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Research Article

Metabolite Profiling and *In-vitro* Evaluation of Cytotoxic Efficacy of *Psychotria dalzellii* Hook.f. on MCF-7 and L929 Cell Lines

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ABSTRACT

In the current study, *Psychotria dalzellii* Hook.f. stem-bark samples were procured from Joida, Western Ghats, Karnataka, India. The collected stem bark samples were air-dried and subjected to soxhlet extraction using petroleum ether, acetone and ethanol as a solvents. The obtained extracts were screened for preliminary phytochemicals which resulted in the existence of flavonoids, alkaloids, glycosides, phenols, tannins, steroids, triterpenoids, saponins, carbohydrates and lignins. Subsequently, the ethanolic stem bark extract of *P. dalzelllii* was subjected to gas chromatography-mass spectrometry (GC-MS) analysis for quantitatively estimate the phytoconstituents. It reveals the presence of 20 bioactive chemicals. Among them, 2-furancarboxaldehyde, 5-(hydroxymethyl)- (59.26%); p-mesyloxyphenol (10.68%); 2-furaldehyde diethyl acetal (4.54%) and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (4.11%) were noted to be the principal compounds detected. Furthermore, *in-vitro* cytotoxic activities on MCF-7 and L929 cell lines were determined by MTT assay. The extract exhibited considerable cytotoxic activity towards MCF-7 cell lines at higher concentration with an IC_{50} value of 440.82 µg/mL and it showed very less toxicity towards L929 normal cell lines. This indicates its potential as a possible source of bioactive constituents for medicinal and pharmaceutical exploration.

Introduction

Cancer is a condition that causes the body cells to multiply out of control and block the development of the cell cycle, giving rise to malignant tumor cells, that have the tendency to spread to other body organs.[1] Currently, it is the top cause of fatalities all over the globe and is taking lives every day.[2] India came in third place after China and the USA. Breast cancer is the most prevalent kind of malignancy and the leading reason for cancer-related fatalities in women throughout the world.[3] An online database called GLOBOCAN 2020 provides data on incidence and mortality rates for 185 countries across 36 different types of cancer.[4] It is estimated that, there were more than 2,261,419 new cases and 684,996 deaths worldwide and in India, the new cases recorded are 1,78,361 and deaths are around 90,408 due to breast cancer. As a result of the increased incidence of cancer-related mortalities provoked researchers to investigate novel anticancer medicines.[5]

The standard cancer treatment procedures relied heavily on conventional therapies, like chemotherapy, surgery, immunotherapy and radiation therapy which are used as single or combinatorial therapy.[6] Although, these therapeutic techniques are effective in treating various types of malignancies, they have limitations, such as recurrence of the malignancy, noncompliance due to significant side effects like pain, exhaustion, anemia, nausea, emesis, hair loss[7] and direct targeting of cell division and DNA replication in malignant and normal cells by alkylating drugs, topoisomerase suppressors and antimicrotubule medicines results in negative side effects.[8] In addition, a small portion of cancerous cells referred as cancer stem cells (CSCs) are unaffected by

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conventional cancer treatment. As a result, it is essential to use a complementary approach to treat cancer.[9]

"Herbal medicine" describes using plants and plant extracts to cure and prevent diseases and disorders.[10] According to the WHO, around 80% of people in developing nations use herbal medicines to address their fundamental medical requirements,[11] due to their safety and effectiveness against some of the most severe forms of diseases.[12] Plant-derived chemicals operate by altering the signaling pathways in malignant cells and these are admitted to simultaneously influence several signaling pathways; as a result, they are quite proficient at prohibiting the unregulated growth of cancerous cells.[13] Several natural anticancer agents are currently used in clinical trials including vinblastine, vincristine, etoposide, paclitaxel, topotecan, camptothecin, irinotecan and taxol are derived from plant sources.[14-16] Nonetheless, there are still a lot of plants with cytotoxic property have yet to be explored.[17]

