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Research Article

COMPREHENSIVE *IN VITRO* EVALUATION OF PHARMACOLOGICAL ACTIVITIES OF SELECTED PLANT EXTRACTS AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY PROFILING OF *FLACOURTIA JANGOMAS* FLOWER EXTRACT

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ABSTRACT

Objective: Continued exploration and bio-evaluation of plants hold good as there are an increasing demand and recognition of natural products in disease management. The aim of this study was to investigate the antioxidant, cytotoxic, and antiproliferative activity of methanolic extracts of different parts of six plants. Six plants - *Couroupita guianensis, Flacourtia jangomas, Lucuma nervosa, Euphorbia milii, Acalypha hispida*, and *Hydnocarpus pentandra* - were chosen for this study.

Methods: The plant parts were extracted with methanol and screened for 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging abilities. The cytotoxic activity of the extracts was investigated on SCC9 and Calu6 through MTT assay, and cell cycle was analyzed by flow cytometry to determine the antiproliferative activity of the extracts. The flower extract of *F. jangomas* was further subjected to gas chromatography-mass spectrometry (GC-MS) analysis for purification of the compounds of interest. A two-way ANOVA was done to estimate the effect of the extract between samples remembered at $p<0.05$ level.

Results: Among all the plant extracts, the extract of *F. jangomas* (flower) showed significant antioxidant potential with IC_{50} values of 11.16±0.54 µg/ml and 12.34±0.37 µg/ml for DPPH and ABTS assays, respectively. GC-MS analysis of the extract revealed the presence of 21 phytocompounds. MTT assay revealed that this extract had promising cytotoxic activity against the two cancer cell lines, Calu6 and SCC9 with IC_{50} values of 43.57±0.04 µg/ml and 53.42±0.15 µg/ml, respectively. The extract treatment caused significant arrest in G_2M phase of cell cycle.

Conclusion: *F. jangomas* flower extract displayed significant antioxidant and antiproliferative activity and can be considered as a potential source of anticancer compounds.

Keywords: Antioxidants, MTT assay, Gas chromatography-mass spectrometry, Calu6, SCC9, *Flacourtia jangomas.*

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INTRODUCTION

Plants play a pivotal role in the life of human beings as they serve as a source of very effective therapeutic remedies for a good number of human health hazards. The medical effectiveness of plants is due to the presence of a wide array of bioactive phytoconstituents that record significant antioxidant activity. Free radicals or reactive oxygen species (ROS) are highly reactive molecules that are produced in cells either as by-products of metabolism or by leakage from mitochondrial respiration and have the potential to cause cell damage at a higher concentration [1,2]. Antioxidants act as free radical scavengers that interact with and neutralize free radicals and nullify the damage caused by them in the biological system [3-5]. Oxidative stress arises as a result of shift in the dynamic balance between production of free radicals and antioxidant defense [5]. It is a contributing factor in the pathology of many diseases such as cancer, muscular degeneration, cardiovascular diseases, and age-related diseases [6,7]. Plants are treasures of reliable, abundant and inexhaustible source of free radical scavenging molecules [8]. Hence, there is a surge in the development of potent antioxidants of plant origin that are less harmful and less expensive than the synthetic antioxidants. For analyzing the various compounds, gas chromatography mass-spectroscopy (GC-MS) is found to be a sophisticated technique [9,10]. GC-MS is a simple and effective tool in identification and quantification of the different phytocomponents [11,12]. This is a well-suited technique in separation of components of a mixture and identifying the unknown compound by matching its spectra with the reference spectra [13].

Many of the antioxidants are found to be anticarcinogens. Owing to the anticancerous compounds present, a wide variety of plants are used in the development of drugs for the prevention of cancer [14-16]. Cancer is one of the critical health problems that is increasing at an alarming rate globally. The disease involves an out of control cell growth that invades the neighboring cells [17]. The different therapies used in the treatment of cancer being expensive and with a lot of side effects, researchers are turning their attention to find out better and reliable therapeutic anticancerous agents of plant origin [18]. Cell-based assays are appropriate methods to seek the effect of new substances on cell proliferation or whether they have cytotoxic effect. MTT assay is one such which is simple and quick and used to assess the cell metabolic activity [18,19].

Considering the worldwide interest in utilizing plants for curative purpose, six plants - *Couroupita guianensis, Flacourtia jangomas, Lucuma nervosa, Euphorbia mili, Acalypha hispida,* and *Hydnocarpus pentandra* were chosen for this study. *C. guianensis* holds antibiotic, antifungal, antiseptic, and analgesic properties [20]. *F. jangomas* is used in traditional medicine as it possesses astringent properties, relieves toothache, and diarrhea [21]. *L. nervosa* is used in folk medicine. *E. milii* is used for the curing many ailments like skin diseases and possesses antimicrobial properties [22]. *A. hispida* is known as cat's tail, which

possesses a lot of medicinal properties and is used as carminative, diuretic, and expectorant [23]. The leaves of this plant are used to treat skin diseases, and flowers are used in curing diarrhea. *Hydnocarpus pentandrus* known as Chaulmoogra is used to treat many diseases [24].

Thus, this study was focused on exploring the antioxidant, antiproliferative, and cytotoxic activities of the methanolic extracts of different parts of the selected plants. Furthermore, the flower extracts of *F. jangomas* were selected and subjected to GC-MS analysis based on the results obtained from this study, and the results of their antifungal activity and phytochemical studies carried out in the previous investigation.

METHODS

Collection of plants

Disease free and healthy parts (Leaves, Bark, Flower, Root) of the six plants; *C. guianensis, F. jangomas, L. nervosa, E. mili, A. hispida,* and *H. pentandra* were collected from Mavelikara, Alappuzha district, Kerala state, India. These were washed with tap water, rinsed with distilled water and shade dried. The dried material was ground to fine powder using grinder mixer and stored in air tight containers. The plants were identified taxonomically and authenticated. The samples taken for the experimental work were A: *C. guianensis* leaves, A1: *C. guianensis* bark, A2: *C. guianensis* flower, A4: *C. guianensis* root, B: *F. jangomas* leaves, B1: *F. jangomas* bark, B2: *F. jangomas* flower, B4: *F. jangomas* root, E: *L. nervosa* leaves, E1: *L. nervosa* bark, E2: *L. nervosa* flower, E4: *L. nervosa* root, F: *E. mili* leaves, F1: *E. mili* bark, F2: *E. mili* flowers, F4: *E. mili* root, G: *A. hispida* leaves, G1: *A. hispida* bark, G2: *A. hispida* flower, G4: *A. hispida* root, H: *H. pentandra* leaves, H1: *H. pentandra* bark, H2: *H. pentandra* flower, and H4: *H. pentandra* root.

