



# Phytochemical Screening, GCMS Profile and Anticancer Potential of *Digera muricata*

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## KEYWORDS

Phytochemicals,  
cell viability  
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MS, cell lines

## ABSTRACT:

**Introduction:** Cancer remains one of the most life-threatening diseases globally, necessitating the discovery of safer and more effective therapeutic alternatives. Natural products derived from medicinal plants have shown significant promise in cancer treatment due to their phytochemical diversity and lower toxicity. *Digera muricata* (L.) Mart., a plant traditionally used for its antioxidant and anti-inflammatory properties, is understudied in terms of its anticancer activity. The presence of bioactive compounds in this plant suggests its potential as a source of anticancer agents.

**Objectives:** The major objectives covered included screening of the phytochemical constituents, GC-MS profile, and determination of the anticancer potential of methanolic extract of *Digera muricata*.

**Methods:** Different extracts of *D. muricata* such as Petroleum ether, Chloroform, Methanol and Aqueous, were subjected to preliminary phytochemical screening to identify the presence of secondary metabolites. GC-MS analysis was performed to identify volatile and semi-volatile constituents. The anticancer activity was evaluated for breast cancer cell lines MCF-7 by using the MTT assay.

**Results:** Phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, tannins, and terpenoids. GC-MS profiling identified multiple bioactive compounds, including phytol, squalene, and hexadecanoic acid, known for their anticancer properties. The methanolic extract exhibited significant cytotoxicity against MCF-7 cells in a dose-dependent manner.

**Conclusions:** *Digera muricata* contains various phytoconstituents with promising anticancer activity. The findings support its potential application as a natural source of anticancer agents, warranting further in vivo and mechanistic studies for drug development.

## 1. Introduction

Cancers have been a major cause of death, globally, where it is usually discovered at an advanced stage. However, if diagnosed in occult stage, it can be cured completely [1,2]. Multiple etiologies are another important concern in this condition like genome abnormalities, metabolic disorders, carcinogens, unhealthy diet etc. [3,4]. Though therapeutic interventions are well devised, problems of adverse effects as well as resistance lead to complexity of the situation [5]. Breast cancer is the most common type of cancer among women all around the world

where new tumours are formed from the breast cancer cells in a short span of time [6].

Today, natural medicines not only provide the primary health-care needs for the majority of the population in developing countries but have attracted more and more attention in developed countries due to soaring health-care costs and universal financial austerity. In USA, approximately 49% of the population has tried natural medicines for the prevention and treatment of diseases. In case of cancers also, adjuvant therapies have been practiced which has increased in last few years [7]. However, more natural moieties are still required to



be approved through clinical trials. Henceforth, in this research, we have explored a natural alternative for possible antiproliferative effect against resistant cell lines.

*Digera muricata* is a herb, a member of the Amaranthaceae family, that grows up to 20–70 cm in the Western Himalayas, primarily in damp regions at elevations of 1000 m [8]. It has a wide range of medicinal attributes like antimicrobial, anthelmintic, antioxidant, antidiabetic, hepatoprotective, and nephroprotective [9]. Kidney stones are treated with its leaves, and its roots are utilized as a galactagogue [10]. The plant's bloom, seeds, and extract are used to cure discharges from the urine [11,12].

The Primary metabolites were studied in numerous samples of solvents, such as Carbohydrates, Proteins, Lipids, Phenols, Chlorophylls, and Amino acids. The plant consists of both  $\alpha$ -spinasterol and  $\beta$ -spinasterol. Analysis of different Tartara fractions indicated the presence of flavonoids, alkaloids, terpenoids, saponins, coumarins, tannins, and cardiac glycosides and anthraquinones. This has found in the hexane extract of this plant rutin and hyperoside flavonoids [12].

The MCF-7 cell line was utilized as a breast cancer model in this investigation. It is one of the most used breast cancer cell lines in cancer research because of its lengthy history and expression of the estrogen receptor (ER). Since MCF-7 cells are one of the rare breast cancer cell lines that also have HR+/HER2, which has been related to aggressive breast tumors, they are an extremely important model for the research of invasive breast cancer [13]. The MDA-MB-231 cell line is also used in this study since, like MCF-7, it is an epithelial adenocarcinoma; however, it is ER negative, insufficiently differentiated, and highly malignant and tumor-promoting. MDA-MB-231 cells are invasive in vitro and create xenografts that spontaneously spread to lymph nodes when they are implanted orthotopically. The MDA-MB-231 cell line was used due to its distinct metastatic characteristics [14].

## 2. Objectives

Screening of the phytochemical constituents, analyze the GC-MS profile, and determine the anticancer potential of *Digera muricata* methanol extracts.

