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INVESTIGATIONS OF INVITRO CYTOTOXICITY AND ANTIOXIDANT ACTIVITY OF BIOACTIVES FROM THE LEAVES OF DILLENIA BRACTEATA

Sandhya Kota[1]*, Senthil Raj R[2]
[1]Research Scholar, Dr. MGR Educational & Research Institute, Velappanchavadi, Chennai-600077, Tamil Nadu, India
[2]Faculty of Pharmacy, Dr. MGR Educational & Research Institute, Velappanchavadi, Chennai-600077, Tamil Nadu, India
*Corresponding author: sandhyakota1986@gmail.com; 8919364830

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Abstract
Cancer remains a significant global health challenge due to limited treatment options and associated side effects. Herbal medicines offer promising alternatives for cancer treatment, given their perceived effectiveness, safety, and reliability. In this study, we investigated the in vitro antioxidant and cytotoxic activities of Dillenia bracteata Wight on Ehrlich ascites carcinoma (EAC) cell lines. The plant material was collected from a local area in Chennai, Tamil Nadu. Various extracts were prepared using pet ether, benzene, ethyl acetate, methanol, and distilled water. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, glycosides, phenols, steroids, flavonoids, gums, mucilages, proteins, volatile oils, fixed oils, and fats in the extracts. Quantitative estimation of secondary metabolites showed that the methanol extract exhibited higher levels of chemical constituents compared to other extracts, particularly in terms of phenols, glycosides, flavonoids, and saponins. Column chromatography of the methanol extract using dichloromethane, acetone, ethanol at various concentrations led to the isolation of an active chemical constituent, which was subjected to further evaluation for antioxidant and cytotoxic activities. The in vitro antioxidant activity of the isolated fraction was assessed using superoxide radical scavenging, hydroxyl radical scavenging, and lipid peroxidation assays. Results indicated significant antioxidant activity of the isolated fraction compared to the methanol extract and standard drugs. Furthermore, the cytotoxicity of the isolated fraction and methanol extract was evaluated using the MTT assay. Both showed significant anticancer activity against EAC cell lines, with IC50 values of 91.55 µg/mL and 92.72 µg/mL, respectively. Morphological changes observed in EAC cell lines treated with the isolated fraction and methanol extract suggested the induction of apoptosis, a crucial mechanism in cancer treatment. In conclusion, this study underscores the importance of exploring natural sources for novel anticancer agents and provides valuable insights for the development of alternative cancer treatment options with minimal side effects. The findings support the potential anticancer properties of Dillenia bracteata and warrant further investigation into its mechanisms of action. Further research is needed to elucidate the exact mechanisms underlying the anticancer activity of Dillenia bracteata and to validate its efficacy in vivo

Keywords:Dillenia, Antioxidant, Cytotoxic activity, Flavonoids, extraction, EAC cell lines

INTRODUCTION

Cancer is one of the most burdening health problem in the world now (Tagne et al., 2014). It is characterized by uncontrolled progression of cell cycles leading to abnormal growth of tissue (Sumitra and Nagani et al., 2013). Cancer develops via altering the oncogenic genes, genes that supress the tumors and genes that repair DNA replication. This alterations are triggered by chemicals, smoking, alcohol, solar radiation, microorganisms and mutations (Mukherjee et al., 2007; Ganapathy et al., 2015). Cancer treatment involves a spectrum of options that includes pharmacotherapy, radiotherapy, harmonal therapy and surgical options where each of them have side effects and adverse drug reactions especially in chemotherapy (Krishnamoorthy and Ashwini, 2011). These side effects on one hand and the harmful effects of cancer on the other hand provoked scientists to probe for alternative therapy options for treating cancer safely and effectively with less side effects and better efficacy (Haghighi et al., 2017). In current scenario, the demand for the herbal formulations has been growing drastically in the world and majority of the industries were focussing on investigating the research on herbal medicine to treat cancer. Thus to enhance the safety of the herbs and application of the medicinal properties of the herbs to treat cancers, extensive research is to be conducted (Chopra et al., 1956).

