancer activity of the compound found to be more potent as compared to the pet-ether extract. The detailed anticancer mechanism of 6 $\alpha$ -hydroxy-4<sup>[14]</sup>, 10<sup>[15]</sup>, guainadien-8 $\beta$ , 12-olide soon will be communicated.

### 5. Conflict of interest statement

We declare that we have no conflict of interest.

**6. Funding** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for profit sectors.

**7. Acknowledgment** Authors acknowledge the support of Professor Kisan Kodam and Ms Priyanka Ladage, Biochemistry Division, Department of Chemistry and CIF, SPPU, Pune, during HPLC analysis.

### 8. References

- Chadwick M, Trewin H, Gawthrop F, Wag staff C. Sesquiterpenoids Lactones: Benefits to Plants and People. 2013; 12780-805.
- Bihani G, Rojatar S, Bhansali S, Kulkarni V, Katte R. Spectral characterization, docking and *in-vivo* anti-inflammatory activity of Isoivangustin, a constituent isolated from methanol extract of *Cyathocline purpurea*. 2015; 7:115-21.
- Tambewagh UU, Kandhare AD, Hoore VS, Kadam PP, Khedkar VM, Bodhankar SL *et al.* International Immunopharmacology Anti-inflammatory and antioxidant potential of Guaianolide isolated from *Cyathocline purpurea*. Role of COX-2 inhibition. International Immunopharmacology. 2017; 52:110-8.
- Ma G, Chong L, Li Z, Cheung AHT, Tattersall MHN. Anticancer activities of sesquiterpene lactones from *Cyathocline purpurea in vitro*, 2009, 143-52.
- Gupta A, Joshi A, Dubey B. Comparative pharmacognostical and phytochemical evaluation of two species of *Cyathocline*, 9633.
- Chintalwar GJ, MAMDAPUR VR, YADAVA VS, PDMANABHAN VM. The crystal and molecular structure of a guaianolide. 1991; 54:1397-9.
- Maobe MAG, Nyarango RM, Box PO. Fourier Transformer Infra-Red Spectrophotometer Analysis of *Warburgia ugandensis* Medicinal Herb used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. 2013; 7:61-8.
- Bihani GV, Rojatar SR, Bodhankar SL. Innovare Academic Sciences. Investigation of *in-vivo* analgesic and anti-inflammatory activity in rodents and *in-vitro* antioxidant activity of extracts of whole plant of *Cyathocline*, 2014, 6.
- Hudson A, Lopez E, Almalki AJ, Roe AL, Calderón AI. A Review of the Toxicity of Compounds Found in Herbal Dietary Supplements. *Planta Medica*. 2018; 84:613-26.
- Kaur V, Hallan SS, Nidhi, Kalia AN, Mishra N. Isolation of embelin from and evaluation of its anti-cancer potential in *Embelia ribes* breast cancer. *Asian Journal of Pharmacy and Pharmacology*. 2015; 1:33-9.
- Oelberg DG, Chari MV, Little JM, Adcock EW, Lester R. Lithocholate glucuronide is a cholestatic agent. *Journal of Clinical Investigation*. 1984; 73:1507-14.
- Bultman SJ. The Microbiome and Its Potential as a Cancer Preventive Intervention. *Seminars in Oncology*. 2017; 43:97-106.
- Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M *et al.* Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice. *Diabetes*. 2009; 58:1509-17.
- Namkung H, Yu H, Gong J, Leeson S. Antimicrobial activity of butyrate glycerides toward *Salmonella*



- typhimurium* and *Clostridium perfringens*. Poultry Science. 2011; 90:2217-22.
15. Huang CB, Altimova Y, Myers TM, Ebersole JL. Short- and medium- chain fatty acids exhibit antimicrobial activity for oral microorganisms. Archives of Oral Biology. 2011; 56:650-4.
  16. Mayer AMS, Gustafson KR. Marine pharmacology in 2001-2: Antitumour and cytotoxic compounds. European Journal of Cancer. 2004; 40:2676-704.
  17. Farrell EK, Chen Y, Barazanji M, Jeffries KA, Cameroamortegui F, Merkler DJ. Primary fatty acid amide metabolism: conversion of fatty acids and an ethanolamine in N<sub>18</sub> TG<sub>2</sub> and SCP cells. Journal of Lipid Research. 2012; 53:247-56.
  18. Mohammad Nasir Abdullah, Bee Wah Yap, Yuslina Zakaria ABAM. Metabolites selection and classification of metabolomics data on alzheimer's disease using random forest. In Soft Computing in Data Science - 2nd International Conference, SCDS, Proceedings. 2016; 652:100-12.
  19. Maccarrone M, Finazzi-Agró A. The endocannabinoid system, anandamide and the regulation of mammalian cell apoptosis. Cell Death and Differentiation. 2003; 10:946-55.
  20. Bluett RJ, Gamble-George JC, Hermanson DJ, Hartley ND, Marnett LJ, Patel S. Central anandamide deficiency predicts stress-induced anxiety: Behavioral reversal through endocannabinoid augmentation. Translational Psychiatry. 2014; 4:e408-5.
  21. Jarbas M, Siqueira Junior, Peters RR, B-FA, R-VR. Effects of valeryl salicylate, a COX-1 inhibitor, on models of acute inflammation in mice. Pharmacol Res. 2003; 48:437-43.
  22. Poddar VI, SS. Update on nonoxynol-9 as vaginal spermicide. The European Journal of Contraception & Reproductive Health Care. 2008; 13:339-50.
  23. Dold M, Samara MT, Li C, Tardy M, Leucht S. Haloperidol versus first-generation antipsychotics for the treatment of schizophrenia and other psychotic disorders. Cochrane Database of Systematic Reviews. 2015; 2017:9-10.
  24. Goggi A, Malpathak N. Antioxidant Activities of Root, Stem and Leaves of *Vernonia cinerea* (L) Less. Free Radicals and Antioxidants. 2017; 7:178-83.
  25. Bhuktar AS. Phytochemical screening of curcuma caulina J Graham rhizome phytochemical screening of *Curcuma caulina* J Graham rhizome extract by using HPLC-MS technique, 2018.
  26. Aravindhana R, Sreelatha T, Perumal PT, Gnanamani A. Synthesis, characterization and biological profile of metal and azo-metal complexes of embelin. Complex Metals. 2014; 1:6-79.