Chemicals used

The chemicals methanol, ethanol, dimethyl sulfoxide (DMSO), ammonium persulfate, Triton was procured from Qualigens; 2,2-diphenyl-1-picrylhydrazyl (DPPH), Quercetin, Colchicine and propidium iodide (PI) were purchased from Sigma-Aldrich, USA. 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) solution, 3-(4, 5-dimethylthiazol2yl)-2, 5-diphenyl tetrazolium bromide (MTT reagent), and RNaseA were procured from HiMedia, Bengaluru.

Extraction of plant parts

The shade dried plant parts were homogenized and pulverized. The powdered samples (100 g) were extracted with 1 L of methanol at room temperature for about 3 days. The resultant mixture obtained was filtered and evaporated using rotary evaporator at 40°C. The dried extract was further used for antioxidant and cytotoxicity assays.

Antioxidant study

DPPH

The DPPH free radical scavenging activity of methanolic extracts of the selected six plants was carried out using 1, 1-diphenyl-2 picrylhydrazyl (DPPH) [25]. 1.8 ml of methanolic DPPH (20 mm) was added to 0.2 ml of different extracts in methanol at varying concentration prepared by dilution method. The absorbance was measured at 510 nm using spectrophotometer followed by incubation in the dark at room temperature for 30 minutes [26]. Quercetin was used as reference standard and methanol was used as blank. The experiment was carried out in triplicates. The % scavenging effect was obtained from the formula: Scavenging effect (%) = $(A_0\text{-}A_1)/A_0 \times 100$, where A_0 was the absorbance of the control reaction and A_1 was the absorbance of the sample of the tested extracts. IC_{50} values lesser than 70 were reported.

ABTS

ABTS assay was done using ABTS radicals [14,27]. The extracts were incubated with ABTS solution (7 mm) and ammonium persulfate (2.45 mm) at room temperature in the dark for 16 hrs. Quercetin was used as standard and following incubation; the absorbance was recorded at 734 nm. The experiments were carried out in triplicates. The percent inhibition was calculated at different concentrations, and the IC_{50} values were calculated by Log-Probit analysis. The percent inhibition was calculated using the formula I (%) = $100 \times (A_0\text{-}A_1)/A_0$; Where A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard. IC_{50} values lesser than 70 are reported.

MTT assay

Cytotoxic activity of methanolic plant extracts against two cancer cell lines (Calu6 and SCC9) was evaluated by 3-(4, 5-dimethylthiazol2yl)-2, 5-diphenyl tetrazolium bromide MTT assay [28]. The cell lines were maintained in DMEM at 37°C in 5% CO_2 for 24 hrs in a humid environment. Varying concentration of plant extracts was added to the cell lines, preincubated for 24 hrs. MTT reagent was added to each sample and further incubated for 2-3 hrs. The plates were read at 590 nm using a microplate reader, and the relative viability of the treated cells is expressed as % cell viability and is calculated using the formula, % cell viability= $(OD_{control} - OD_{sample})/OD_{control} \times 100$. The experiments were done in triplicates, where control is the sample without the treatment.

Cell cycle analysis

To estimate the effect of *F. jangomas* flower extract on the cell cycle, both SCC 9 and Calu6 (1×10^6 cells each) cell lines were treated with the 200 µg/ml of methanolic extract, B2. 20 µm colchicine was used as positive control and 1% DMSO as negative control. After overnight incubation with fixing solution (20% PBS in 70% ethanol) at 4°C, cells were washed with 500 µl of PI (0.05 mg/ml PI; 0.05 mg/ml RNaseA [pH 7.2]; 0.1% triton). The percentage of cells in various stages was analyzed using FACS Caliber (BD Biosciences, San Jose, CA).

GC-MS analysis

The methanolic extract of the flower of *F. jangomas* was subjected to GC-MS analysis to identify the phytochemical components. The analysis was performed on a GC CLARUS 500 PerkinElmer system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Restek Rtx^R- 5, (30 m × 0.25 mm 5% diphenyl/95% dimethyl polysiloxane), operating in electron impact mode at 70 eV. Helium was used as carrier gas at a constant flow of 1 ml/minute and an injection volume of 1.0 µl with injector temperature of 280°C. The oven temperature was programed from an initial temperature of 40°C (isothermal for 5 minutes), with an increase of 6°C/minutes, to 280°C, ending with a 15 minutes isothermal at 280°C. Mass spectra were taken at 70 eV. The total GC/MS running time was 60 minutes [29].

RESULTS

DPPH assay: The antioxidant assays revealed substantial free radical scavenging potential in most of the plant extracts. Out of the 24 plant extracts, 15 extracts exhibited DPPH free radical scavenging activity. *F. jangomas* flower extract showed maximum scavenging activity (89.34%) at 100 µg/ml followed by the leaf extract of *H. pentandra* (85.33%) when compared to the standard Quercetin (93.45%) (Fig. 1). The IC_{50} value of *F. jangomas* flower (11.16±0.54 µg/ml) was found to be the nearest to Quercetin (5.23±0.24 µg/ml). The extracts of *H. pentandra* (leaf), *A. hispida* (bark), *A. hispida* (root), *A. hispida* (flower), *H. pentandra* (flower), *Couroupita guianenesis* (root), and *A. hispida* (leaf) exhibited DPPH scavenging ability in a range from 85.33% to 76.78%. All the parts of *A. hispida* possessed potent scavenging ability. The IC_{50} values of all extracts ranged between 11.16±0.54 µg/ml and 63.11±0.07 µg/ml (Fig. 2). A two-way ANOVA between the concentration and different samples was conducted to compare the effect of samples on DPPH scavenging activity. There was a significant effect of different samples remembered at $p<0.05$ level. The significant effect on the DPPH scavenging activity between the samples and varying concentrations [$F(5, 75) = 244.61$, $p=0.00513$] and [$F(15,75)= 11.21$, $p=0.00374$].