The plant Dillenia bracteata Wight belonging to the family Dilleniaceae which is usually called as fish bone plant and locally known as Chiruthaeku, Bettadakanugalu, Colikkay in Telugu, Kannada and Tamil respectively. Thus it is basically distributed throughout the southern India. Dillenia bracteata Wight is 30-60 ft tall with tomentose shoots and scarred branches. Its leaves are simple, ovate-elliptic or obovate-elliptic, 16-46 x 5-19 cm. Flowers are yellow, solitary or clustered, with lanceolate or spathulate bracts. The calyx has 5 ovate sepals. The corolla has 5 obovate yellow petals. Stamens are numerous with linear anthers. Carpels are numerous with curved styles. Fruits are pseudocarps, subglobose, orange when ripe, enclosed by enlarged sepals. Fruiting carpels are 1-2 seeded, ovoid, dark brown to black, about 5 mm. The plant was traditionally used to treat rheumatoid arthritis, dysentry, hepatitis, inflammations, wounds, diabetes and some gastrointestinal disorders. Various reports have been published proving the anti-inflammatory, antibacterial, antihemorragical and immunogenic activities of the plant. Studies showed the anti cancer activity and antiulcer activity too (Shama et al., 2019). Thus this research was aimed to investigate the phytochemistry of the plant along with proving the invitro cytotoxic and antioxidant potential of the isolated fraction from the extract of dried leaves of the plant.

MATERIALS AND METHODS

Plant material

Fresh leaves of Dillenia bracteata Wight was collected local area in Chennai, Tamil Nadu during March. The plant species was identified and authenticated by duly by a certified botanist and a voucher specimen of the identified plant species was deposited in the library for future reference.

Chemicals and Reagents

All Chemicals solvents and reagents used in the study were of analytical grade and were procured from Sigma Aldrich India Ltd, India and Himedia Laboratories Ltd., Mumbai.

Cell Cultures

The Ehrlich ascites carcinoma (EAC) were obtained from National Centre for Cell Science (NCCS), Pune and were maintained in the intraperitoneal region of the swiss albino mice and incubated till further use.

Physico chemical constants

The plant parts were dried at room temperature for 5 days, properly ground and the powder is passed through sieve no. 80. It was evaluated for physical constants like moisture content, ash values, extractive values (Kokate et al., 1995).

Extraction

The dried leaf material was coarsely powdered and defatted and extracted using soxhlet apparatus in successive extraction method using Pet ether, Benzene, Ethyl acetate, Methanol and Distilled water (lin et al., 2016, Avinash et al., 2012). The resultant solution as filtered and the filtrate was evaporated to dryness using rotary vacuum evaporator. The final yield of the extract was calculated per dry weight of powdered leaf.

Preliminary phyto chemical screening

The dried extracts were tested for alkaloids, carbohydrates, glycosides, phenols, steroids, flavoniods, gums and mucilages, proteins, volatile oils, fixed oils and fats and saponins (Harbone, 1998; IP, 1966).

Quantitative Estimation of Secondary Metabolites

The estimation of total phenol, flavonoid contents in the extracts of leaves of *D.bracteata* were performed by using Folin –Ciocalteu's assay method (VYA Barku *et al.,* 2013; Madaan R *et al.,*2011 and John *et al.,* 2014) Alkaloid content, glycoside content and saponin contents by using standard methods described by Edeoga HO *et al.,* 2005.

Isolation of active chemical constituent

The thick methanol extract (MEDB-75g) was weighed and silica gel medium was added (100-200 mesh size). Combination of Dichloromethane and Acetone were used in varying ratios (9:1-1:9 and 100% acetone) were used to elute the extract. Fractions from the column were collected in 200mL portions. Based on the Rf values of TLC, the resultant fractions were combined with comparable fractions. After this fraction 7 which gave the highest yield was further fractioned using dichloromethane: acetone: ethanol (9:1:1, 6:2:2, 5:3:2, 3:3:4 and 1:4:5). The Rf values are shown in table.