## 3. Methods

### Collection and Authentication

The aerial parts of *Digera muricata* were collected from different local areas of Lucknow and selected plant sample were authenticated from Plant Diversity, Systematics and Herbarium Division of CSIR-National Botanical Research Institute (CSIR-NBRI) Lucknow. (Ref no. PDSH/LWG/Authentication/2022-23/08).

### Morphological and Microscopical Evaluation

Morphological studies of leaf, fruit and bark were carried out [15,16]. Transverse sections of the fresh leaf and bark was prepared for the microscopic studies and examined under compound and projection microscope [17,18].

### Physicochemical and fluorescence analysis

Physicochemical examination was carried out in accordance with Indian Pharmacopoeia, which includes loss on drying, Total ash, acid insoluble ash, water soluble ash, sulphated ash, pH, extractive values and foaming index. The powder sample was subjected to several chemical reagent treatments under both daylight and UV light (254 nm and 365 nm) in order to perform fluorescence studies [19].

### Extraction of Plant Material

The entire aerial plant material was first cleaned with distilled water and allowed to air dry. Then, a blender was used to coarse ground it. Using soxhlet method, the powder sample was extracted using petroleum ether, chloroform, methanol, and water according to increasing polarity of solvents. The extractive values of these four crude extracts were determined. For analysis, the methanolic extract was thus kept at ambient temperature [20].

Hot maceration and cold maceration methods were also adopted for comparative study of difference in extractive values obtained from different extracts.

### Preliminary Phytochemical Screening of different extract of *Digera muricata*

Standard operating protocols were followed in the preliminary phytochemical screening process was performed for various extracts to identify different metabolites such as alkaloids, flavonoids, tannins, phenols, glycosides etc [21].

### Total Flavonoid content of Methanolic Extract of *Digera muricata*

The total flavonoid content was measured by a colorimetric assay. Rutin was used as standard for the calibration curve. Total flavonoids content of the extract was expressed as mg rutin equivalents per gram of sample (mg/g). 100 micro liters of extract was added to 4 ml of distilled water. Then, 0.3 ml 5% sodium nitrite was added. After few min, 0.3 ml of 10% aluminum chloride was added. After few minutes of rest 2 ml of 1 M sodium hydroxide was added to the mixture. Then the mixture was diluted by the addition of 3.3 ml distilled water and mixed thoroughly. The absorbance was determined at 510 nm versus a blank [22].



### Total Phenolic content of Methanolic Extract of *Digera muricata*

Folin Ciocalteu reagent was used for determination of total phenolics content. Gallic acid was used as standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg/g). To put it briefly, 0.5 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent. The solution was kept at 25°C for 5-8 min before adding 2 ml of sodium carbonate solution 7.5 % and adjusting the volume to 8 ml with water. After 2 h, the absorbance was measured at 725 nm [22].

### GC-MS Analysis

500 µg of sample (1mg/ml) was taken in a separating funnel and shaken by adding 10 ml of water and ethyl acetate in the ratio of 1:4 (add 2.5 ml water to 7.5 ml Ethyl Acetate). Upper layer was collected and concentrated to 1 ml in the rotary evaporator. 50 µl N, O-Bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS) was added and then finally 10µl of Pyridine was also added. Samples were further heated at 60°C for 30 minutes. For BSTFA+TMCS, make 100µl solution of 99µl of BSTFA and 1µl of TMCS. Samples were transferred in GC vial and dried using nitrogen gas. Finally, samples were dissolved in methanol before GC-MS analysis [23].

### In-vitro studies by using methanolic extract of *Digera muricata*

It was discovered through different analytical estimations such as TFC and TPC that methanolic extract has greater potential. Therefore, in vitro research using *Digera muricata* methanolic extract (DMME) was conducted on the breast cancer cell lines MCF-7.