ABTS assay: Out of the 24 extracts subjected to ABTS assay, only 15 extracts showed antioxidant activity. Among the tested samples the flower extract of *F. jangomas* was found to be more effective followed by leaf extracts of *H. pentandra*. Their activity was compared to Quercetin, the standard antioxidant used in the study (Fig. 3). At 100 µg/ml, the inhibition of the the flower extract of *F. jangomas* was 79.41% (IC_{50}: 12.34±0.37 µg/ml), and the inhibition of Quercetin was 94.56% (IC_{50}: 6.48±0.21 µg/ml). The extracts of *H. pentandra* (leaf), *Couroupita guianenesis* (root), *H. pentandra* (root), *A. hispida* (leaf), *A. hispida* (bark) displayed ABTS scavenging ability in a range from 76.54% to 70.8%. The IC_{50} values of the 15 plant extracts ranged between 12.34±0.37 µg/ml and 45.33±0.24 µg/ml (Fig. 4). The scavenging ability of the extracts and the positive control was found to be dose dependent. A two-way ANOVA between the concentration and different samples was conducted to compare the effect of samples on ABTS scavenging activity. There was a significant effect of different samples remembered at p<0.05 level. The significant effect on the ABTS scavenging activity between the samples and varying concentrations [F(5, 75) = 258.498, p = 1.07E-05] and [F(15,75)= 4.695, p=0.00374].

MTT assay

Among all the tested extracts, more potent activity was exhibited by *F. jangomas* flower and *H. pentandra* leaves as shown in (Fig. 5). In case of SCC9 cell lines, only five extracts were found to have promising cytotoxic effect. The IC_{50} values of *H. pentandra* leaves, *F. jangomas* flower, *F. jangomas* root, *H. pentandra* root and *C. guianensis* root were found to be 37.62±0.26 µg/ml 53.42±0.15 µg/ml, 57.28±0.05 µg/ml, 87.45±0.56 µg/ml and 104.23±0.34 µg/ml respectively for SCC9 cell lines. These extracts have shown a dose-dependent manner with 70.32%, 71.52%, 59.37%, 67.23%, and 63.52% of cytotoxicity at 320 µg/ml. However, in contrast to SCC9 cell lines, only two extracts showed significant cytotoxicity on Calu6 cell lines. The IC_{50} values of *F. jangomas* flower and *H. pentandra* leaf samples were found to be 43.57±0.04 µg/ml and 53.42±0.15 µg/ml, respectively (Fig. 6)

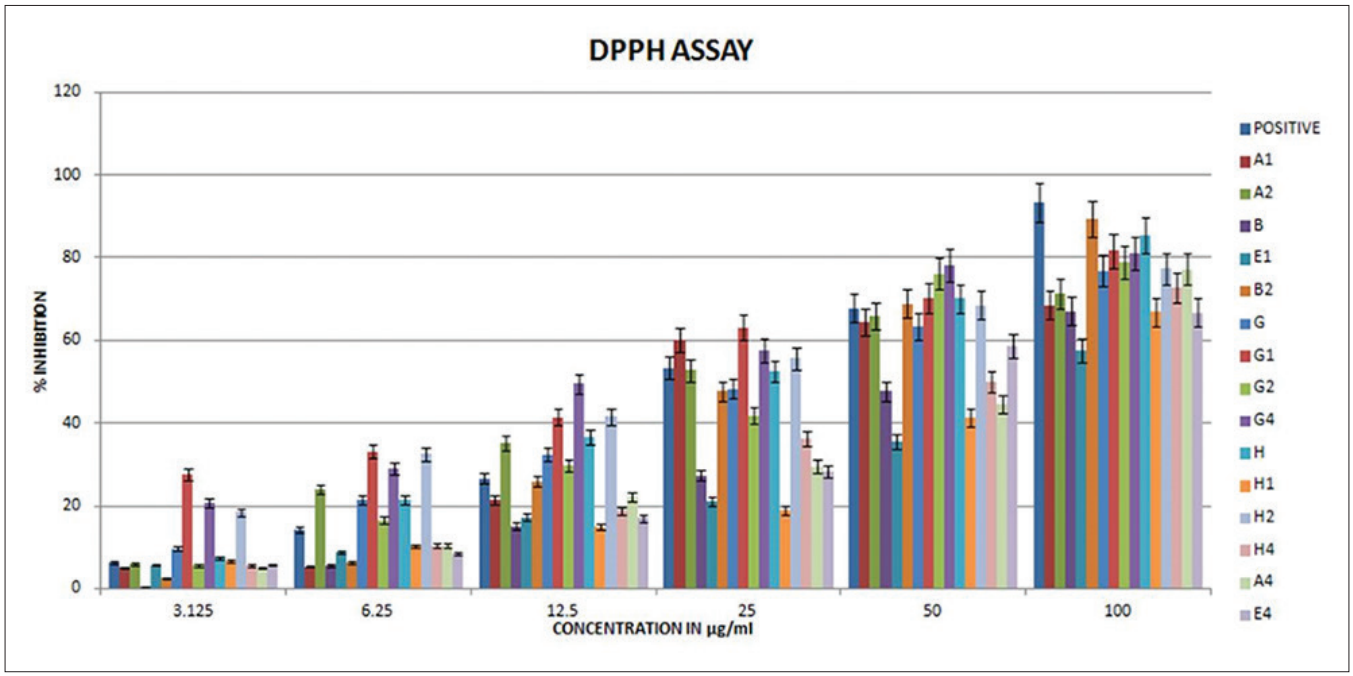

Fig. 1: The percent inhibition values of 2,2-diphenyl-1-picrylhydrazyl assay. All the values were average of triplicates. The values were expressed in % ± s.e. All the values are significantly different at p<0.05 compared to positive control; Quercetin