In vitro antioxidant activity

The in vitro antioxidant activity of the methanol extract and the isolated fraction was carried out using the following three assay methods in accordance with previously reported procedures with minor modifications (Li et al., 2017).

Superoxide radical scavenging activity

The reaction mixture containing phosphate buffer (0.5 mL, 100mM, pH 7.4), 1.0 mL of NADH (0.4mM), 1.0 mL of NBT (0.156mM), 0.1 mL of PMS (0.06mM) and 3 mL of the isolated fraction, extract and standard of various concentrations (10- 50 µg/mL, in 90% ethanol). After incubation at 25 ºC for 1 h, the absorbance of the reaction mixture was measured at 560 nm against an appropriate blank to determine the quantity of formazan formed (More & Makola, 2020).

Hydroxyl radical scavenging activity

The reaction mixture containing 0.1 mL of 2-deoxy-2- ribose (10mM), 0.33mL of phosphate buffer (50mM, pH 7.4), 0.1 mL of FeCl3 (0.1 mM), 0.1 mlethylenediamine tetra-acetic acid (EDTA) (0.1mM), 0.1 mL of H_2O_2 (mM), 0.1 mL of ascorbic acid (1mM) and 1.0 mL of various concentrations (5-50 µg/mL) of the isolated fraction, extract and standard. After incubation for 45 min at 37 ºC, 1.0 mL of 2.8% (v/v) TCA, and 1.0 mL of [thiobarbituric acid, TBA, 0.5% (v/v) in 0.025 mol/L NaOH solution containing 0.2% (w/v) of butylated hydroxyl anisole, BHA] were added in the reaction mixture, and the mixture was incubated at 95ºC for 15 min to develop the pink chromogen. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution (Sabir et al., 2020).

Lipid peroxidation scavenging activity

Reaction mixture (0.5 mL) containing rat liver homogenate (0.1 mL, 25% w/v) in Tris-HCl buffer (40 mM, pH 7.0), KCl (30 mM), FeCl3 (0.16 mM) and ascorbic acid (0.06 mM) was incubated for 1 h at

37 ºC in the presence and absence of the isolated fraction, extract and standard drugs at various concentrations (50- 250 µg/mL). The lipid peroxide formed was measured by TBARS formation (Karthik et al., 2019). For this incubation mixture 0.4 mL was treated with sodium dodecyl sulphate

(8.1%, 0.2 mL), TBA (0.8%, 1.5 mL) and acetic acid (20%, 1.5 mL, pH 3.5). The total volume was then made upto 4.0 mL by adding distilled water and kept in a water bath at 100 ºC for 1 h. After cooling, 1 mL of distilled water and 5.0 mL of a mixture of n-butanol and pyridine (10:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The butanol-pyridine layer was removed and its absorbance at 532 nm was measured to quantify TBARS.

In all the three above methods, the %inhibition of scavenging activity was calculated using the following equation.

$$Percent\ inhibition\ (\%) = (A_{control}-A_{test})/A_{control}\times 100\ (1)$$

Where, Acontrol is the absorbance of the control and Atest represents the absorbance of a test substance (Ethanol Extract / standard drug).

Determination of Invitro Cytotoxicity using MTT Assay (Mosmann and Tim, 1983).

The isolated fraction, Methanol extract of Dillenia were tested for *in vitro* cytotoxicity, usingEAC cell lines by MTT assay. Briefly, the cultured EAC Cells were harvested and pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200µL) into the 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the extract, isolated fraction samples in a serum-free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After incubation, MTT (20 µL of 5 mg/ml) was added into each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC_{50} value was calculated using the formula:

Cell viability % = Test OD/Control OD X 100

RESULTS

Physicochemical constants

The Physicochemical constants of the leaf powder like ash values and moisture content were illustrated in table 1. The extractive values and the physical parameters were given in table 2.

