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Marine halophyte derived polyphenols inhibit glioma cell growth through mitogen-activated protein kinase signaling pathway

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ABSTRACT

Plants that are pharmacologically significant require intensive phytochemical characterization for bioactive profiling of the compounds, which has enabled their safe use in ayurvedic medicine. The present study is focused on the phytochemical analyses, quantitative estimation and profiling of secondary metabolites of leaf extract, as well as the antioxidant and cytotoxic activity of the potent halophytes such as *Avicennia marina, Ceriops tagal, Ipomoea pes-caprae,* and *Sonneratia apetala.* The in vitro antioxidant property was investigated using DPPH, ferric reducing antioxidant capacity (FRAP) assay. Bioactive compounds such as phenols, flavonoids, saponin and alkaloids were quantitatively estimated from the extracts of *A.marina, C.tagal, I.pes-capra* and *S.apetala*, which possessed higher phenol content than the other studied halophytes. The extracts at 200 μg/ml revealed higher antioxidant activity than the standard ascorbic acid and it functions as a powerful oxygen free radical scavenger with 77.37%, 75.35% and 72.84% for *S.apetala, I.pes-caprae* and *C.tagal* respectively and with least IC_{50} for *I.pes-caprae* (11.95 μg/ml) followed by *C.tagal* (49.94 μg/ml). Cell viability and anti-proliferative activity of different polyphenolic fractions of *C.tagal* (CT1 and CT2) and *I.pes-caprae* fraction (IP) against LN229, SNB19 revealed *Ipomoea* as the promising anti-cytotoxic fraction. IP-derived polyphenols was further subjected to apoptosis, migration assay, ROS and caspase − 3 and − 7 to elucidate its potentiality as a therapeutic drug. IP-polyphenols was found to have higher percentage of inhibition than the CT1 and CT2 polyphenols of *C.tagal* on comparison with TMZ. All the above-mentioned in-vitro analysis further validated the ability of IP-polyphenols inducing cell death via ROS-mediated caspase dependent pathway. Further, proteomic and phospho-proteomic analysis revealed the potential role of IP-polyphenols in the regulation of cell proliferation through MMK3, p53, p70 S6 kinase and RSK1 proteins involved in mitogen-activated protein kinase signaling pathway. Our analysis confirmed the promising role of *I.pes-caprae* derived polyphenols as an anti-metastatic compound against GBM cells.

1. Introduction

Natural products from traditional Indian medicine play an important source of new drugs and drug intermediates. Halophytes are widely used as folk medicine to treat various diseases, microbial infections, thus remains a major source to cure minor and major ailments [1]. About 65% of Indian population depends on traditional medicines [2], primarily for health care benefits as well to gain economic values [3]. The marine halophytes contain rich bioactive compounds; yet, the usage of these medicinally significant plants demands effective separation and therapeutic validation.

The present study was aimed at investigating the therapeutic value of traditional and medicinally important halophytic plants in and around the coastal regions. Earlier, we have reported the systematic

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documentation to conserve these medically important plants [4]. The present investigation isolated bioactive compounds from the selected halophytes, *Avicennia marina, Ceriops tagal, Ipomoea pes-caprae,* and *Sonneratia apetala* using HPLC and HPTLC. Secondary metabolite profiling was also performed using GC-MS to identify the significant compounds and their structures.

Bioactive compounds are used to prevent various diseases, especially cancer by regulating the expression and activity of transcription factors, growth factors, inflammatory mediators, and cell cycle intermediates. The polyphenols from plant sources are known to be the potent antioxidants which may exert chemo-preventive role towards degenerative diseases [5]. Among the various kinds of natural antioxidants in marine halophytes, polyphenolic compounds constitute the core potent secondary metabolite owing to their multiple applications as additives in food industry, cosmetics, pharmaceutical and medicinal industries [5]. The polyphenol consumption could reduce the risk of developing breast cancer among postmenopausal women [6]. Polyphenols have the ability to disturb or disorganize series of oxidative reaction in constituents of cells and thus prevents various degenerative diseases and different types of cancer [7].

The glioblastoma multiforme (GBM) is a highly malignant type of astrocytoma and is most commonly diagnosed in older adults [8]. Despite intensive research, GBM, a malignant brain tumor is difficult to treat due to high resistance to conventional therapeutic approaches. Resveratrol is a plant polyphenol, that can cross the blood-brain barrier and has shown promising results both in-vivo and in-vitro against brain cancer treatment, yet needs clinical validation [9]. The current treatment of GBM involves surgery followed by radiation and chemotherapy. Temozolomide (TMZ), an orally administered DNA alkylating/methylating agent is the most commonly used drug against GBM that targets the proliferating cells. Higher TMZ doses may lead to systemic toxicity, BBB impermeability and development of refractory tumors [10]. Therefore, natural product-based therapy has gained much popularity due to its efficacy and less toxicity. Thus, the present investigation is focused on isolating and validating the therapeutic potential of significant bio-active compounds, especially polyphenols from the marine halophytes against GBM.

2. Materials and methods

2.1. Plant materials

Ten different halophytes selected for the present study were *Arthrocnemum indicum, Avicennia marina, Cressa cretica, Ceriops tagal, Sonneratia apetala, Salicornia brachiata, Sesuvium portulacastrum, Suaeda maritima, Suaeda monoica* and *Ipomoea pes-caprae.*

2.2. Chemicals

Wagner's reagent, Sodium hydroxide, Ferric chloride, Ninhydrin reagent, α-naphthol solution, Nitric acid, Sulfuric acid, Hydrochloric acid, Folin–Ciocalteu reagent, Sodium carbonate, Gallic acid, Vanillin reagent, Diosgenin, Ammonium hydroxide, sodium nitrite, aluminum chloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), Potassium ferricyanide, Phosphate buffer (pH 7.4), Trichloroacetic acid, Rutin, Ascorbic acid, Silica gel G, hexane, methanol, TMZ (Temozolomide), Methanol (HPLC grade, Merck), Cyclohexane, ethyl acetate, Formic acid, Ferric chloride, Silica plate 60 F_{254} (Merck), Whatman No.1 filter paper.

2.3. Sample collection

The halophytes were collected from the natural environment distributed along the coastal areas of Tamil Nadu such as Perungulam and Mandapam coast of Ramanathapuram district, Parangipettai, Pachyankuppam, Killai and Samiyaarpettai of Cuddalore district. The collected plants were taxonomically identified with the voucher specimen assigned and the herbarium was deposited in the Research Department of Zoology and Biotechnology, Lady Doak College, Madurai, India [4].

2.4. Preparation of plant extracts

The collected and pre-processed leaf samples were finely powdered and about 10 g of each sample was extracted with 250 ml of different solvents such as ethanol, methanol, and water at 40°C for 3 h using a hot plate magnetic stirrer and re-extracted using the respective solvents. The samples were then cooled and centrifuged at 4500 rpm for 15 min to recover the supernatant. The coarse filtrate was filtered using Whatman No.1 filter paper and evaporated to dryness under vacuum using rotary evaporator. The filtrate was then stored at 4°C for further analysis [11].

2.5. Qualitative phytochemical screening

Preliminary screening of the ten different phytochemicals Alkaloids, flavonoids, tannins, terpenoids, phytosterol, saponin, phenol, proteins and amino acids, carbohydrates and steroids was done to assess the presence of secondary metabolites using the following standard protocols [12].

2.6. Quantitative estimation of leaf extracts of halophytes

2.6.1. Determination of total phenol content (TPC)

Folin-Ciocalteu colorimetric method with modification was performed to determine the total phenolic content of the leaf extracts [13]. Briefly, 100 μl of the crude extract (1 mg/ml) and the gallic acid as standard was mixed thoroughly with 1 ml of Folin–Ciocalteu reagent (1:2 w/v) for 5 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate (Na_2CO_3). The mixture was then allowed to stand for 60 min at room temperature, and the absorbance at 715 nm was measured using UV-Vis Spectrophotometer. The total phenol content was expressed as gallic acid equivalents (mg GAE/g extract) derived from the standard curve.

2.6.2. Determination of total flavonoid content

The total flavonoid content was estimated using the procedure as described [14]. A total of 1 ml of the crude leaf extract and the rutin as standard was diluted with 200 μl of distilled water followed by the addition of 150 μl of sodium nitrite (5% w/v) solution. This mixture was incubated for 5 min followed by the addition of 150 μl of aluminum chloride (10% w/v) solution and allowed to stand for 6 min. To this, 2 ml of sodium hydroxide (4% w/v) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and incubated for 15 min at room temperature. Appearance of pink color indicates the presence of total flavonoids content and the absorbance was measured at 510 nm. The total flavonoids content was expressed as rutin equivalent (mg RE/g extract) using the standard curve.