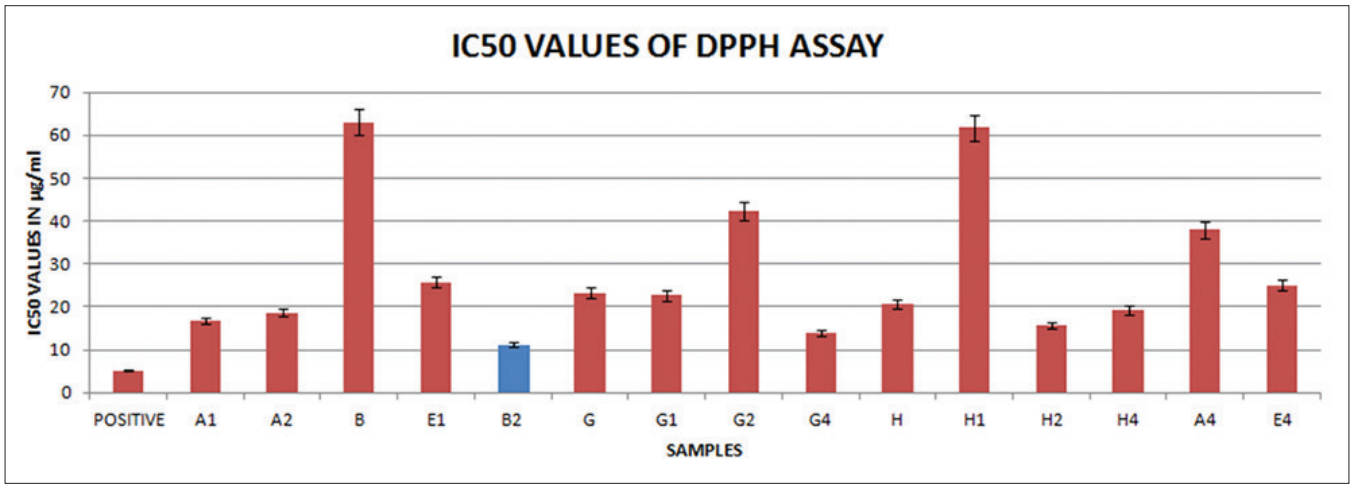

Fig. 2: The IC_{50} values of 2,2-diphenyl-1-picrylhydrazyl assay. All the values were average of triplicates. Quercetin is used as positive control. The values were expressed in % ± s.e

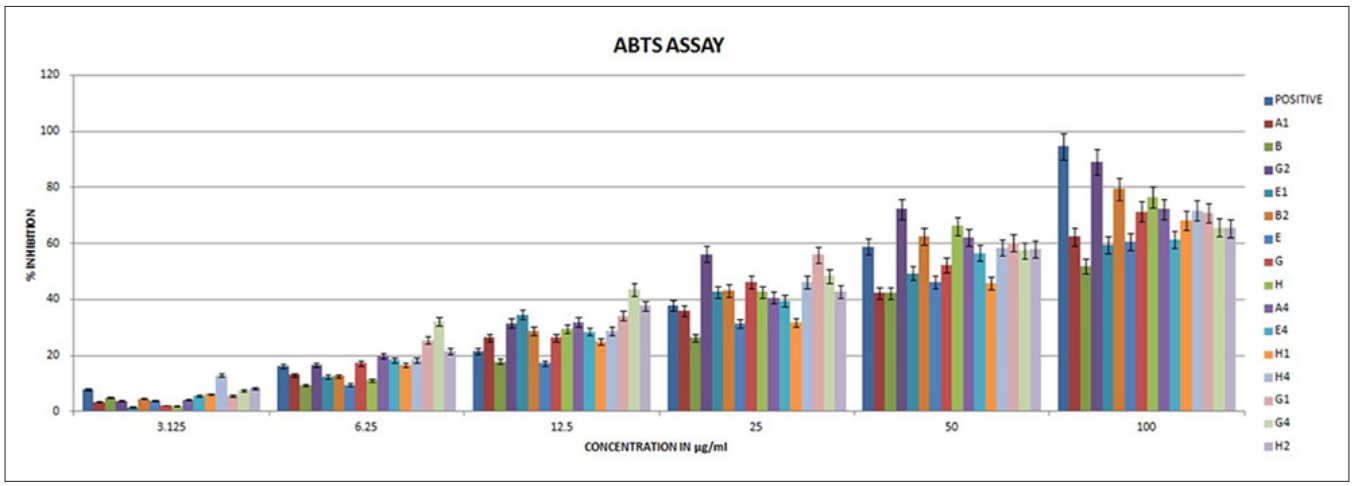

Fig. 3: The percent inhibition of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid assay. All the values were average of triplicates. The values were expressed in % ± s.e. All the values are significantly different at p<0.05 compared to Quercetin