Psychotria L. is the most extensive genus in the Rubiaceae family; more than 1600 species were recorded.[18] Several species of the genus *Psychotria* are employed in folklore practices for treating a variety of ailments.[19,20] *Psychotria dalzellii* Hook.f. is a large erect shrub or small tree commonly known as Dalzell's psychotria. *P. bracteate* Wight & Arn. and *Uragoga dalzellii* (Hook.f.) Kuntze are the synonyms. This plant is endemic to the Western Ghats and fairly common in forests as undergrowth.[21] It has been ethnomedicinally reported that the stem part is used to treat pruritus.[22] Eye drops prepared from freshly squeezed *P. dalzellii* leaf juices help in treating migraine headaches.[23] According to earlier studies, this plant showed anti-diabetic and antioxidant properties.[24] To date, no reports have been found regarding anticancer activity from stem bark extract of *P. dalzellii.* As a result, the current work was undertaken to identify various chemical constituents found in stem bark extract of *P. dalzellii* and to assess the cytotoxic efficacy towards MCF-7 cells and mouse fibroblast normal cells.

Materials And Methods

Collection of Plant Materials and Taxonomical Identification

The *P. dalzellii* fresh stem bark samples was collected from Joida located in the Western Ghats of Karnataka, India. The plant materials were identified and authenticated by the Taxonomist and the voucher samples were deposited in the Department of Applied Botany, Kuvempu University, shankaraghatta, Shivamogga, Karnataka, with voucher specimen number (KU/BOT/RN/TAB-01).

Preparation of the Plant Extracts

The retrieved stem bark materials were washed under tap water and then it was chopped; dried in the shade and pulverized utilizing a mill grinder to a coarse powder and then, it was subjected to soxhlet extraction using petroleum ether, acetone and ethanol solvents. The extracted materials were air-dried and kept in airtight containers at 4°C and then it was utilized as a test sample for further studies.

Preliminary Biochemical Profiling

Using standard methods,[25,26] the obtained *P. dalzellii* stem-bark extracts were subjected to qualitative phytochemical screening to identify different classes of active phytoconstituents such as phenols, flavonoids, alkaloids, glycosides, tannin, terpenoids, saponins, steroids, lignins and carbohydrates.

Metabolite Profiling by GC-MS analysis.

The standard GC-MS model was used to perform the GC-MS profiling of *P. dalzellii* stembark ethanolic extract at SAIF laboratory, IIT Bombay. The protocol followed was Dandekar *et al.* (2015).[27]

Instrument details

The GC instrument utilized was an Agilent 7890, the detector was a flame ionization detector (FID) and the GC ran for a total of 35 minutes. The Joel Accu time of flight analyzer (TOF) GCV instrument for MS, with a mass range of 10 to 2000 amu and a resolution of 6000 was employed. GC-MS profiling was carried out using a split-less injection method (spilt 20:80-8-200-5M-8-260-10M-10-280-HP5-ETOH) of 1.0-µL of the sample in ethanol on a Hewlett Packard 6890 (USA) With a mass detector attached, the gas chromatograph has an internal capillary column made of cross-linked 5% phenyl methyl siloxane HP-5 MS (length 30 mm, internal diameter 0. 32 mm, film 0. 25 m).

GC-MS operating parameters

The starting column temperature was 35°C with a retention period of 3 minutes. The temperature was set to increase by 8°C every minute, reaching 280°C at the end. Injecting 1-µL of the sample into the port allowed it to quickly evaporate and migrate down the column at a flow rate of 1-mL/min utilising helium as the carrier gas. At 70 eV, the MS spectrum was recorded. Following the column separation, the components were recognized and subjected to additional analysis by FID.

In-vitro Cytotoxicity Determination by MTT Assay

Cell line culturing

The cell lines MCF-7 and L929 were procured from NCCS, in Pune, India. The cells were cultured in DMEM coupled with 10% heat-inactivated FBS, 1% penicillin-streptomycin and 1% nonessential amino acid. Cells were incubated at 37°C in 5% CO_2 and 95% atmospheric humidity. The cells were counted and their viability was assessed using a hemocytometer after trypsinization. The MTT experiment was carried out with 20,000 cells per well in 200 µL cell suspension plated into 96-well plates.