2.6.3. Determination of total saponin content

Total saponin content was determined based on vanillin-sulfuric acid colorimetric reaction [15], with minor modifications. About, 100 μl of

the crude leaf extract and the diosgenin as standard was mixed with 250 μl of (8%) vanillin reagent (0.8 g of vanillin in 10 ml of 99.5% ethanol). Then, 2.5 ml of 72% sulfuric acid (w/v) was added and mixed well. This solution was kept in water bath at 60°C for 10 min. It was cooled in ice cold water and the absorbance was read at 544 nm using UV-Vis spectrophotometer. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from the standard curve.

2.6.4. Determination of alkaloids

Five grams of powdered leaf samples was stirred gently with 200 ml of glacial acetic acid, covered and kept still stand for 4 h. The filtered solution was reduced and concentrated to one quarter on a water bath. To this sample, concentrated ammonium hydroxide was added drop wise until the precipitate formed. The whole solution was allowed to settle and the precipitate was collected by filtration and washed with aqueous ammonium hydroxide. The filtrate was then dried and weighed [16]. The percentage of total alkaloid content was calculated using the following equation.

$$\text{Percentage of total alkaloids}(\%) = \frac{Weight of the residue}{Weight of the sample taken} \times 100 \quad (1)$$

2.7. In vitro antioxidant activities of potent leaf extracts

2.7.1. DPPH radical scavenging activity

The ability of crude leaf extracts to scavenge the 2,2-diphenyl-1-picryl-hydrezyl (DPPH) radicals was assessed using the modified method [17]. DPPH (100 μM) dissolved in methanol was used as a free radical and 100 μl of DPPH solution was added to different concentrations of the extracts (5 μg, 25 μg, 50 μg, 100 μg and 200 μg/ml) and the standard. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. Control was also prepared as above but without sample extracts. Then absorbance was measured at 517 nm using plate reader (Labtronics (LT-1260), India). The lower absorbance value indicates the higher radical scavenging activity. Ascorbic acid was used as the standard antioxidant. The ability of DPPH radical scavenging activity was calculated using the following equation.

$$\text{DPPH scavenging effect}(\text{inhibition}\%) = 1 - \frac{A1}{A0} \times 100 \quad (2)$$

where, A0 is the absorbance of the control, A1 is the absorbance of the sample extracts. The IC_{50} (the microgram of extract to scavenge 50% of the radicals) value was calculated using linear regression analysis.

2.7.2. Reducing power activity

Reducing power ability of the crude leaf extract was determined by modified protocol [18]. Varying concentration of the extract ranging from 100 μg, 200 μg, 300 μg, 400 μg and 500 μg/ml was mixed with 1 ml of phosphate buffer (pH 7.4) and 1 ml of freshly prepared potassium ferricyanide (1%). The mixture was incubated in water bath at 50 °C for 20 min followed by the addition of 1 ml of 10% trichloroacetic acid and centrifuged at 3000 r/min for 10 min. Then, 2 ml of supernatant was mixed with equal volume of distilled water and 500 μl of freshly prepared 1% ferric chloride. The absorbance was read at 700 nm, where the higher absorbance of the extract is directly proportional to the higher reducing power. The results were compared with the standard, rutin.

2.8. Partial purification of polyphenolic compound using column chromatography

Air dried leaf powder of *Avicennia marina, Ceriops tagal, Ipomoea pes-caprae* and *Sonneratia apetala* was extracted using suitable solvent and vacuum dried before loading into the column. Dried leaf extract was mixed with silica gel to make fine powder for easy distribution of sample. Gradient solvent system from non-polar to high polar solvent system provides best elution and best separation of various organic compounds from any plant-based organic extract. Hence, gradient solvent system including hexane, hexane: ethyl acetate, ethyl acetate, ethyl acetate: methanol and methanol in different ratio and volume based on the amount of sample was used. Completely dried leaf extract sample (5 g) was loaded on a glass column (60 ×3 cm) packed with silica gel G (40 g, 60–120 μm mesh) as stationary phase. When the sample had adsorbed to the silica gel, small amount of cotton was placed at the top to cover the sample. The mobile phase was poured continuously on the top of the column by aid of a funnel. Solvents of different polarities such as hexane, methanol was passed through column at uniform rate under gravity to fractionate the sample extract [19]. Each fraction was collected separately in a test tube and numbered consecutively for further confirmation on thin layer chromatography. Fractions were collected and pooled together on the basis of similar retention factor (Rf) and the solvent was removed by freeze drying and the sample was lyophilized, weighed and stored for further analysis.

2.9. HPTLC fingerprinting of purified fractions from selected halophytes

The HPTLC analysis was performed following the protocol of Kandler et al. [20], on aluminum plates pre-coated with silica gel 60 F254, Merck, Germany. The samples were applied using Camag syringe (Switzerland), mounted on Linomat V applicator. Application of bands of each extract was carried out using spray technique. The purified samples were applied as 3 μg/ml concentration from 1 mg/ml stock on the plate of 10 × 10 cm as bands of 8 mm width. The plates were then developed in a CAMAG twin trough chamber previously equilibrated with a mobile phase for 20 min. Phenols were separated using the solvent system cyclohexane: ethyl acetate: formic acid (4:6:1 v/v/v). The plate was developed up to 7 cm, air dried, viewed and scanned at wavelength (λ_{max}) of 254, 366 and 540 nm using CAMAG TLC Scanner 4 which was programmed through vision CATS software (Version 3.1).

2.10. Cytotoxicity assays

2.10.1. Cell culture

Human glioblastoma cell lines, LN229, SNB19 and mouse embryonic fibroblast (MEF) cells obtained from Dr.Kirsi Granberg (Faculty of Medicine and Health Technology, Tampere, Finland) were used to test the anticancer effect of purified fractions of *Ceriops tagal*, CT1, CT2 and *Ipomoea pes-caprae*, IP fraction. The LN229 cell was established from a patient with right frontal parieto-occipital glioblastoma with mutated p53 (TP53) and homozygous deletions in the p16 and p14ARF tumor suppressor genes. The SNB19 has mutation in phosphatase and tensin homolog (PTEN), telomerase reverse transcriptase (TERT) and also in TP53. MEF cells exhibit an E10.5 genotype with Vin + /+ (vinculin), and has p53 function, providing a preliminary model for comparing p53 activity, and was obtained from Wolfgang H. Ziegler (Hannover Medical School, Hannover, Germany) [21,22]. The Dulbecco's Modified Eagle Medium-high glucose (DMEM) (Biowest, #L0102–500) supplemented with 10% Fetal Bovine Serum (FBS) (Biowest, #S181H-500), 100 U/ml Penicillin, 0.1 mg/ml Streptomycin (Sigma-Aldrich, #P4333), 0.025 mg/ml Ampicillin B (Sigma-Aldrich, #A9528), 0.05% 1x Trypsin/EDTA (ThermoFisher Scientific, #25300), was used to grow theses cell lines. Cell cultures were incubated at 37 °C supplemented with 5% CO_2 in a humidified incubator. Cultures were passaged at 70% confluence, following standard cell passaging protocol.

2.10.2. Dose dependent assay

Dose-dependent inhibition assay was done using the IC_{50} concentration for the fraction C1, C2 and IP fraction, where the percentage of growth inhibition is correlated with dosage of the compound [23]. The LN229 and SNB19 cells were seeded on 12-well plates with a density of 1×10^5 cell/well. After 48 h, the cells were treated with 0.5 μg, 1.0 μg, 5 μg, 10 μg and 15 μg/ml concentrations of C1, C2, IP fractions and

TMZ. TMZ was used as a positive control and DMSO (0.1%) as negative control. Cell viability was quantified after 48 h using the trypan blue exclusion method. The live and dead cell populations were counted using a Countess II FL Hemocytometer (ThermoFisher Scientific, #A25750) and the percentage of cell growth inhibition was calculated using the equation given below.

$$\textbf{Cellgrowthinhibition}(\%) = \frac{\textbf{\textit{MeanNo.of untreatedcells(DMSOcontrol)}} - \textbf{\textit{MeanNo.of treatedcells}} \times \mathbf{100}}{\textbf{\textit{MeanNo.of untreatedcells(DMSOcontrol)}}} \quad (3)$$

Three biological and two technical repeats were maintained to obtain the statistical data. Semi-log dose-response curves were plotted to calculate the half-maximal inhibitory concentration (IC_{50}) of the compounds specific for LN229, SNB19 cells. The cytotoxicity of the C1, C2 and IP fraction at a concentration of 1.0 μg was evaluated in non-cancerous cell line, MEF. The compound that showed higher cytotoxic effect against LN229, SNB19 with minimum effect on MEF cells were selected for further in-vitro analysis.