Table 1. Physicochemical parametres of the leaves of Dillenia bracteata Wight

S. No.	Parameters	Average % W/W
1.	**Ash values**	
	a) *Total ash*	6.18±0.43
	b) Acid insoluble ash	3.25±0.55
	c) Water soluble ash	1.83±0.34
	d) Sulphated ash	0.21±0.11
2.	**Moisture content**	9.46±1.22

Values were expressed as Mean ±SD

Table 2. Extract parameters of leaves of Dillenia bracteata Wight

Solvent	Extractive value %w/w	Colour	Consistency
Pet. Ether soluble Extractive	3.96	Black green	greasy
Benzene soluble extractive	1.02	Dark green	greasy
Ethyl acetate soluble Extractive	5.88	Black green	greasy
Methanol soluble Extractive	24.73	Black brown	non greasy
Aq soluble Extractive	25.97	Brown	sticky non greasy

Preliminary phytochemical screening revealed the presence of polyphenols, alkaloids, steroids, carbohydrates, proteins, gums and mucilages, saponins etc.

Table 3. Preliminary phytochemical screening of various extracts of Dillenia bracteata Wight

Sl. No.	Test	Pet. Ether	Benzene	Ethyl acetate	Methanol	Water
1.	Carbohydrates	-	-	-	+	+
2.	Alkaloids	-	+	-	+	+
3.	Glycosides	-	-	-	+	+
4.	Tannins	-	-	-	+	+
5.	Steroids	+	-	-	-	-
6.	Triterpenoids	-	-	-	+	-
7.	Volatile oils	-	-	-	-	-
8.	Fats and fixed oils	-	-	-	-	-
9.	Flavanoids	-	-	-	+	+
10.	Polyphenols	-	-	-	+	+
11.	Saponins	-	-	-	+	+
12.	Aminoacids	-	-	-	+	+

13.	Gums and mucilages	-	-	-	-	+

"+" representsPresence **"-"** representsAbsence

Quantification of secondary metabolites

The estimation of the amount of secondary metabolites in various extracts was determined and tabulated in table 4. Pet ether extract has a total phenol content of 34.25 mg/g, total flavonoid content of 14.35 mg/g, total saponin content of 0.99 mg/g, total glycoside content of 3.09 mg/g, and total alkaloid content of 2.90 mg/g. Benzene extract has least amount of all the components compared to the other extracts followed by Ethyl acetate extract. Methanol extract exhibits significantly higher values for all components compared to the other extracts. Distilled water extract also shows relatively high values, especially for total phenol and flavonoid content following the methanol extract. Hence, the methanol extract was used to isolate various fractions using coloum chromatography.

Table 4. Amount of secondary metabolites in various extracts ofDillenia bracteata Wight

Sl. No.	Extract	Total Phenol content mg/g	Total Flavonoid content mg/g	Total saponin content mg/g	Total Glycoside content mg/g	Total alkaloid content mg/g
1.	**Pet ether**	34.25±3.09	14.35±1.97	0.99±0.15	3.09±0.34	2.90±0.93
2.	**Benzene**	12.33±3.61	7.94±1.11	0.31±0.10	0.65±0.11	2.76±0.81
3.	**Ethyl acetate**	30.91±3.08	17.22±2.08	1.26±0.42	1.01±0.14	0.87±0.06
4.	**Methanol**	320.85±16.27	196.55±15.45	22.10±2.07	35.37±5.83	5.66±0.88
5.	**Distilled Water**	297.86±11.39	136.64±13.28	29.12±3.91	31.86±4.87	4.68±1.63