Treatment groups

MCF-7 cell lines were treated with ethanolic stem bark extract of *P. dalzellii.* Prior to the experiment, the desired concentrations of the extract were made in dimethyl sulfoxide. The reactant solution mixtures were diluted with medium and cells were treated with varying doses (31.25–500 µg/mL) of extract and incubated for 24 hours. The effect of cytotoxicity was compared with cisplatin, a commonly used standard medicine. The trials were divided into two treatments. Negative control; untreated cancer cells. Positive control; cells treated with cisplatin (10 µM). The same treatments were followed for normal cell line L929.

Determination of cell viability

The 96-well plates were removed after 24 hours of the incubation period and add the MTT reagent to a final dose of 0.5 mg/mL of overall volume. Again 96 well plates were placed in the incubator for 3 hours. After incubation, the formazan produced was solubilized by adding 100 µL DMSO solution. The suspension was placed on a gyratory shaker for 5 minutes, an ELISA reader was used to measure the absorbance between 570 and 630 nm. IC_{50} value was ascertained.

Results

Qualitative Assessment of Phytoconstituents

The *P. dalzellii* stem bark extract of three solvents contained a wide range of phytochemical constituents, as shown in Table 1. The ethanolic extract has shown the presence of alkaloids, flavonoids, glycosides, tannins, phenols, saponins, triterpenoids, steroids, lignin and carbohydrates. Whereas, phytoconstituents like tannins, glycosides, steroids, and lignin were detected in acetone extract. The petroleum ether extract contained only saponins. Since the ethanolic extract showed the presence of maximum phytochemical components, these bioactive compounds might be responsible for its medicinal properties and hence, the ethanolic extract was used for further pharmacological investigation.

Quantitative GC-MS Analysis

The GC-MS profiling of the stem-bark ethanolic extract of *P. dalzellii* showed the existence of 22 peaks indicating the presence of twenty phytocompounds (Figs 1 and 2). The mass spectral fingerprint of all constituents was identified by comparing their recorded spectra to the NIST library V-11 data bank mass spectra provided by the software. The compound names with their retention time (RT), molecular formula (MF), molecular weight (MW), peak area percentage and their properties are represented in Table 2.

The results revealed the existence of 2-furan-carboxaldehyde, 5-(hydroxymethyl)- (59.26%) as a predominant compound followed by p-mesyloxyphenol (10.68%); 2-Furaldehyde diethyl acetal (4.54%); 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (4.11%); 1-Cyclohexyl-2,2-dimethyl-1-propanol acetate (3.97%); Silane, dimethyldecyloxyhexadecyloxy- (2.49%); Dodecane (2.43%); Benzene, 1-(chloromethyl)-2-fluoro- (2.02%); Octadecane, 9-ethyl-9-heptyl- (1.80%); Hexadecane, 1-iodo (1.30%); 1,7-Dimethyl-4-(1-methylethyl)cyclodecane (1.17%); Oxalic acid, hexyl neopentyl ester (1.10%); Tridecanol, 2-ethyl-2-methyl- (1.04%); Octadecanoic acid, 2-oxo-, methyl ester (0.98%); n-Hexadecanoic acid(0.94%); Sulfurous acid, butyl heptadecyl ester (0.93%); Dodecane, 1-iodo- (0.77%); Methoxyacetaldehyde diethyl acetal (0.69%); Decane, 3-bromo- (0.39%) and 3-Hexanone, 2,5-dimethyl- (0.24%) in ethanolic stem bark extract of *P. dalzellii*.