2.11. GC-MS analysis of bioactive compounds

The profiling of secondary metabolites from the methanol extract of *Ceriops tagal* (CT1 fraction) and *Ipomoea pes-caprae* (IP fraction)that showed the best cytotoxicity results were selected for GC-MS analysis (Agilent technologies, mass hunter software). Experimental conditions of GC-MS system include, TR 5-MS capillary standard non-polar column with the dimension of 30 Mts, ID: 0.25 mm, and 0.25 μm film thickness was used. Flow rate of mobile phase with helium as carrier gas was set at 1.0 ml/min. In the gas chromatography, temperature program with 40 °C was raised to 250 °C at 5 °C/min and 1 μl injection volume was used. Samples dissolved in chloroform were run fully at a range of 50–650 *m/z* and the results were compared using Mass hunter software [24] and the structures were drawn using SwissADME [25].

2.12. Caspases 3/7 activity assay

The Caspases 3/7 Activity was performed in LN229 and SNB19 cells. The cells were seeded on 96-well plates in complete medium with an initial density of 1×10^4 cells/well and incubated overnight at 37 °C with 5% CO_2. The cells were treated with IC_{50} concentration of the C1 and IP fraction along with DMSO (0.1%) and untreated cells and incubated for 5 h. The cells were allowed to equilibrate at room temperature for 30 min and 100 μl of Caspase-Glo reagent (Glo® 3/7 Assay kit, Promega, Madison USA) was added to the treated, untreated, and blank wells. The plates were placed on an agitator for 30 s at 300–500 rpm and incubated for 1 h in dark conditions. After incubation, the luminescence signal was measured using a Luminometer plate reader (Tecan, Spark). The fold change in caspase 3/7 was calculated using the following equation.

$$\%ChangeofActivity = \frac{(F_{test} - F_{blank})}{(F_{control} - F_{blank})} * 100 \quad (4)$$

where F_{test} is the luminescence from the treated wells, $F_{control}$ is the luminescence from the untreated wells, and F_{blank} is the luminescence from the unstained wells.

2.13. Reactive oxygen species (ROS) assay

LN229 and SNB19 cells were seeded in 12-well plates with an initial density of 1×10^5 cells/well in complete medium and incubated overnight at 37 °C with 5% CO_2. After incubation, the medium was replaced with serum-free medium upon treatment. The treatment conditions were maintained as mentioned by Doan et al., 2020. The cells were treated with the IC_{50} concentration of polyphenol fractions of CT1, IP. In addition, 30% H_2O_2 was used as a positive control. After the treatment, the plate was incubated for 5 h at 37 °C with 5% CO_2. Cells were trypsinized and centrifuged at 3000 rpm for 10 min. The supernatant was discarded, and the cells were resuspended in 2 μM 2′,7′-dichlorodihydrofluoresceindiacetate (H_2DCFDA) [27]. Further, the cells were incubated for 10 min in dark condition, washed with 500 μl of pre-warmed PBS and finally centrifuged at 3000 rpm for 10 min. Cells were recovered in a 100 μl serum free medium and incubated in a 96 black well plate for 20 min at 37 °C with 5% CO_2. Fluorescent intensity was measured using Luminometer plate reader (Tecan, Spark) at 485 nm and 538 nm. The fold change of intracellular ROS was calculated using the Eq. (4) as mentioned above.

2.14. Apoptosis

LN229 and SNB19 cells at the initial density of 5×10^5 were seeded in 6 well plates and treated with IP fraction as described by Doan et al., 2020. The induction of apoptosis was done using Dead Cell Apoptosis Kit with Annexin-V/FITC and PI (ThermoFisher Scientific, Waltham, MA, USA) following the manufacturer's protocol.

2.15. Migration assay

Migration was done in both SNB19 and LN229 cells to investigate the chemotactic capability and migration of cells through the extracellular matrix using the IC_{50} concentration of IP polyphenol fraction. The experiment was performed as previously described [26]. The assay was performed in 6-transwell plates ($n = 6$) with the pore size of 8 μM (Corning Life Science, St. Louis, MO, USA).

2.16. Protein array

Human Phospho-MAPK Array is used to simultaneously detect the relative levels of phosphorylation of 26 kinases including nine MAPKs (R&D systems, Minneapolis, MN, USA). The array can capture 26 different MAPK antibodies in duplicates on nitrocellulose membrane. LN229 cells at a density of 1×10^7 cells/ml were treated with IC_{50} concentration of IP fraction and DMSO as the control for 48 h. The procedure was performed according to the manufacturer's protocol. Briefly, the cell lysates along with the cocktail of biotinylated detection antibodies were incubated with Proteome Profiler Human Phospho-MAPK Array. Streptavidin-HRP and chemiluminescent detection reagents are added which produce the signals at each spot that correspond to the amount of phosphorylated protein. Images were captured using XENOGEN (Vivo Vision IVIS Lumina, Männedorf, Switzerland) and the data was analyzed using ImageJ software.

2.17. Statistical analysis

The data analysis was performed using GraphPad prism software 8.0 (Graphpad software Inc. San Diego, CA, USA). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by

Table 1
Qualitative phytochemical screening of halophytes: Qualitative phytochemical screening showed the presence of active pharmacological components such as alkaloid, flavonoid, tannin, terpenoids, phenol, carbohydrates, proteins, amino acids, saponins and steroids. Extraction was done using ethanol (E), methanol (M) and aqueous (A) from the selected halophytes. Symbols represents the presence of secondary metabolites based on the color change, (-): Negative test, (+): Weak positive test (if the reagent has slight opacity), (++): Positive, (+++): Strongly positive. The halophytes selected for the study were represented as follows. *Arthrocnemum indicum* (AI), *Avicennia marina* (AM), *Cressa cretica* (CC), *Ceriops tagal* (CT), *Ipomoea pes-caprae* (IP), *Salicornia brachiata* (SB), *Sesuvium portulacastrum*(SP), *Sonneratia apetala* (SA), *Suaeda maritima* (SMA), *Suaeda monoica* (SMO).

S. NO	Plants	Alkaloids			Flavonoids			Tannin			Terpenoids/ Triterpenes			Phenol			Carbohydrate			Proteins			Amino acid			Saponin			Steroids		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
1	***Arthrocnemum indicum***	++	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	+	-	-	-
2	***Avicennia marina***	++	++	++	++	++	.	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	++	-	++	-	-	-
3	***Cressa cretica***	++	++	++	++	++	+	-	.	-	-	.	+	++	++	-	-	-	-	-	+	+	-	-	-	++	.	++	-	-	-
4	***Ceriops tagal***	++	+	+	+	++	++	-	+	+	-	+	+	+	++	-	-	-	-	+	+	+	-	+	++	+	+	+	-	-	-
5	***Ipomoea pes-caprae***	++	++	+	++	++	++	-	+	+	-	+	+	++	++	+	-	-	+	-	+	+	-	++	++	-	-	-	-	-	-
6	***Salicornia brachiata***	++	++	++	+	+	++	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	+	-	++	-	-	-
7	***Sesuvium portulacastrum***	++	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	++	+	-	-	-	-
8	***Sonneratia apetala***	++	++	++	+	+	+	-	-	-	+	+	-	++	++	++	-	+	-	-	-	+	-	-	-	+	+	++	+	+	-
9	***Suaeda maritima***	++	++	+	+	++	+	-	-	-	-	+	-	+	++	-	-	-	-	-	+	+	-	+	-	+	.	+	-	-	-
10	***Suaeda monoica***	++	++	++	+	+	+	-	+	-	-	-	+	++	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-

Tukey Post-Hoc test for the quantitative estimation and Dunnet's for the in-vitro analysis. The results were expressed as mean±standard deviation (S.D.) of triplicate determinations (n = 3). The value of *$p < 0.05$, * *$p < 0.01$were considered significant and ns as non-significant. The IC_{50} for the in-vitro studies were calculated using dose-response curve.

3. Result and discussion

3.1. Qualitative phytochemical screening

In our previous study, we have surveyed the halophytes with ethnomedicinal value along the coastal regions of Tamil Nadu (Murugesan et al., 2020). Out of 20 different plants, the following halophytes such as, *Arthrocnemum indicum, Avicennia marina, Cressa cretica, Ceriops tagal, Ipomoea pes-caprae, Salicornia brachiata, Sesuvium portulacastrum, Sonneratia apetala, Suaeda maritima, Suaeda monoica* were selected for the present study based on the availability, high medicinal significance and the traditional uses among the local inhabitants (Supplemental file 1). The preliminary phytochemical screening of ethanol, methanol and aqueous extracts of halophytes were tabulated (Table 1)**.** The leaf extracts were qualitatively analyzed for the presence of ten different phytochemicals such as phenols, flavonoids, terpenoids, tannin, saponin, alkaloids, carbohydrate, protein, amino acids and steroids. Among all these halophytes, phenolic compounds were present in both methanol and ethanol extracts except the aqueous extract of *Avicennia marina, Cressa cretica, Ceriops tagal, Sesuvium portulacastrum, Suaeda monoica* and *Suaeda maritima*. Similarly, flavonoids were present in both methanol and ethanol extracts of all the plants except the aqueous extract of *Avicennia marina*. Saponins were present in the aqueous extracts of almost all the extracts except *Ipomoea pes-caprae, Sesuvium portulacastrum* and *Suaeda maritima*, whereas in ethanol extracts, except *Arthrocnemum indicum* and *Ceriops tagal,* all other extracts contain saponin. Similarly, alkaloids were present in all the three extracts of halophytes, while steroids which have cholesterol reducing property are present only in ethanol and methanol extract of *Sonneratia apetala*.