The methanol extract of the leaves of D. bracteata was subjected to column chromatography using various ratios of solvents and they eluted 19 fractions out of which similar fractions were combined and finally made as 9 fractions named as fraction 1-9. All the fractions were collected and filtered. They were evaporated using a rotary evaporator and the dry powder was weighed and noted in table 5. Fraction 7 gave the highest weight thus it was subjected to further fractionation using DCM, acetone and ethanol as solvents. This eluted 5 fractions of which fraction 2 gave more contents in terms of weight. This was subjected to further fractionation using methanol and chloroform and yielded 2 fractions, Fraction 2a and 2b. Fraction 2a resulted in more weight and further analysis showed no separate constituents. Thus this fraction was considered as isolated fraction and used for further analysis.

Table 5. Isolated fractions from the methanol extract of leaves of Dillenia bracteata Wight

Fraction	Elute	Ratios (v/v)	Weight (g)
Fraction 1	DCM+Acetone	9:1	1.24
Fraction 2	DCM+Acetone	8:2	0.81
Fraction 3	DCM+Acetone	7:3	1.17
Fraction 4	DCM+Acetone	6:4	5.05
Fraction 5	DCM+Acetone	4:6	2.95

Fraction 6	DCM+Acetone	3:7	6.32
Fraction 7	DCM+Acetone	2:8	19.88
Fraction 8	DCM+Acetone	1:9	14.49
Fraction 9	Acetone	1	10.03
Isolate 1	DCM+Acetone+Ethanol	9:1:1	1.08
Isolate 2	DCM+Acetone+Ethanol	6:2:2	7.82
Isolate 3	DCM+Acetone+Ethanol	5:3:2	2.84
Isolate 4	DCM+Acetone+Ethanol	3:3:4	1.95
Isolate 5	DCM+Acetone+Ethanol	1:4:5	2.26
Isolate 2a	Methanol+Chloroform	7:3	5.03
Isolate 2b	Methanol+Chloroform	5:5	2.11

In vitro antioxidant activity

The isolated fraction exhibited the lowest IC50 value (18.437) compared to the methanol extract (26.384) and the standard antioxidant (23.433). A lower IC50 value indicates better antioxidant activity, suggesting that the isolated fraction is more effective in scavenging superoxide radicals compared to the methanol extract and the standard antioxidant. Similarly, the isolated fraction showed the lowest IC50 value (16.174) compared to the methanol extract (23.665) and the standard antioxidant (22.098). This indicates that the isolated fraction has stronger hydroxyl radical scavenging activity compared to the methanol extract and the standard antioxidant. For lipid peroxidation inhibition, again, the isolated fraction exhibited the lowest IC50 value (27.808) compared to the methanol extract (33.941) and the standard antioxidant (29.833). A lower IC50 value in this assay indicates better inhibition of lipid peroxidation, suggesting that the isolated fraction is more effective in preventing lipid peroxidation compared to the methanol extract and the standard antioxidant.

These results demonstrate that the isolated fraction possesses potent antioxidant activity as evidenced by its superior performance in all three assays compared to both the methanol extract and the standard antioxidant. This suggests that the isolated fraction may contain bioactive compounds with strong antioxidant properties. The higher potency of the isolated fraction in scavenging superoxide and hydroxyl radicals, as well as inhibiting lipid peroxidation, highlights its potential therapeutic value in combating oxidative stress-related diseases.