In-vitro cytotoxicity of ethanolic stembark extract of *P. dalzellii* against MCF-7 cells was assessed by the MTT assay. At various concentrations of extract (31.25, 62.25, 125, 250, and 500 µg/mL) the cytotoxicity effect was determined. After the treatment of 24 hours incubation, the results revealed a dose-dependent cytotoxic effect on the tested MCF-7 cancer cells. The ethanolic stem bark extract of *P. dalzellii* showed considerable cytotoxicity on MCF-7 cells at the higher concentration of extract (500 µg/mL) with IC50 value of 440.82 µg/mL. It exhibited very little or negligible cytotoxicity on L929 cell lines (Table 3).

Discussion

Plants are the primary source of several effective medicines.[28] Due to the presence of important phyto-metabolic constituents medicinal plants exhibit pharmacological activities.[29] For this reason, a thorough understanding of phytochemicals is critical for drug discovery and the development of new therapeutic agents to combat serious illnesses.[30] Nevertheless, investigating medicinal plants also renders it easier to understand plant toxicity and aids in safeguarding people and animals from natural toxic chemicals.[31]

Table 1: Preliminary qualitative phytochemical screening of *P. dalzellii* stem bark extracts

Constituents	*Petroleum ether*	*Acetone*	*Ethanol*
Alkaloids	-	-	+
Flavonoids	-	-	+
Phenols	-	-	+
Glycosides	-	+	+
Tannins	-	+	+
Saponins	+	-	+
Triterpenoids	-	-	+
Steroids	-	+	+
Lignin	-	+	+
Carbohydrates	-	-	+

The qualitative results are represented by (+) for the presence and (–) for the absence of these phytochemical classes.

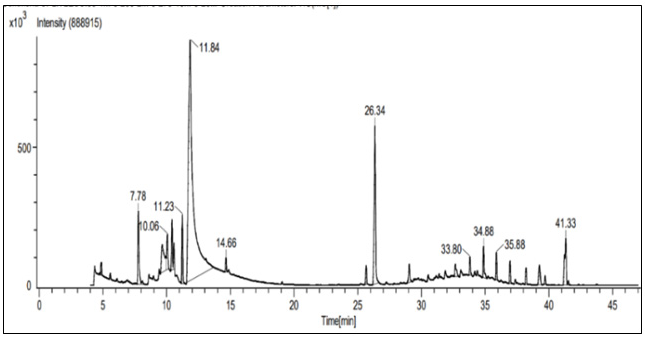

Fig. 1: GC-MS chromatogram showing separated bioactive chemicals from ethanolic stembark extract of *P. dalzellii*

The current preliminary qualitative phytochemical study of *P. dalzellii* showed the existence of various chemical compounds in selected extracts of the plant (Table 1). These phytochemicals were screened by different biochemical tests. These phytochemicals are reported to have many biological properties. Based on previous reports, alkaloids possess antioxidants, muscle relaxants, antibiotics, anticancer, antihyperlipidemic, antiobesity and are also responsible for antiprotozoal properties.[32,33] Terpenoids reported to have anti-carcinogenic, anti-ulcer, hepaticidal, antioxidant, antibacterial, diuretic,[34] anticancer,[35] antiviral, and anti-inflammatory properties.[36] Flavonoids possess antimicrobial, anticancer, anti-inflammatory, anti-allergic, antidiabetic and anti- aging properties.[37,38] Tannin has anti-inflammatory, antioxidant, antiseptic, antimicrobial and anti- diabetic properties.[39] Yadav *et al.* (2011) have reported that steroids are known to possess cardiotonic and antimicrobial activities.[40] The glycosides reported have anticancer properties. Besides, saponins are a specific glycoside class with antibacterial, hypolipidemic, anti-diabetic and anti-cancer activities, in addition to having soap-like properties.[37] Lignin is used as a food additive and it also has anticancer and antidiabetic properties.[41]

GC-MS incorporates two analytical methods to produce a single strategy for assessing a combination of chemical components. The mixtures of various components are separated using gas chromatography and each component is then individually analyzed using mass spectroscopy.[57] It is a powerful technique that gives reliable proof for the qualitative and quantitative identification of phytoconstituents.[58] GC-MS profiling of ethanolic stembark extract of the *P. dalzellii* showed 22 peaks that indicate the existence of 20 phytometabolic compounds (Fig. 1). Most of the phytoconstituents identified in this plant have well-known biological properties and some identified compounds are unknown for their biological uses (Table 2). Thus, chemical profiling by GCMS analysis is essential for understanding the nature of active principles in medicinal plants. However, the isolation of specific bioactive compounds and subjecting them to biological