It was evident from the preliminary qualitative data, that the polarity of the solvent plays a significant role in extracting lipophilic and hydrophilic compounds and hence different metabolites were extracted in different solvents. Several studies show that methanol and ethanol can dissolve polar compounds such as, phenols [28], flavonoids [29], sugar, amino acids, glycosides, anthocyanin, terpenoid, saponin, tannin and polyphenol [30]. The polyphenol content may also vary according to the biotic conditions such as species, organ, physiological state and abiotic conditions like season, climate, and temperature [31]. Therefore, based on the primary qualitative phytochemical screening of secondary metabolites, all these 10 halophytes were subjected for further quantitative analysis.

3.2. Quantitative estimation of secondary metabolites

Total phenolic content (TPC), Total flavonoid content (TFC) and Total saponin content (TSC) of ethanol, methanol and aqueous extracts for 10 different halophytes were performed.

3.2.1. Total phenolic content

The total phenolic content was expressed in milligram of gallic acid equivalents per gram of extract. Total phenol content ranged between 1.29 and 42.58 mg GAE/g extract. Among the different extracts of these plants, methanol extract exhibited higher phenolic content than ethanol and aqueous extracts, which might be due to the difference in the polarity of the solvents used. Thus, the phenolic content was found to be more in the leaf extracts of *Ipomoea pes-caprae* followed by *Sonneratia apetala, Ceriops tagal* and *Avicennia marina* with the concentration of 42.58 mg GAE/g, 30.2 mg GAE/g extract, 27.39 mg GAE/g and 16.42 mg GAE/g extract respectively. Reduced level of phenolic content was observed in aqueous extract of *Arthrocnemum indicum* (1.29 mg

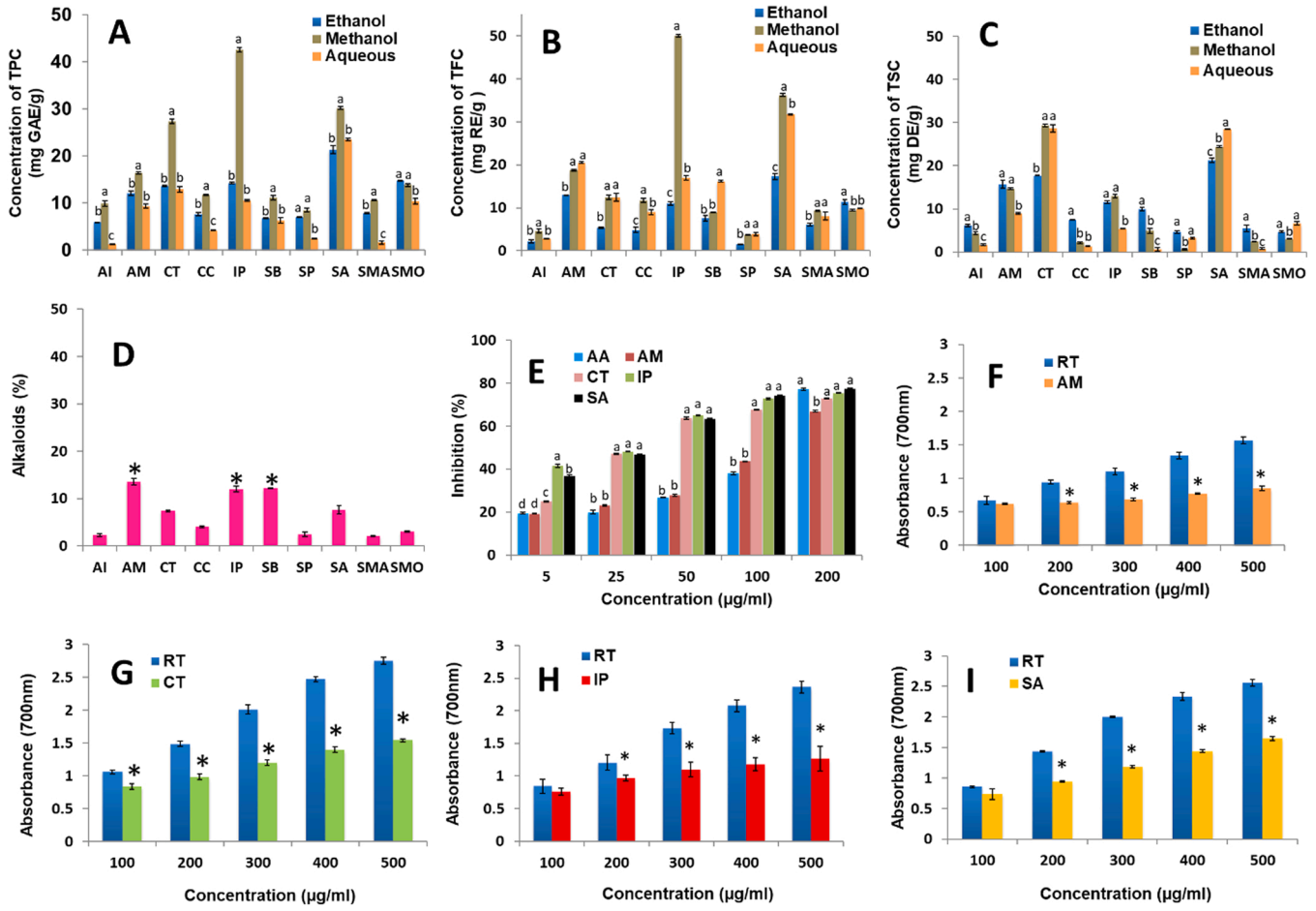

Fig. 1. Quantitative estimation and in-vitro antioxidants assay of potential secondary metabolites from halophytes. Quantitative estimation of (A) total phenol content (TPC), (B) total flavonoid content (TFC), (C) total saponin content (TSC) was estimated from 10 different halophytes. The lowercase different letters refer significant statistical results (Tukey test) for TPC, TFC and TSC with $p < 0.05$. The lowercase same letters represent non-significance; (D) total alkaloid (%) was estimated for all the 10 halophytes; (E) In-vitro antioxidant DPPH assay was determined from the top potential halophytes *Avicennia marina* (AM), *Ceriops tagal* (CT), *Ipomoea pes-caprae* (IP), *Sonneratia apetala* (SA) and Ascorbic acid (AA) as standard. *$p < 0.05$ for AA vs. SA; **$p < 0.01$ for AM vs. CT; **$p < 0.01$ for AM vs. SA (Tukey test); (F, G, H & I) Reducing power assay for these selected halophytes was performed with Rutin (RT) (µg/ml) as standard with *$p < 0.05$ (Two-way ANOVA). All the experiments were performed in biological triplicates (n = 3) and technical repeats (n = 3). The values were represented as mean ± SD.

GAE/g extract) (Fig. 1A).

3.2.2. Total flavonoids content

The total flavonoid content was expressed in milligram of Rutin (RE) equivalents per gram of extract. The total flavonoid content also varied between 1.44 and 50.74 mg RE/g extract in all the plants analyzed. High level of flavonoids was observed in methanolic extract of *Ipomoea pes-caprae* with the concentration of 50.74 mg RE/g extract and 36.22 mg RE/g extract for *Sonneratia apetala*. The aqueous extracts of *Sonneratia apetala* has 31.71 mg RE/g extract and *Avicennia marina* has 20.5 mg RE/g extract while least level was observed in *Sesuvium portulacastrum* with 1.44 mg RE/g extract (Fig. 1B).

3.2.3. Total saponin content

The total saponin content was expressed in milligram DE (diosgenin) equivalents per gram of extract. The total saponin content was found to be higher in methanol, aqueous, and ethanol extract of *Ceriops tagal* with about 29.33 mg DE/g, 28.66 mg DE/g, 17.73 mg DE/g extract respectively and *Sonneratia apetala* with 24.42 mg, 28.6 mg, 21.21 mg DE/g extract respectively (Fig. 1C).