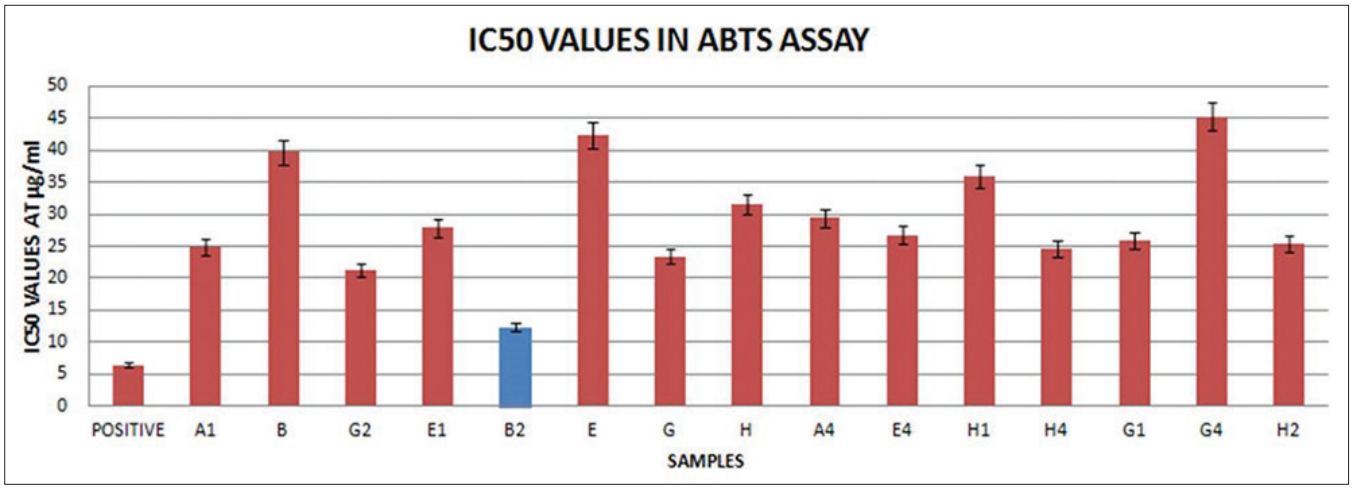

Fig. 4: The IC_{50} values of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid assay. All the values were average of triplicates. Quercetin is used as positive control. The values were expressed in % ± s.e.

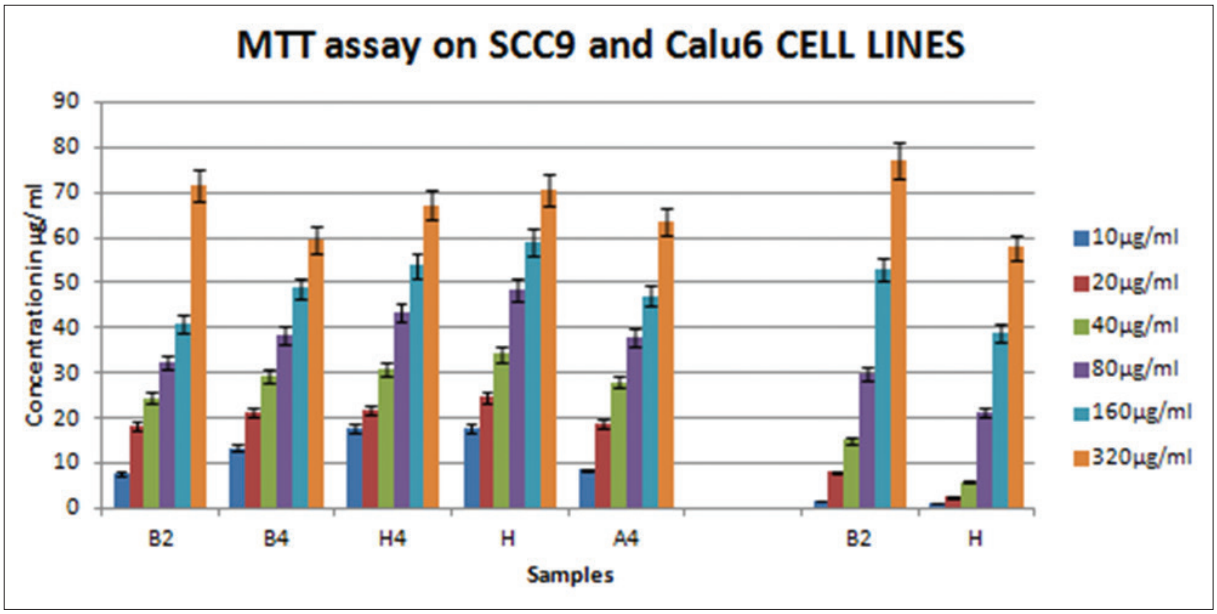

Fig. 5: The percent inhibition values of the extracts obtained in MTT assay on SCC9 and Calu-6 cell lines. All the values were average of triplicates. The values were expressed in % ± s.e.

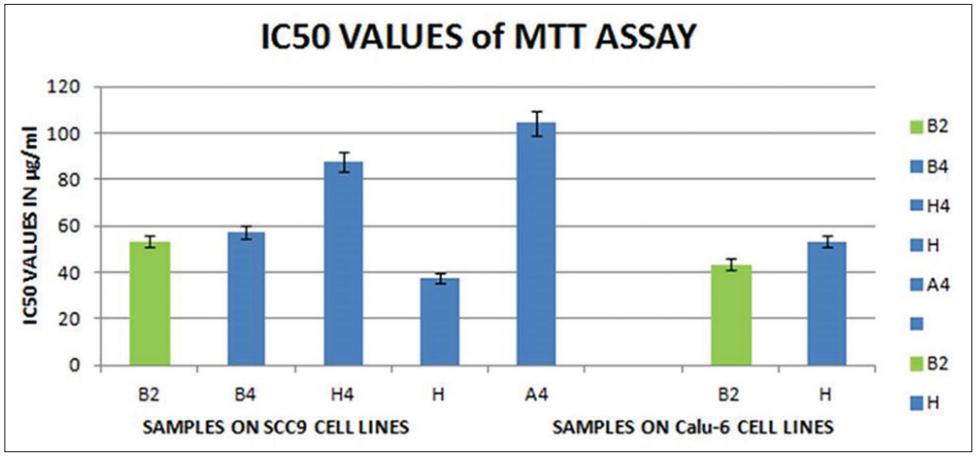

Fig. 6: IC_{50} values of the extracts obtained in MTT assay. All the values were average of triplicates. B2 and H were proved to be effective