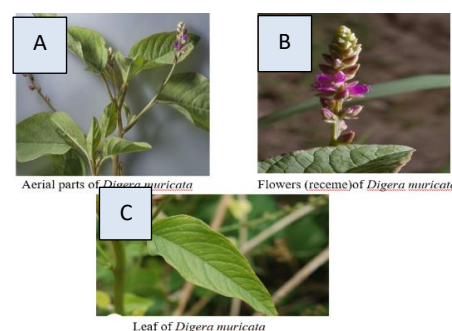
### Cytotoxicity Assay

The *in-vitro* cytotoxic activities of DMME were investigated using an MTT assay. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide. MTT dye colorimetric assay was performed to evaluate the MCF-7 cell viability. The viable cancer cells were seeded at a density of  $1 \times 10^4$  (100 µl/well) in 96 well plates in 100 µl DMSO media for one day and incubated in a humidified atm of 95% air and 5% CO<sub>2</sub> at 37°C for 24h to generate cell monolayer treatment with various concentrations *viz.* 10, 50, 100, 250, 500 & 1000 µg/ml of DMME was carried out by incubating MCF-7 cells for 24 h. They re-incubated for 3 hours at 37°C till a formazan blue color crystal formed after mixing 10 µl of MTT dye solution (5 mg/ml stock solution) after 21 hours of disclosure. After discarding the culture media from every well, 100 µl of DMSO was added to stop formazan crystals from forming. The formula used to determine the absorbance at 540 nm using a microplate reader (Elisa plate reader) [24,25].

$$\% \text{ Cell viability} = \frac{[\text{Treated Cells}]}{[\text{Control Cells}]} \times 100$$

## 4. Results

### Morphological Evaluation of Aerial Parts of *Digera muricata*

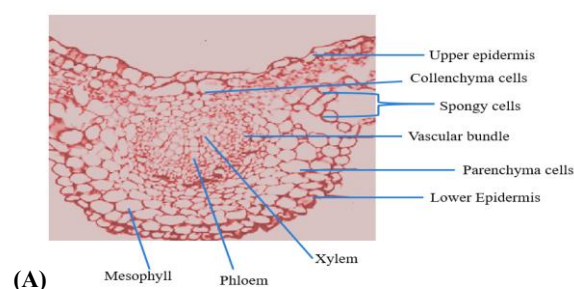


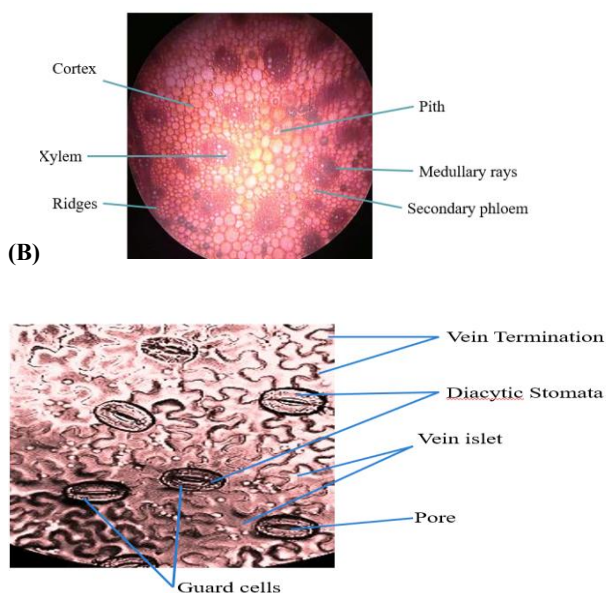
**Fig.1** Morphological characters of *D. muricata* (A) Aerial parts (B) Flowers (C) Reticulate leaves

**Table:1** Morphological characters of different aerial parts of *Digera muricata*

Parameter	Description		
	Leaf	Flower	Stem
Colour	Green	Whitish pink	Green
Odour	Pungent	Odourless	Odourless
Taste	Mucilaginous	Mucilaginous	Mucilaginous
Size	2-8 × 0.3-5 cm	20-30 cm raceme	12-14 cm
Fracture	-	-	Smooth
Texture	Smooth and glabrous	Smooth	Smooth
Venation	Reticulate	-	-
Apex	Acute	-	-
Lamina	Broadly ovate	-	-

### Microscopical Evaluation of different parts:





**Fig.2** Microscopical features of *D. muricata* (A) T.S of Leaf (B) T.S of Stem (C) Anisocytic stomata

S.No.	Leaf constants	Quantity in leaves
1.	Stomatal number (upper epidermis)	3-7
2.	Stomatal number (lower epidermis)	5-9
3.	Stomatal Index	4-8
4.	Vein islet number	7-12
5.	Vein termination number	4-8
6.	Palisade ratio	5-8
7.	Mean length of xylem vessels	247.5 $\mu$ m
8.	Mean length of fibers	375.7 $\mu$ m

**Table:2** Quantitative microscopy of *Digera muricata*

**Table:3** Physicochemical Evaluation of powdered aerial parts *Digera muricata*

Parameters	Estimated values in <i>D. muricata</i> aerial parts (% w/w)
Loss on Drying	5.27 $\pm$ 0.05