3.2.4. Percentage of alkaloids

High quantity of alkaloid was obtained in *Avicennia marina* (13.6%), *Salicornia brachiata* (12.2%) and *Ipomoea pes-caprae* (12%), while low amount was observed in *Suaeda maritima* (2%) (Fig. 1D).

Although quantitative estimation of phenol, flavonoid, saponin, and alkaloid revealed the presence of these secondary metabolites, significant amount of phenolic content was observed in *Ipomoea pes-caprae, Sonneratia apetala, Ceriops tagal* and *Avicennia marina.* The phenol serves as the major class among all the secondary metabolites. The hydroxyl group of phenols confers scavenging capability which acts as a potent antioxidant [32]. The ability of phenolic compounds with potential antioxidant activity to quench the free radicals produced due to oxidative stress, focused our further analysis on the phenolic compounds of these four plants. Hence, these selected halophytes have been further validated for the probable mechanism of phenolic compounds by which they act to exert its pharmacological properties.

3.3. In vitro antioxidant activities of potent halophyte leaf extracts

3.3.1. DPPH radical scavenging activity

DPPH radical scavenging activity is the most widely used and sensitive method to determine the antioxidant activity [33]. DPPH is a stable free radical, which is used to quantify the percentage of antioxidants produced by the plants to quench the free radical. The DPPH activity was performed for the methanol extracts of *Avicennia marina, Ceriops tagal, Ipomoea pes-caprae* and *Sonneratia apetala* based on the results obtained from the above-mentioned quantitative estimation.

Ascorbic acid was used as a standard. The percentage of inhibition was found to be increased as the concentration of the plant extract increased from 5 to 200 μg/ml. The spectrophotometric analysis revealed that *Ipomoea pes-caprae, Sonneratia apetala* and *Ceriops tagal* possess significant antioxidant capacity. At 200 μg/ml concentration, the % of inhibition for positive control was 77.01%, whereas *Sonneratia apetala* exhibited 77.37% and *Ipomoea pes-caprae* showed 75.35% while, 72.84% and 66.95% of inhibition was observed for *Ceriops tagal* and *Avicennia marina* respectively. The IC_{50} value was found to be lower for *Ipomoea pes-caprae* (11.95 μg/ml) followed by *Sonneratia apetala* (26.23 μg/ml), *Ceriops tagal* (49.94 μg/ml) and *Avicennia marina* (123.01 μg/ml) at a concentration of 200 μg/ml of methanolic extracts (Fig. 1E). It was clearly evident from the previous qualitative and quantitative data, that a strong relationship between the phenol content and the antioxidant activity was observed, and thus possess strong scavenging ability for free radicals due to their hydroxyl groups [34,35].

3.3.2. Reducing power assay

Reducing power assay serve as a significant reflection of the antioxidant activity. Compound with higher reducing power reduces the oxidized intermediate and serves as the primary and secondary antioxidants [36]. In the present study, *Avicennia marina, Ipomoea pes-caprae, Ceriops tagal* and *Sonneratia apetala* showed higher reducing potential. Methanolic extracts of *Sonneratia apetala* exhibited higher reducing potential (Fig. 1F) followed by *Ceriops tagal* (Fig. 1G), *Ipomoea pes-caprae* (Fig. 1H) and *Avicennia marina* (Fig. 1I). A higher absorbance value was observed as the concentration of the plant leaf extracts increases, which in turn confirms the concentration-dependent reducing power. Thus, these selected halophytes are believed to contain good reducing capability with potent antioxidant activity, which has been further subjected to pharmacological analysis.

3.3.3. Chromatography analysis of polyphenolic compounds from the top leading halophytes

Based on the qualitative and quantitative phytochemical analysis, *Avicennia marina, Ceriops tagal, Ipomoea pes-caprae* and *Sonneratia apetala* were selected for further chromatographic analysis. Crude extracts of all the four halophytes were subjected to HPLC analysis and the chromatogram confirmed the presence of phenolic compounds at 280 nm (Fig. 2A). The HPLC chromatogram of *Avicennia marina* showed 3 peaks with retention time (RT) 3.306, 5.766, 6.643, 2 major peaks for *Ceriops tagal* at RT 3.168 and 3.338 among 6 peaks while *Ipomoea pes-caprae* and *Sonneratia apetala* showed 2 major peaks with RT 3.112, 3.331 and RT 3.142, 3.337 among each 7 peaks respectively. The crude extracts of all the four halophytes were subjected to partial purification of phenolic compounds using column chromatography (Fig. 2B). The purified phenolic fractions with similar Rf value identified through thin layer chromatography (TLC) was considered as single fraction (Supplemental file 2). Further, 1 fraction of *Avicennia marina* (AM), 2 of *Ceriops tagal* (CT), 1 of *Ipomoea pes-caprae* (IP) and 2 of *Sonneratia apetala* (SA) were subjected to phenol fingerprinting using HPTLC and was developed using phenol specific solvent system (cyclohexane: ethyl-acetate: formic acid 4:6:1 v/v/v) with detectable signals quenching at UV 254 nm, 366 nm (Fig. 2C). The corresponding densitogram of AM showed 4 peaks with Rf between 0.02 and 0.94, CT1 with 6 peaks between Rf max 0.037 and 0.937 and CT2 with 3 peaks between Rf max of

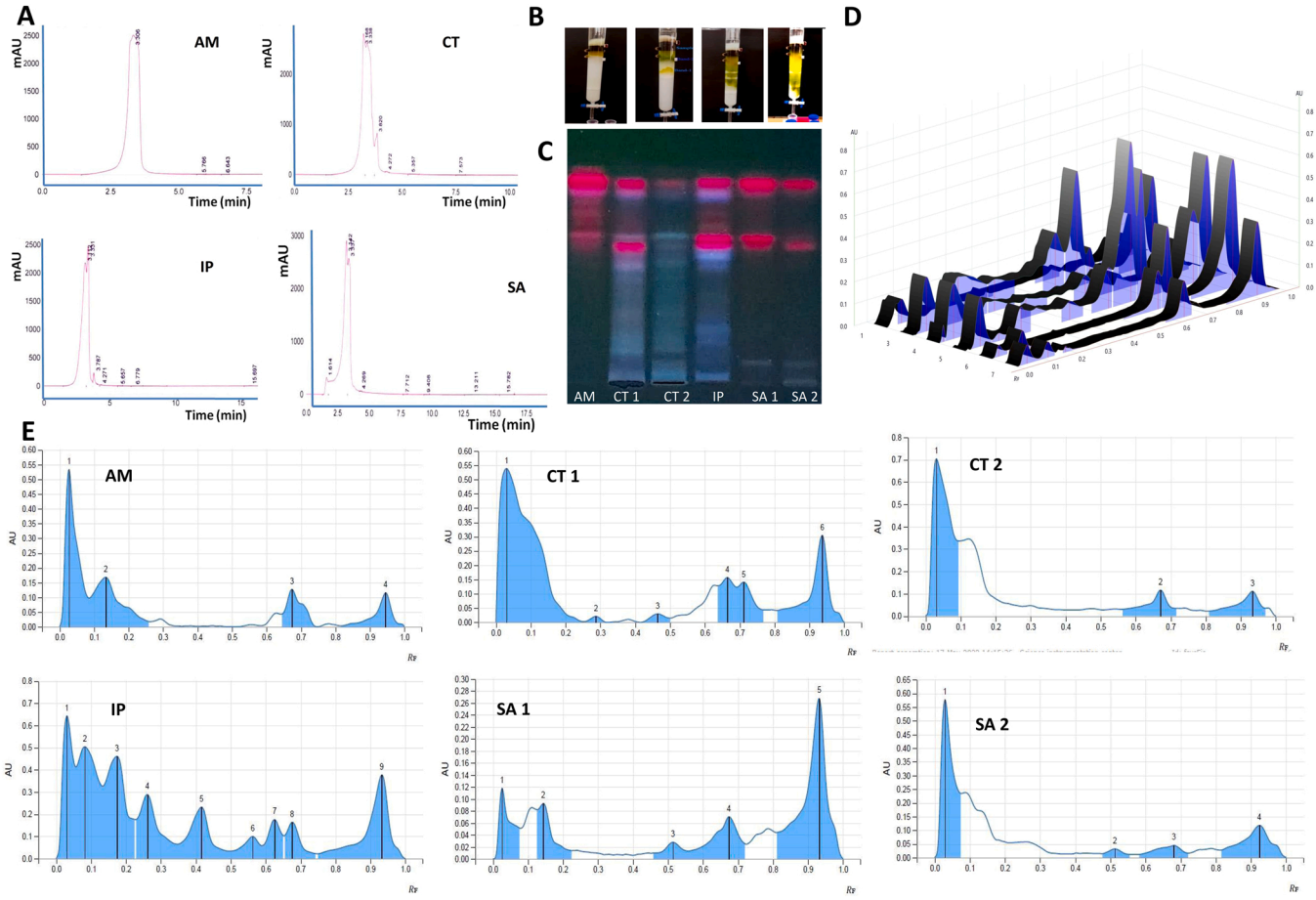

Fig. 2. Screening of phenolic compounds from the potential halophytes using chromatographic analysis. HPLC analysis of crude (A) AM, CT, IP and SA (B) partial purification of secondary metabolites using column chromatography. HPTLC fingerprint chromatogram of purified fractions from (C) AM, CT1, CT2, IP, SA1 and SA2. (D) Isometric view of the purified fractions from HPTLC (E) Densitogram with the corresponding Rf value of the purified fractions.