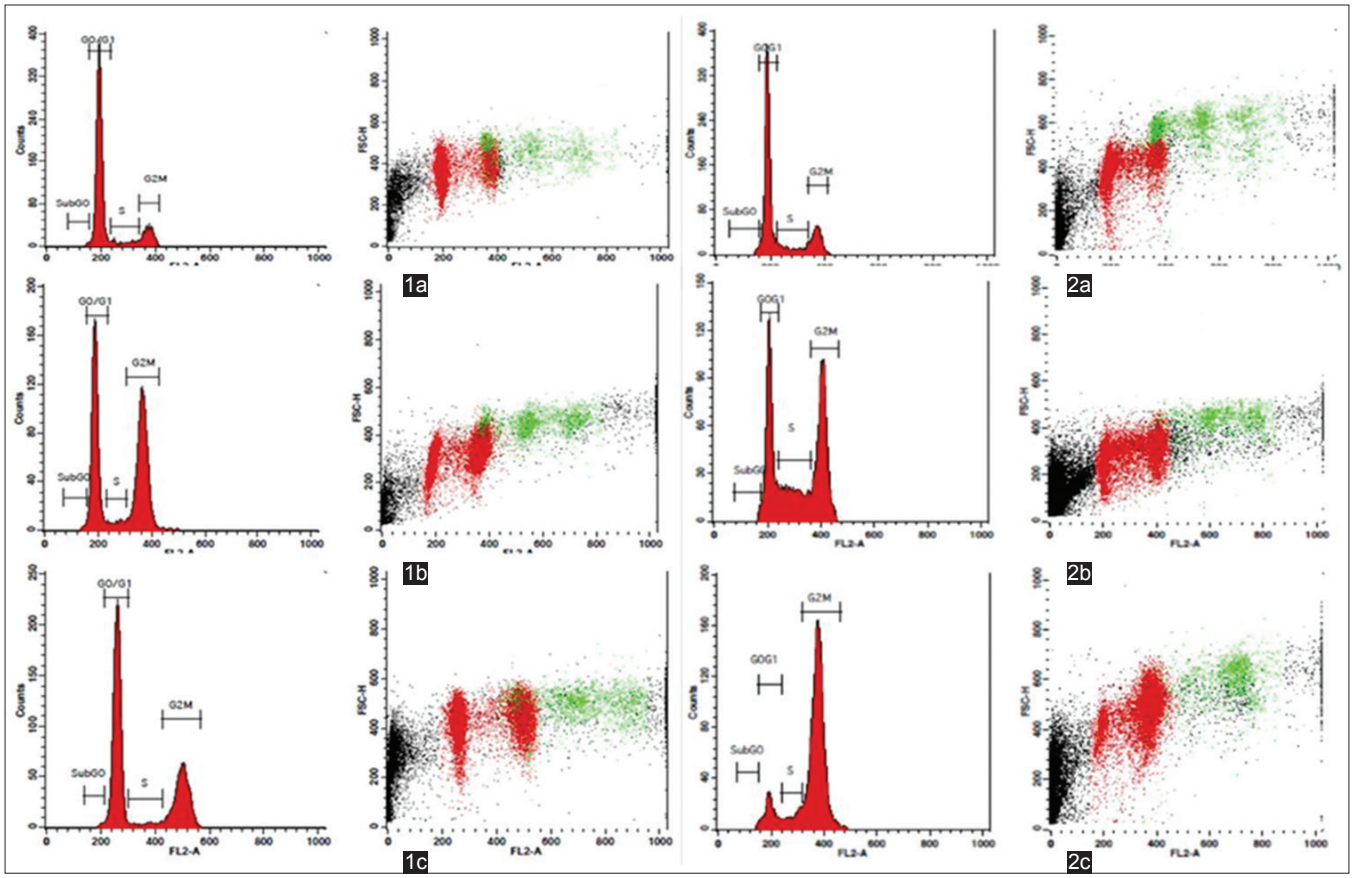

Fig. 7: Flow cytometry plots of the sample B2 against SCC9 and Calu6 cell lines, (1a) SCC9 cell lines treated with 1% dimethyl sulfoxide as control, (1b) SCC9 cell lines treated with 20 µM of colchicine, (1c) SCC9 cell lines treated with 200 µg/ml of extract, (2a) Calu6 cell lines treated with 1% dimethyl sulfoxide as control, (2b) Calu6 cell lines treated with 20 µM of colchicine, (2c) Calu6 cell lines treated with 320 µg/ml of extract

and their percentage of cytotoxicity were found to be 77.1% and 57.78% in case of Calu6 cell lines. A two-way ANOVA between the concentration and different samples was conducted to compare the effect of samples on cell cytotoxicity. There was a significant effect of different samples remembered at $p<0.05$ level. The significant effect on the cytotoxic activity between the samples and varying concentrations [$F(5, 30) = 115.011$, $p = 1.07E-05$] and [$F(6, 30)= 9.2575$, $p=0.00374$].

Cell cycle analysis

The flow cytometry results of the sample *F. jangomas* flower extract against SCC9 cell lines indicate that the extract treatment at 320 µg/ml has significantly arrested at 33.82±0.05% in G_2M phase of cell cycle, compared to negative control (14.45±0.51%) and positive control (50.33± 0.05%) in G_2M phase (Fig. 7). At 160 µg/ml, the extract did not show arrest in G_2M phase. The results of the same sample against Calu6 cell lines showed that the extract at 320 µg/ml has significantly arrested at 80.83±0.02% in G_2M phase of cell cycle, compared to negative control (18.73±0.24%) and positive control (42.86±0.03%) at G_2M phase (Fig. 8).

GC-MS analysis

About 21 compounds were identified from the GC-MS analysis of the sample B2 extract exhibiting various phytochemical activities. These may

be the contributing factors responsible for the antifungal activity found in the extract against *Candida tropicalis* [30]. The compounds were identified and authenticated using their MS data by comparison with those of the NIST Mass Spectral Library. The retention time and percentage peak of various bioactive compounds are presented in Fig. 9. Table 1 reveals the major phytoconstituents present In the flower extract were Methyl Benzoate/ Niobe Oil, Benzohydrazine/Benzoyl Hydrazide, Dimethylbutane, Isobutyl Pentyl Ester, Oxalic Acid, Butyl Propyl Ester, 2-Heptyl-1,3-Dioxolane, 1,3-Dioxolane, acetic acid, 3-[1,3]Dioxolan-2-Yl]Propyl Ester, 2,2'-Bis[1,3-Dioxolane], Isopropoxy-1,1,7,7, 7-Hexamethyl-3,5,5-Tris(Trimethylsiloxy) Tetrasiloxane, 2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-Yloxy)-2,4,4, 6,6,8,8,10,10 Nonamethylcyclopenta siloxane, 1,3-Dioxacyclopentane, 1,3-Dioxolane, Methyl Decanoate/Methyl Caprate, Methyl Tridecanoate, Alpha Methyl Valeric Acid, Methyl Myristate, Acetylhydrazide, Isonitropropane, 1,2-Diacetylhydrazine, 1-Nitropan.