Table 6. Invitro antioxidant activity of isolated fraction and methanol extract of leaves of Dillenia bracteata Wight

Compound	IC_{50} (µg/ml)		
	Superoxide radical scavenging	**Hydroxyl radical scavening**	**Lipid peroxidation**
Isolated Fraction	18.437**	16.174*	27.808*
Methanol Extract	26.384	23.665	33.941
Standard	23.433*	22.098	29.833*

The values were significant at ***p<0.001, **p<0.05, *p<0.01 compared to the extract treated group

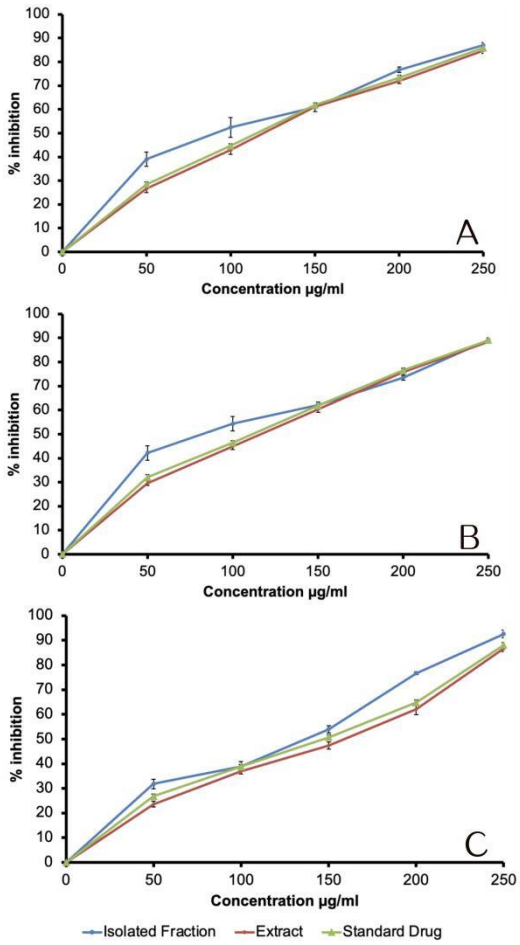

Figure 1. Invitro antioxidant activity of methanol extract and isolated fraction of leaves of Dillenia bracteata Wight

A. Superoxide radical scavenging activity, B. Hydroxyl radical scavenging activity, C. Lipid peroxidation scavenging activity

Invitro cytotoxicity activity

Invitro cytotoxicity of the isolated fraction and methanol extract of D.bracteata had been tested on the EAC cell line in various concentrations ranging from 1 to 1000µg/ml and the respective IC_{50} values were calculated and tabulated in table 7. the IC_{50} value of the extract was 92.72 where as the isolated fraction showed 91.55µg/ml indicting that the isolated fraction was slightly potent comparable to extract. The extract and fraction also induced morphological changes in the cells. the control group cells showed normal cell architecture. methanol and extract treated cells showed condensation of the nucleus and death in the cells induced by apoptosis.

Table 7. Invitro cytotoxicity of methanol extract and isolated fraction

Concentration (µg/ml)	% Cell viability	
	Methanol Extract	Isolated Fraction
1	99.723±0.134	99.723±0.134
5	99.580±0.190	99.580±0.190
10	97.086±4.377	97.086±4.377
20	96.856±4.265	96.856±4.265
40	91.586±0.550	91.233±0.777
60	82.221±0.543	82.221±0.543
80	63.302±0.710	63.302±0.710
100	42.446±0.822	41.741±0.426
150	35.988±1.252	35.018±0.429
200	25.089±0.520	25.089±0.520
250	14.384±0.656	14.120±0.299
300	10.371±0.203	10.017±0.421
500	7.906±0.309	7.597±0.480
1000	3.062±0.138	2.930±0.160
IC_{50}	92.72 µg/ml	91.55 µg/ml