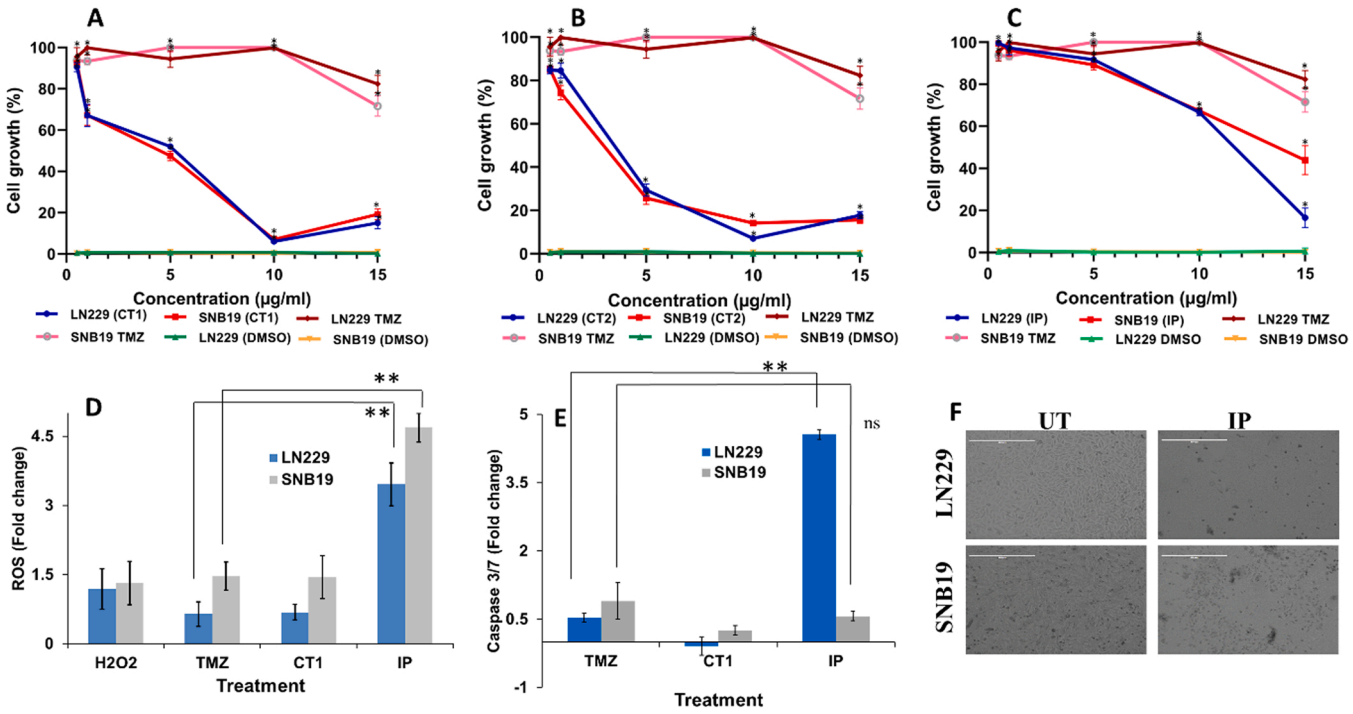

Fig. 3. Dose dependent cytotoxicity analysis of CT1, CT2 and IP fractions in GBM cells. Cytotoxicity effect of (A) CT1 fraction (B) CT2 fraction and (C) IP fraction at varying concentration (0.5 µg, 1 µg, 5 µg, 10 µg and 15 µg) in GBM cell lines, LN229 and SNB19. DMSO was used as the negative control with temozolomide (TMZ) as positive control; *$p < 0.05$ for drug treated vs DMSO (Dunnet's Test) (D) Fold change of Reactive oxygen species (ROS) was analyzed for GBM cells treated with CT1 and IP fraction with H_2O_2 as positive control; (E) Measurement of Caspase 3/7 for CT1 and IP fraction against GBM cell lines; F: Representative phase contrast microscopic image of IP and untreated (DMSO) cytotoxicity in LN229 & SNB19 cells. The values are expressed as mean ± standard error using biological and technical replicates (n = 6), **$p < 0.01$, ns= non-significance (Dunnet's Test).

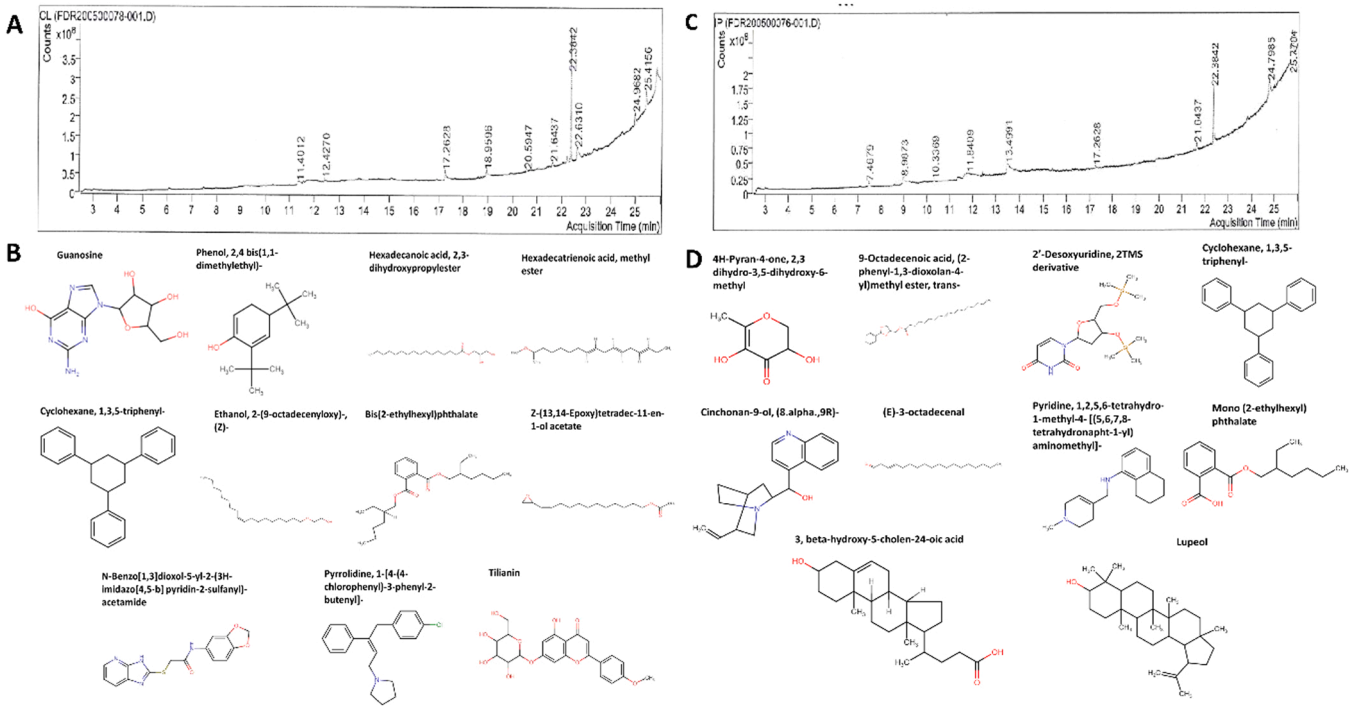

Fig. 4. Secondary metabolite profiling of purified CT and IP using GC-MS analysis. Gas chromatogram of (A) CT1 and (C) IP fraction shows the presence of secondary metabolites using Mass Hunter (Ver.12.0). The structures of the identified compounds for (B) CT1 and (D) IP structures were drawn using SwissADME.

0.03 and 0.93 respectively. IP fractions showed 9 peaks with Rf max 0.03 and 0.93 while SA1 showed 5 peaks with Rf max between 0.026 and 0.932 (Fig. 2D and E). Visualization at 366 nm unveils notable blue/violet bands for CT1, CT2 and IP with Rf values between 0.03 and 0.9, which confirms the presence of phenolic compounds.