DISCUSSION

The luxuriant world of plants we see has an immense storehouse of reliable and abundant sources of phytochemical and highly potent antioxidants to substitute the harmful man-made synthetic antioxidants. As far as disease management is concerned, when highly sophisticated medicines fail perhaps the green world dominates offering a solution for many of the ailments ranging from fungal and bacterial infections to cancer [6,31]. Plants have bestowed us with abundant sources of phytochemical that has a pivotal role in the development of novel and effective curative compounds. They are natural therapeutic tools that play a key role in fighting diseases. Many of them are still unexplored; not subjected to biological screening to understand their efficacy [32].

The plants chosen in this study throws light on their medicinal properties and were found to be promising in terms of their antioxidant and cytotoxic activity. The present findings divulge with very supportive literature. The infusions got from different parts of *C. guianensis* were used to cure hypertensions, tumors, inflammations, etc. [33]. The *F. jangomas* plant extracts showed a noteworthy inhibition of DPPH radical scavenging activity in concentration dependent manner with IC_{50} value of 11 µg/mL and also significant cytotoxicity where brine shrimp lethality bioassay was used [34]. Promising antifungal activity was exhibited by the flower extract of *F. jangomas* against *Candida*

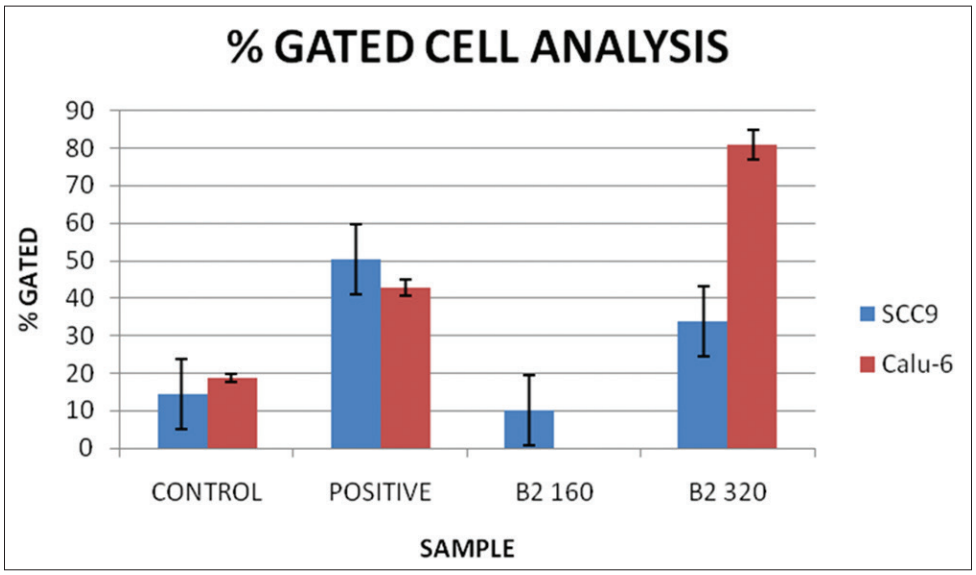

Fig. 8: The % cell viability values at G2M phase. All the values were average of triplicates. The values were expressed as %± s.e. The cell viability assay was done on both SCC9 and Calu-6 cell lines

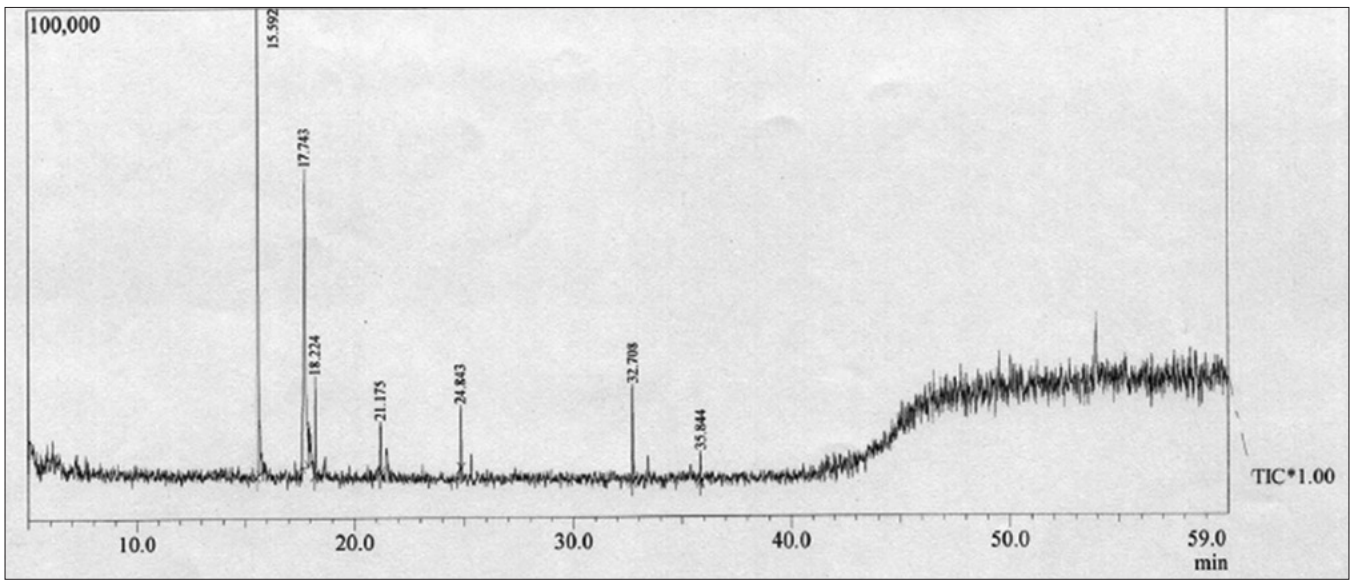

Fig. 9: The gas chromatography-mass spectrometry spectral peaks of the sample B2

Table 1: The phytochemical constituents obtained on GC chromatogram with NIST library match