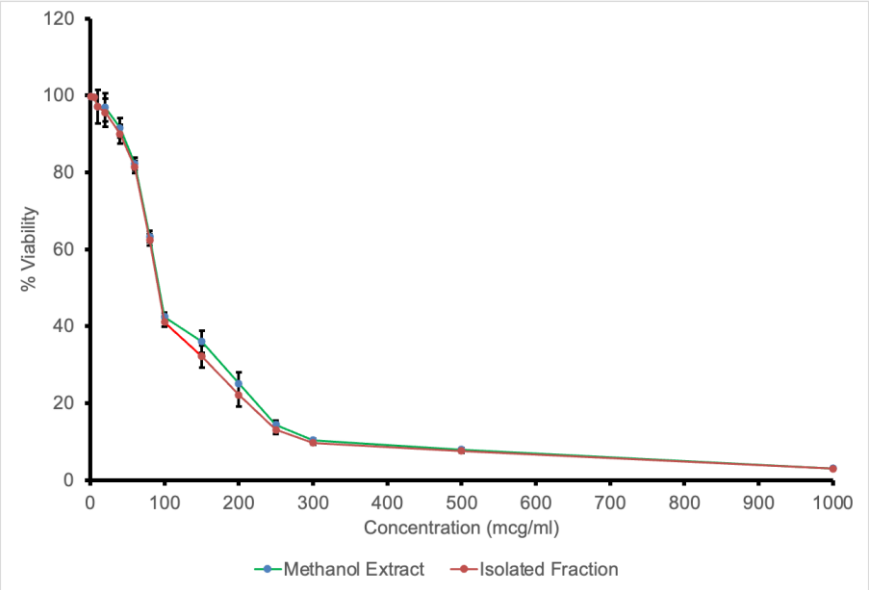

Figure 2. % cell viability of EAC cell lines with Methanol extract and isolated fraction

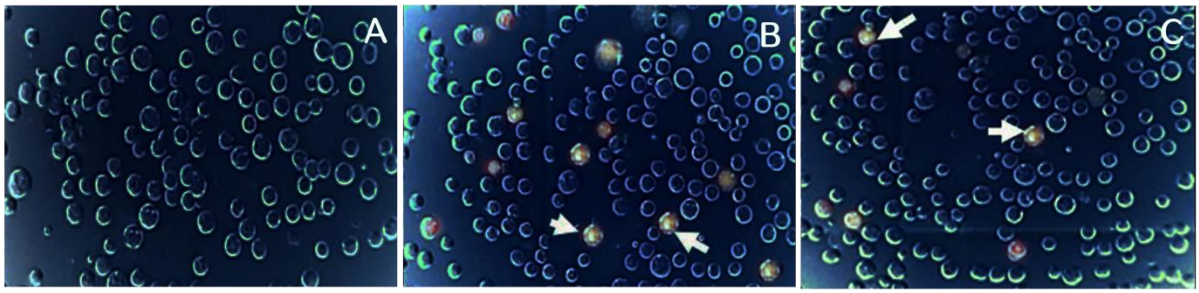

Figure 3. Morphology of EAC cell lines treated with B. methanol extract and C. isolated fraction A. Control

Green nucleus indicating healthy cells; Arrows indicate formation of apoptotic bodies, condensation of nucleus with orange fluorescence, blebbing of membranes, orange/red stained nucleus indicates dead cells, dark green colour inside apoptotic cells indicates fragmented chromatin

DISCUSSION

Cancer is the most fatal problem in the world causing mortality and due to the poor treatment options with more side effects, curing this is becoming a challenge (Mamun et al., 2016). Therefore in search for the alternative options, herbal medicines pose themselves as effective, safer and reliable options for treating cancer. Thus in this research, Dillenia bracteata had been investigated for invitro antioxidant and cytotoxicity activity on EAC cell lines. The plant was previously investigated for anticancer activity in various methods and proven to show its activity against diabetes, inflammations and wounds (Shama et al., 2019). The results of the current study also enhance those studies. In this study various extracts of the plant had been prepared using pet ether, benzene, ethylacetate, methanol and water. The preliminary phytochemical screening which indicated the presence of glycosides, flavonoids, phenols, alkaloids etc in the extracts but The quantification of secondary metabolites was performed which showed the methanol extract showed more number of chemical constitutents compared to other extracts followed by aqueous extract. Benzene showed least amount of chemical consitituents indicating that the bioactive compounds present in the plant leaves were polar and soluble in polar solvents like methanol. With respect to the phenols, glycosides, flavonoids and saponins, methanol extract showed a signifincatly higher amount of constituents compared to other extracts.