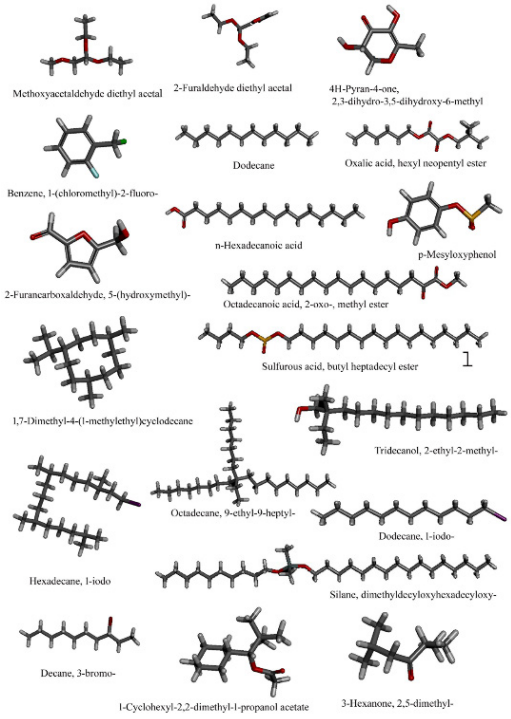

Fig. 2: Structure of certain major chemical constituents identified in stem bark ethanolic extract of *P. dalzellii*

activity will provide comprehensive evidence.

Several members of the *Psychotria* genus were found earlier to have anticancer activity towards various malignant cells.[59,60] *P. dalzellii* was previously found to possess antioxidant activity[24] and the plants having anti-oxidant properties exhibited anticancer features by preventing the colonization of numerous human malignant cells.[61] The most significant phytochemicals identified in the GC-MS study show anti-cancer properties (Table 2). Thus, in the present study, the ethanolic stembark extract of the plant was tested for *in-vitro* cytotoxicity against MCF-7 and L929 cell lines using MTT cell viability test. The MTT test is regarded as a rapid and reliable method to appraise cell viability and death using colorimetric analysis. Here, the results showed considerable *in-vitro* growth inhibition effect on breast cancer lines with IC_{50} values of 440.82 µg/mL and had very less effect on the proliferation of normal cell line (L929) at a higher concentration of 500 µg/mL. The concentration of the extracts was assessed in duplicate by serial dilutions at varying concentrations (31.25, 62.5, 125, 250, and 500 µg/mL), (Table 3 and Fig. 3). These findings reveal the time and dose-dependent increase in cytotoxicity and it was compared with the standard cisplatin with a very low concentration (10 µM). Cisplatin, a commercially available anticancer medication, targets only malignant cells while having less effect on non-cancerous cells. Since cancer cells lack a robust vascular system in comparison to normal cells, medicines utilized in the treatment of cancer increase the retention effect and permeability, passively targeting the cancer

Table 2: List of identified phytochemicals in crude ethanolic stembark extract of *P. dalzellii*