3.3.4. Dose dependent cytotoxicity analysis of purified polyphenols of Ceriops tagal and Ipomoea pes-caprae

Cytotoxicity analysis revealed that the extracts of CT fraction 1, CT fraction 2 and IP fraction inhibited the cell growth in both LN229 and SNB19 cell lines with the least cytotoxicity values of 1 μg/ml when compared with the vehicle control, DMSO. Results showed that at 15 μg/ml concentration, all the three fractions exhibited least % of cell growth which was about 15.08%, 17.74% and 1.87% respectively for LN229. Similarly, the % of cell growth for SNB19 was found to be 15.6%, 15.6% and 13.59% for all the three fractions, respectively (Fig. 3A–C). Out of two CT fractions 1 and 2 and IP fraction, CT 1 and IP fraction showed better cytotoxicity effect on LN229 and SNB19 than CT2. Hence, further analysis on investigating the capability of ROS activating caspase dependent apoptotic signaling pathway was examined in both GBM cell lines for CT1 and IP fractions.

As shown in Fig. 3D, exposure of LN229 and SNB19 cell lines with IC_{50} concentration of CT1 and IP fraction resulted in ROS production with the fold increase of 0.685, 1.441 and 3.456, 4.687 for the above-mentioned cell lines respectively. On the other hand, we also examined caspase 3/7, a key effector caspase in both the cell lines. CT1 fraction showed a downregulation to about − 0.092 in caspase expression in LN229 with fold change of about 0.256 in SNB19 cell lines. Also, IP fraction showed an increased caspase expression of about 4.56 for LN229 and 0.567 for SNB19 (Fig. 3E). Thus, these CT1 and IP fractions were further subjected to GC-MS analysis to elucidate the functional compounds present.

3.3.5. GC-MS analysis of purified polyphenols from Ceriops tagal and Ipomoea pes-caprae

The purified fraction with high polyphenolic content from *Ceriops tagal,* CT1 and *Ipomoea pes-caprae*(IP) were subjected to GC-MS analysis. Fig. 4A and C represents the chromatogram from the purified fraction of *Ceriops tagal* and *Ipomoea pes-caprae* respectively. CT1 fraction of *C.tagal* showed the presence of 12 compounds with the major compounds including Guanosine (RT-11.4012); Phenol, 2,4-bis (1,1-dimethylethyl) (RT-12.4270); Hexadecanoic acid, 2,3-dihydroxypropyl ester (RT-17.2624); Hexadecatrienoic acid, methyl ester (RT-18.9596); 2-heptadec-5″-en-1″-yloxy tetrahydrofuran (RT-20.5947); Cyclohexane, 1,3,5-triphenyl- (RT-21.6437); Ethanol, 2-(9-octadecenyloxy)-, (*Z*)- (RT-22.2299); Bis (2-ethylhexyl)phthalate (RT-22.3842); Z-(13,14-epoxy) tetradec-11-en-1-ol acetate (RT-22.6310); N-Benzo [1,3] dioxol-5-yl-2-(3 H-imidazo[4,5-*b*]pyridine-2-ylsulfanyl)-acetamide(RT-24.9682); Pyrrolidine, 1-[4-(4-chlorophenyl)− 3-phenyl-2- butenyl]- (RT-25.4156); Tilianin (RT-25.8398) (Fig. 4B).

Likewise, GC-MS analysis of *Ipomoea pes-caprae* showed the presence of 11 bioactive compounds, which includes 4 H-Pyran-4-one, 2,3 dihydro-3,5-dihydroxy-6-methyl (RT-7.4679); 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans- (8.9873); 2′-Desoxyuridine, 2TMS derivative (RT-10.3369); Cinchonan-9-ol, (8.alpha.,9 R)- (RT-11.8409); (E)− 3-octadecenal (RT13.4991); Pyridine, 1,2,5,6-tetrahydro-1-methyl-4- [(5,6,7,8-tetrahydronapht-1-yl) aminomethyl]-(RT-17.2628); Cyclohexane, 1,3,5-triphenyl- (RT-21.6437); Mono (2-ethylhexyl) phthalate (RT-22.3842); 3, beta-hydroxy-5-cholen-24-oic acid (RT-24.7985); and Lupeol (RT-25.7704) (Fig. 4D).

3.3.6. IP derived polyphenols prevents cell migration by inducing cell death

From the above in-vitro analysis, it was evident that IP fraction could induce caspase dependent cell death significantly than CT1 fraction, and hence IP fraction was further subjected to apoptotic and migration assay. Hypodiploid DNA content or apoptotic cells were high in IP induced LN229 and SNB19 cells which was examined using Annexin V-FITC/PI staining. In IP induced LN229 cells, percentage of cells entering

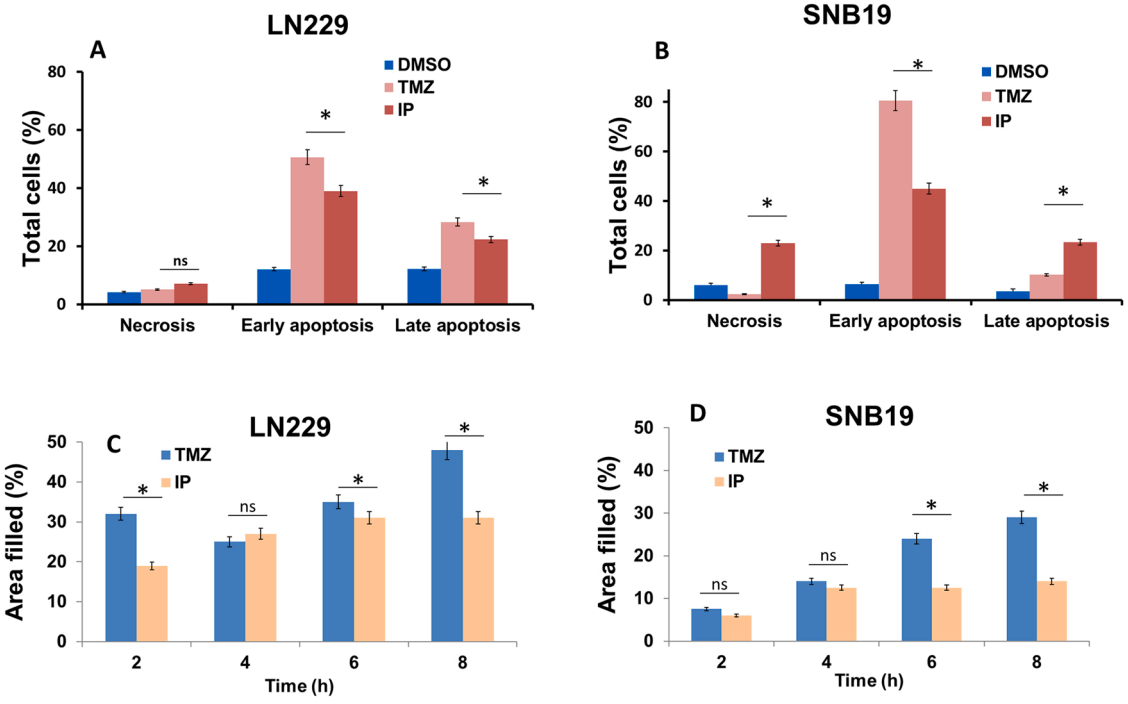

Fig. 5. Effect of IP fraction in inducing apoptosis and preventing migration of GBM cells. The percentage of cells entering apoptosis and necrosis was analyzed in IP treated (A) LN229 cells and (B) SNB19 cells using Annexin V/PI double staining method. The ability of IP fraction in preventing the migration of (C) LN229 cells and (D) SNB19 cells were analyzed and the % of area filled was represented as bar diagram. DMSO was used as a negative control with TMZ as positive control. The values are expressed as mean ± Standard error of biological triplicate samples (n = 3) and experimental repeats (n = 3), *p < 0.05, ns=non-significant (ANOVA).