Coloumn chromatography was conducted with the methanol extract using dichloromethane and acetone in varied proportions to elute various fractions that resulted in 19 fractions out of which similar fractions were combined and finally 9 fractions were obtained. based on the amount of fraction, Fraction 7 was subjected to re-elution using dichloromethane, acetone and ethanol which resulted in 5 other fractions from which fraction 2 was re-eluted to give two fractions. the first and large fraction was selected and filtered off the coloumn and recrystallized.

The isolated fraction and the methanol extract were subjected to invitro antioxidant study using superoxide, hydroxyl and lipid peroxidation scavenging activities. The results indicated that the isolated fraction showed a significant activity compared to the extract and standard drug also. The presence of flavonoids and polyphenols can be attributed to the antioxidant activity of the plant. Further investigations were carried out to prove the cytotoxicity of the plant on the EAC cell lines. The results of the study indicated that the isolated fraction showed a significant anticancer activity comparable to the methanol extract which is evident from the IC_{50} of 91.55 and 92.72 µg/ml respectively. Figure 2

shows the comparison of the anticancer activity against EAC cell lines and the lower the IC_{50} higher the activity (Rieser et al., 1996). Thus in further studies, this IC_{50} can be considered while investigating the cytotoxicity of the compounds or the methanol extract.

On the other hand EAC cell lines had been the most widely used cell lines in cancer research and as the extract and the isolated fraction exhibited significant antioxidant activity the assumptions of the anticancer activity of the plant leaves was supported by the investigation (Ozaslan et al., 2011). Morphological changes shown in figure 3 indicated the cells reacted to the extract and isolated fraction showed significant condensation of the nucleus, chromosomal fragmentation compared to round shape and regular size of normal cells indicating active apoptosis. As the phase is important to control the cancer growth and cell cycle, induction of this phase is a crucial stage in the cancer treatment (Brown and Attardi, 2005). This cell shrinkage and condensation are features of apoptosis and so it can be advocated that the mechanism of action of the isolated fraction in inhibition of cancer was by induction of apoptosis (Sun et al., 1994). Thus in further research, the exact mechanisms of anticancer activity of the isolated fraction to be investigated so as to enhance the cancer treatment and also to quantify the activity in terms of identification of mutations, gene expression etc involved in cancer development.

CONCLUSION

In conclusion, the investigation into the potential anticancer properties of leaves of Dillenia bracteata highlights its promising role as an alternative therapeutic option against cancer. The in vitro antioxidant and cytotoxicity studies conducted in this research underscore the significant activity of the isolated fraction, particularly when compared to the methanol extract and standard drugs. The presence of flavonoids and polyphenols in the plant extract suggests their contribution to the observed antioxidant and anticancer effects.

Moreover, the morphological changes observed in EAC cell lines treated with the extract and isolated fraction indicate a potential mechanism of action involving apoptosis induction. This is a crucial aspect in cancer treatment, as the inhibition of cancer growth and induction of apoptosis can impede tumor progression. Moving forward, further investigations are warranted to elucidate the exact mechanisms underlying the anticancer activity of the isolated fraction. Understanding these mechanisms can not only enhance cancer treatment strategies but also facilitate the identification of potential molecular targets for therapeutic intervention. Additionally, quantifying the activity of the isolated fraction in terms of mutation identification, gene expression analysis, and other molecular pathways involved in cancer development will provide valuable insights into its therapeutic potential. Overall, the findings presented in this study underscore the importance of exploring natural sources, such as Dillenia bracteata, for their potential anticancer properties. Further research in this direction holds promise for the development of novel and effective cancer treatment options with minimal side effects, ultimately contributing to the global effort to combat this fatal disease.

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