S. No.	*Retention time (minutes)*	*Average%*	*The name of a chemical compounds*	*Molecular formula*	*Molecular weight*	*Properties*
1	4.86	0.69	Methoxyacetaldehyde diethyl acetal	$C_7H_{16}O_3$	148	No significant report
2	7.78	4.54	2-Furaldehyde diethyl acetal	$C_9H_{14}O_3$	170	Flavouring agent[Pub Chem]
3	9.64	4.11	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	Antioxidant,[42] anticancer,[43] antimicrobial,[44] antiproliferative, anti-inflammatory, antipyretic, anti-arthritic, antidiabetic and automatic nerve activity[45]
4	10.06	2.02	Benzene, 1-(chloromethyl)-2-fluoro-	C_7H_6ClF	144	Anti-inflammatory, anti-cancer, antimicrobial, anti-allergic and to have an analgesic effect[Pub Chem]
5	10.42	2.43	Dodecane	$C_{12}H_{26}$	170	Antimicrobial, cytotoxic activity[46] and antineoplastic (ovarian cancer)[Pub Chem]
6	10.57	1.10	Oxalic acid, hexyl neopentyl ester	$C_{13}H_{24}O_4$	244	No significant report.
7	11.23	3.34	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	Food additives as a flavouring agent,[47] antimicrobials, preservatives,[48] antioxidant, anti-hypoxic, antiallergic, anti-inflammatory, antisickling, antihyperuricemic, antiproliferative and cytotoxic property[49]
8	11.84	55.27	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	Food additives as a flavouring agent,[47] antimicrobials, preservatives,[48] antioxidant, anti-hypoxic, antiallergic, anti-inflammatory, antisickling, antihyperuricemic, antiproliferative and cytotoxic property[49]
9	14.66	0.65	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	Food additives as a flavouring agent,[47] antimicrobials, preservatives,[48] antioxidant, anti-hypoxic, antiallergic, anti-inflammatory, antisickling, antihyperuricemic, antiproliferative and cytotoxic property[49]
10	25.65	0.94	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antioxidant, hypocholesterolemic, anti-inflammatory nematicidal, hemolytic, pesticide and antiandrogenic[38]
11	26.34	10.68	p-Mesyloxyphenol	$C_7H_8O_4S$	188	Antioxidants, anticancer,[50] anti-inflammatory,[51] skin depigmentation, and also treating liver spots[Pub Chem]
12	29.04	1.17	1,7-Dimethyl-4-(1-methyl ethyl) cyclodecane	$C_{15}H_{30}$	210	Cytotoxic[52]
13	32.66	0.98	Octadecanoic acid, 2-oxo-, methyl ester	$C_{19}H_{36}O_3$	312	Antibacterial[53]

14	33.80	0.93	Sulfurous acid, butyl heptadecyl ester	$C_{21}H_{44}O_3S$	376	Increases aromatic amino acid decarboxylase activity, arachidonic acid inhibitor and acidifier[54]
15	34.88	1.80	Octadecane, 9-ethyl-9-heptyl-	$C_{27}H_{56}$	380	Anticancer [Pub Chem]
16	35.88	1.30	Hexadecane, 1-iodo	$C_{16}H_{33}I$	352	Anti-atopic dermatitis[55] and flavouring agent[56]
17	36.95	1.04	Tridecanol, 2-ethyl-2-methyl-	$C_{16}H_{34}O$	242	No significant report
18	38.21	0.77	Dodecane, 1-iodo-	$C_{12}H_{25}I$	296	No significant report
19	39.26	2.49	Silane, dimethyldecyloxyhexadecyloxy-	$C_{28}H_{60}O_2Si$	456	No significant report
20	39.70	0.39	Decane, 3-bromo-	$C_{10}H_{21}Br$	220	No significant report
21	41.33	3.97	1-Cyclohexyl-2,2-dimethyl-1-propanol acetate	$C_{13}H_{24}O_2$	212	No significant report
22	41.52	0.24	3-Hexanone, 2,5-dimethyl-	$C_8H_{16}O$	128	No significant report

Table 3: *In-vitro* cytotoxic activity of *P. dalzellii* ethanolic stem bark extract against human breast cancer and mouse fibroblast cell line

S. No	*Sample Conc. (µg/mL)*	*MCF-7 cell lines*			*L929 cell lines*		
		Percentage of cell viability	*IC_{50} value (µg/mL)*	*Standard cisplatin, (10 µM) cell viability %*	*Percentage of cell viability*	*IC_{50} value (µg/mL)*	*Standard cisplatin, (10 µM) cell viability %*
1	Untreated	100 ± 00			100 ± 00		
2	31.25	96.21 ± 0.03			98.59 ± 007		
3	62.5	85.05 ± 0.16	440.82	27.32 ± 0.01	91.16 ± 0.005	NA	40.97 ± 0.015
4	125	81.94 ± 0.02			84.93 ± 0.001		
5	250	73.61 ± 0.06			75.03 ± 0.019		
6	500	42.66 ± 0.00			70.44 ± 0.011		

Note: Values are expressed in Mean ± Standard Error of Mean; The experiments were duplicated (n = 2). *NA = Not available.