R_{index}	Compound name	Mole formula	Mole weight
GC chromatogram with NIST library match of peaks with R.T 15.592 minutes			
1060	Methyl benzoate/Niobe oil	$C_8H_8O_2$	136
1437	Benzohydrazine/Benzoyl hydrazide	$C_7H_8N_2O$	136
GC chromatogram with NIST library match of peaks with R.T 18.225 minutes			
533	Dimethylbutane	C_6H_{14}	86
1385	Isobutyl pentyl ester	$C_{11}H_{20}O_4$	216
1250	Oxalic acid, butyl propyl ester	$C_9H_{16}O_4$	188
GC chromatogram with NIST library match of peaks with R.T 21.175 minutes			
1235	2-Heptyl-1,3-dioxolane	$C_{10}H_{20}O_2$	172
578	1,3-Dioxolane	$C_3H_6O_2$	74
1204	Acetic acid, 3-[1,3]dioxolan-2-yl] propyl ester	$C_8H_{14}O_4$	174
1057	2,2'-bis[1,3-dioxolane]	$C_6H_{10}O_4$	146
GC chromatogram with NIST library match of peaks with R.T 24.842 minutes			
1648	Isopropoxy-1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576
1716	2-(2',4',4',6',6',8',8'-heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethyl cyclo pentasiloxane	$C_{16}H_{48}O_{10}Si_9$	652
578	1,3-dioxacyclopentane	$C_3H_6O_2$	74
1770	1,3-dioxolane	$C_{10}H_{16}BrCl_3O_2$	352
GC chromatogram with NIST library match of peaks with R.T 32.708 minutes			
1282	Methyl decanoate/methyl caprate	$C_{11}H_{22}O_2$	186
1580	Methyl tridecanoate	$C_{14}H_{28}O_2$	228
910	Alpha methyl valeric acid	$C_6H_{12}O_2$	116
1680	Methyl myristate	$C_{15}H_{30}O_2$	242
GC chromatogram with NIST library match of peaks with R.T 35.842 minutes			
863	Acetylhydrazide	$C_2H_6N_{20}$	74
637	Isonitropropane	$C_3H_7NO_2$	89
1219	1,2-diacetylhydrazine	$C_4H_8N_2O_2$	116
701	1-nitropan	$C_3H_7NO_2$	89

tropicalis [30]. Flowers of *E. mili* exhibited significant antioxidant activity by reducing the levels of ROS and antitumor properties against breast cancer and colon cancer in mice by inducing apoptosis [22]. Significant antioxidant and anticancer properties were shown by the plant *A. hispida*. The cytotoxicity analysis using the Brine Shrimp Lethality test indicated very low level of toxicity [35]. Seeds of *H. pentandra* has hydnocarpin, a flavonolignan, which possess potent antimicrobial and anticancer activity [24].

The antioxidant activity of plant extracts was determined by DPPH and ABTS assays. The results of both assays as shown in Figs. 1-4 highlighted the antioxidant capacity of *F. jangomas* flower extract to be the best of all extracts followed by *H. pentandra* leaf extracts. A comparative analysis of antioxidant efficacies of the 4 parts of each plant shows that all the parts of *A. hispida* possess significant scavenging activity while in other plants, 2 parts each exhibited good antioxidant activity. In case of MTT assay Figs. 5 and 6 threw light on the fact that more potent cytotoxicity was shown by *F. jangomas* flowers and *H. pentandra* leaves and the activity was found to be dose dependent. The extracts of *E. mili*, *A. hispida,* and *L. nervosa* have not shown any cytotoxic activity. Taking into consideration the potency of *F. jangomas* flower extract with regard to its antioxidant activity, cytotoxic activity and also the results of antifungal studies done earlier, the extracts were subjected to cell cycle analysis and GC-MS analysis.

As depicted in Figs. 7 and 8, the extract showed effective cell cycle arrest at G_2M phase and in GC-MS analysis, the plant confirmed of the various phytochemical constituents as presented in Table 1 which were or being used as antifungal agents. Fig. 9 revealed the retention time and the spectral peaks of the bioactive compounds. Ethyl-2-hydroxy-4-methyl benzoate found in the isolated compounds of *Clerodendrum phlomides* has shown strong antimicrobial activity than the other compounds and this may be owing to the presence of esters and phenolic hydroxyl group. The similarity in antimicrobial activity of this compound with parabens may be attributable to the similar structure it shares with that of parabens [36]. Compounds derived from the reaction of 1-phenyl-3 methyl-4-benzyl-pyrazol-5-one and benzoyl hydrazide in ethanol is found to have high biological activity particularly strong antimicrobial activity against Gram-positive bacterial strains taken for study.

Promising antifungal potential was shown by propyl, butyl, pentyl esters against *Cryptococcus neoformans*. Myristic acids are found to have potent antibacterial and antifungal agents [37,38]. Fairly high percentage of methyl myrstitate that has strong antifungal and antibacterial principles is found in the leaves of *Excoecaria agallocha* [39]. Hydrazide hydrazone derivatives were found to exhibit marked antibacterial and antibacterial activity against *Streptococcus faecalis, Candida pseudotropicalis, Candida albicans,* and *Streptococcus aureus* [40-42]. It's reported that hydrazone derivatives possess noticeable anticonvulsant, antimicrobial antiproliferative, antitumour activities [29,43,44].

CONCLUSION

Our current work unveil the pharmacological efficacy of different plant extracts among which *F. jangomas* flower extract was considered to be promising in being a potent source of natural antioxidants and also possessing significant cytotoxic and antiproliferative activity. We report for the first time the isolation of bioactive compounds of *F. jangomas* flower extract and the antiproliferative potential of the extract on Calu6 and SCC9 human cancer cell lines. Further, this plant can be taken up as a promising candidate in future research for the development of new anticancer drugs.

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