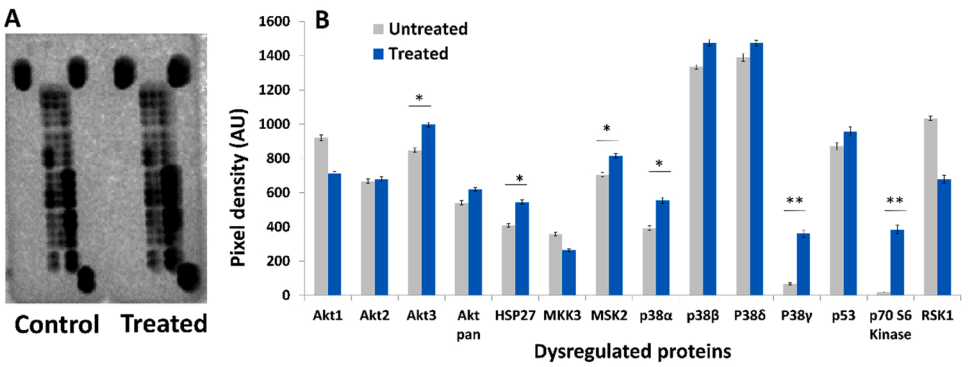

Fig. 6. Proteome profiling of MAPkinase-associated proteins in IP fraction treated GBM cells. (A) Protein array images showing the relative level of expression of total 26 apoptosis-associated proteins upon IP treatment in LN229 cells. DMSO was used as control. (B) The integrated pixel density of 14 apoptotic proteins showing up regulation and down regulation of Akt1, MMK3, p53, p70 S6 kinase and RSK1 upon IP treatment compared with control. The data were shown as mean ± SD, ** $p < 0.01$, *$p < 0.05$ (ANOVA).

necrosis was found to be 7.1%, 39% in early apoptosis and 22.3% in late apoptosis (Fig. 5A). Similarly, for IP induced SNB19 cells, 23% of cells entered necrosis, 45% in early apoptosis and 23.31% in late apoptosis (Fig. 5B). Nevertheless, the proportion of cells entering all stages of apoptosis was not significant in treated LN229 cells than the TMZ treated cells. In contrast, the proportion of cells significantly increased only in necrosis and late apoptosis stages.

Likewise, we have also performed wound healing assay to investigate the effect of IP induced GBM cell lines in inhibiting the migration ability for up to 8 h. A line was drawn with the freehand micro tip to measure the entire area for the migrated and invaded cells. In LN229 and SNB19 cells after treatment with IP fraction, the percentage of invaded cells decreased when compared with TMZ. After 8 h of treatment, the percentage of invaded area was found to be increased to 48% and 29% for TMZ, while reduced to 31% and 14% for IP in both LN229 and SNB19, respectively (Fig. 5C and D). These data confirmed that IP fraction could comparatively reduce the cell migration by enhancing cell death.

3.3.7. IP-polyphenols regulates proteins related to MAPkinase pathway

Proteome profiling was performed in IP induced GBM cell lines to identify the altered proteins associated with MAPkinase pathway. The proteomic array was analyzed using ImageJ to extrapolate the weak and strong signals from the images. The relative expression was quantified from the sample and control using ratiometric analysis (Fig. 6A). Various MAPKinase genes such as MMK3, p53, p70 S6 kinase and RSK1 were regulated upon treatment with IP fraction. MKK3, mitogen-activated protein kinase 3 was found to be down-regulated whose expression was closely associated with poor prognosis and clinic-pathological features of glioma [37]. Also, p53, a tumor suppressor and transcription factor found to be downregulated in glioma condition, was observed to be upregulated in treated GBM cell lines. Likewise, P70 S6 Kinase was upregulated upon treatment whose deregulation has been associated to various pathologies, including metabolic disorders and cancers. GBM cells treated with IP fraction downregulated RSK1 expression, which aberrantly expressed in high grade glioma with immune infiltration (Fig. 6B) [38].

4. Discussion

Secondary metabolites help the plant to adapt them to the natural environment and also determine the bioactive potential. Bioactive characters are formed by antioxidant, antimicrobial, anti-inflammatory, antiviral and other chemotherapeutic actions of their secondary metabolites [39]. Halophytes are the salt-tolerant plants inhabiting extreme environmental condition that exhibits diverged metabolic responses than the conventional plants. The present study systematically validated the presence of various metabolites like alkaloid, saponin, flavonoids, and phenolic compounds. We have mainly focused our research on four important plants *Avicennia marina, Ceriops tagal, Ipomoea pes-caprae* and *Sonneratia apetala* based on the bioactive compounds identified from preliminary phytochemical screening. All these plants were found to contain higher amount of phenolic compounds as the key bioactive compounds, which was also pre-clinically evaluated for its anti-metastatic properties against GBM cells.

Polyphenol compounds act as an antioxidant and have the ability to chelate metal ions involved in the production of free radicals. They also function as an anti-aging, anti-inflammatory, antioxidant and anti-proliferative agents [40,41]. Polyphenols typically possess one or more phenyl rings and hydroxyl group that makes them capable of detoxifying harmful oxidants either by donating hydrogen or electron [42]. Likewise, the presence of flavonoids potentially prevents the oxidative cell damage and possesses anticancer activity. Also, the presence of o-diphenol group, the double bonds of the benzene ring and the double bond of the oxo functional group (-C═O) in the plants provides higher antioxidant activity of the flavonoids [43].

Other significant secondary metabolite that was identified in these halophytes was saponins that possess significant pharmacological properties such as anti-inflammatory, immuno-stimulant, hypocholesterolemic, hypoglycemic, anti-fungal and cytotoxic activities [44]. The presence of carbohydrate portion of the molecule classifies it as a surface-active agent and thus makes it hydrophilic in nature [45]. It is found that saponins exhibit numerous medicinal properties such as hemolytic factor, anti-inflammatory, antibacterial, antiviral, anticancer and cytotoxic activity [46–48]. In addition, saponins are reported to exhibit cholesterol-lowering action in animals and human [49]. Similarly, alkaloids are significant in many biological activities such as antioxidant, anti-carcinogenic, anti-diabetic, antimicrobial activity etc., [50]. Flavonoids are reported to have antioxidant effects, anti-inflammatory, cytotoxic activities [51]and also have been shown to inhibit the initiation, promotion, and progression of tumors [52].

Halophytes produce high level of free radical-scavenging secondary metabolites in order to adapt themselves in harsh environmental conditions. The selected halophytes were found to possess higher phenolic content and thus exert strong antioxidant activity which attributes the free radical scavenging property. The hydroxyl groups and the substitutions on the aromatic rings of the phenolic compounds confer the free radical scavenging property [53].

The halophytes with potent cytotoxicity activity such as *Ceriops tagal, Ipomoea pes-caprae* has been subjected for further GC-MS analysis, which revealed the presence of significant secondary metabolites like n-hexadecanoic acid, benzofuran and phytol. Phytol is proven to exhibit antioxidant and antinociceptive effects and it is used as schistosomicide drug. Phytol is a precursor of synthetic vitamin E and vitamin K which has cytotoxic effect against breast cancer cell lines (MCF7) [54,55]. Hexadecanoic acid, a methyl ester has been found to exhibit anti-inflammatory, antioxidant, hypocholesterolemic, antibacterial activity [56].

Protein array data revealed the regulation of a few promising genes of MAPKinase pathway involved in the GBM disease progression. p53, a tumor suppressor protein observed to be up regulated in treated GBM

cells, controls numerous genes involved in cell cycle arrest, cell senescence, DNA repair, metabolic adaptation and cell death [57]. Similar result was also observed when GBM cells treated with *Ipomea* leading to apoptosis. Likewise, RSK1, p90 ribosomal S6 kinase (RSK) family was found to be downregulated, which is aberrantly expressed in subpopulation of glioblastomas than in low grade glioma. RSK plays vital role in several cancers, controlling proliferation, mRNA translation and survival [58]. Thus, our data have provided insights on the effect of *Ipomea* in regulating the key genes involved in the MAPkinase pathway, which confirms the ability of *Ipomea* as the potential pharmacological agent against GBM treatment.

5. Conclusion

The current study gives a glimpse of the active secondary metabolites present in the marine halophytes. It was identified that from all the 10 different plants under investigation, four plants were found to contain higher quantity of secondary metabolites. HPTLC profiling reveals the presence of polyphenols as the promising metabolites in those four different halophytes including *Avicennia marina*, *Ceriops tagal*, *Ipomoea pes-caprae* and *Sonneratia apetala*. Secondary metabolite profiling through GC-MS identified potential polyphenolic compounds like n-hexadecanoic acid, benzofuran and phytol, which was further validated for its anti-GBM therapeutic ability. Purified polyphenolic fraction from *Ipomoea pes-caprae* through column chromatography was found to have least IC_{50} value and has the ability to induce apoptosis in GBM cells. Also, IP derived polyphenols were able to regulate crucial genes involved in MAPkinase pathway, such as MMK3, p53, p70 S6 kinase and RSK1. Thus, all these data revealed that *Ipomoea pes-caprae* polyphenols could be further pre-clinically validated for its therapeutic value against anti-GBM therapy.

CRediT authorship contribution statement

MM performed biochemical assays, in-vitro studies and data analysis. AM performed cell culture studies. AM, MK, SN, PR and TR managed all studies. TR and MK involved in cell cultures studies. AM conceived the project. All the authors contributed to writing the manuscript.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114288.

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