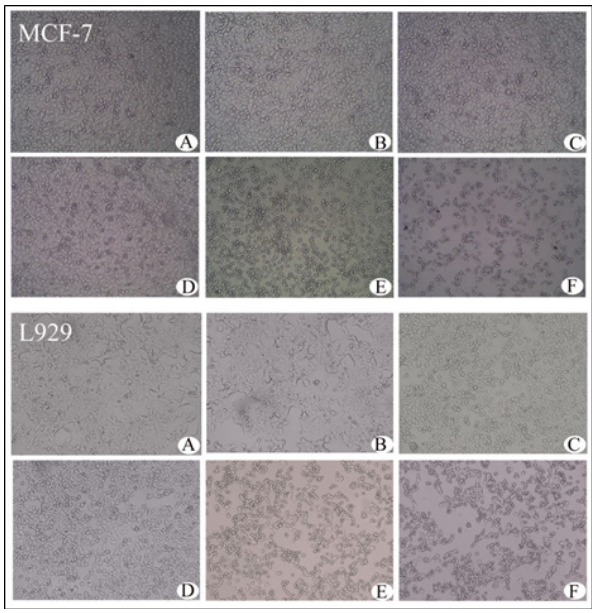

Fig. 3: The cytomorphological variations caused by different concentrations of ethanol extract of *P. dalzellii* on MCF-7 and L929 cell lines. Concentrations of extract (A) 31.25 µg., (B) 62.5 µg., (C) 125 µg., (D) 250 µg., (E) 500 µg., (F) Cisplatin at 10 µM

cells' leaky vasculature. As a result, medications can more easily reach cancer cells and kill them. However, occasionally, they can also infiltrate healthy cells and have hazardous effects.[62] Likewise, The microscopic images of the cell lines showed the cytotoxicity of *P. dalzellii* and cytomorphological changes that occurred in MCF-7 cells by increasing the concentration of the crude extract of the plant which resulted in an intracellular suicide program that possesses cell shrinkage, cell rounding, and a reduction in the number of viable cells, all of which are signs of apoptosis.[63,64] These variations are depicted in Fig. 3. Thus, the ethanolic stem bark extract of *P. dalzellii* may inhibit the fundamental capabilities that malignant cells need to grow and proliferate. Meanwhile, the ability of cancer cells to grow and proliferate by forming colonies and the capacity of cancer cells to migrate in order to metastasize. Thus, the ethanolic stem bark extract of *P. dalzellii* may have the potential to inhibit both necessary characteristics of MCF-7. The findings also validated the differential impacts induced by the extracts and standard drug cisplatin in cancer and normal cell lines.

It is concluded from the above results that, the preliminary phytochemical investigation of *P. dalzellii* shows the

existence of various make it as bioactive compounds and these compounds are known for their potential therapeutic properties. Furthermore, GC-MS analysis revealed 20 specific chemical constituents in the plant ethanolic stembark extract. The *in-vitro* cytotoxic studies towards the MCF-7 cell lines revealed that the ethanolic extract of *P. dalzellii* has considerable anticancer activity (IC_{50} value = 440.82 µg/mL) and the extract has very less cytotoxicity to the normal L929 cell lines. Overall, the findings of this study highlight the promising bioactive nature of *P. dalzellii* and its potential in cancer cell apoptosis. Further studies, including the isolation of pure compounds, *in-vivo* research and clinical studies, are essential to evaluate the safety, efficacy, and therapeutic potential of *P. dalzellii* extracts for cancer treatment.

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