Ash Values	Total Ash	Acid Insoluble Ash	Water Soluble Ash
	11.45 $\pm$ 0.15	2.33 $\pm$ 0.35	4.18 $\pm$ 0.13
Extractive Values	Cold Extraction	Hot Extraction	Successive Extraction
✓ Petroleum Ether	0.39 $\pm$ 0.52	0.44 $\pm$ 0.11	0.43 $\pm$ 0.12
✓ Chloroform	0.51 $\pm$ 0.23	0.68 $\pm$ 0.09	0.35 $\pm$ 0.08
✓ Methanol	1.56 $\pm$ 0.49	2.64 $\pm$ 0.06	1.10 $\pm$ 0.09
✓ Water	2.74 $\pm$ 0.05	3.86 $\pm$ 0.21	1.34 $\pm$ 0.32
pH	Methanolic Extract		Aqueous Extract
	5.0		4.5
Foaming Index	Height of foam in every tube is more than 1cm		
Swelling Index	Up to 10ml		

**Table:4** Fluorescence analysis of powder of aerial parts of *Digera muricata*

S.No.	Test	Day light	UV (254nm)	UV (365nm)
1.	Powder + 50% HCL	Slight brown	Black	Green
2.	Powder + 50% HNO <sub>3</sub>	Dark orange	Dark blue	Green
3.	Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Black	Black	Brown



4.	Powder Petroleum ether	+	Colorless	Bluish black	Green	<b>Saponin Glycosides</b>	Foam test	+	+	+	+
							Haemolytic test	-	-	-	+
5.	Powder +Chloroform		Green	Brown	Green	<b>Anthraquinone Glycosides</b>	Borntra ger's test	+	-	+	+
6.	Powder Methanol	+	Shiny green	Brown	Bright green		Modified Borntra ger's test	-	+	+	+

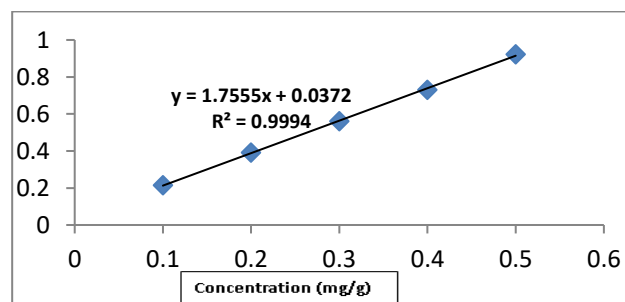
**Table: 5 Preliminary Phytochemical Screening of aerial parts of *Digera muricata***

Phytochemical Analyzed	Test Performed	Results			
		Petroleum ether extract	Chloroform extract	Methanolic Extract	Water Extract
<b>Carbohydrates</b>	Molish test	-	+	+	+
	Benedict's test	-	+	+	-
	Fehling's test	-	-	+	+
<b>Proteins and Amino Acids</b>	Xantho proteic test	+	-	+	+
	Ninhydrin test	+	-	+	+
	Millon's test	-	-	+	+
<b>Alkaloids</b>	Mayer's test	+	+	+	-
	Wagner's test	+	-	+	-
	Dragendroff's test	-	+	+	+
	Hager's test	-	-	+	+

<b>Flavonoids</b>	Shinoda test	+	+	+	+
	Liebermann burchard's test	+	+	-	-
<b>Phytosterols</b>	Salkowski's test	+	-	+	-
<b>Phenols</b>	Ellagic acid	+	-	+	+
	Ferric chloride test	-	+	+	+
<b>Tannins</b>	Gelatin test	-	+	+	+

**Total flavonoid content (TFC) in Methanolic extract of *Digera Muricata***

The calibration curve of standard sample quercetin has been shown in Fig.3 showing R<sup>2</sup> =0.9994. The sample i.e. methanolic extract of *Digera muricata*, was found to have the total flavonoid content as 16.20 ± 0.68mgQE/gm

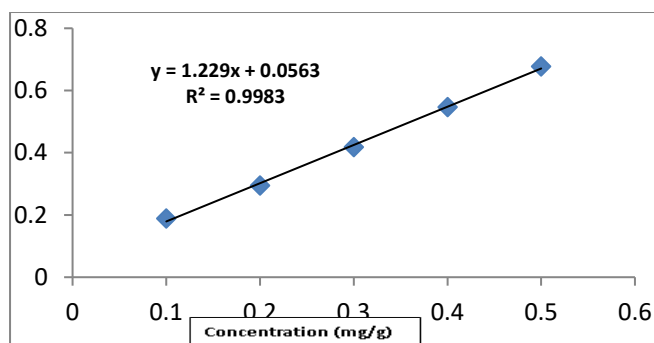


**Fig.3** Calibration curve of Quercetin standard for estimation of total flavonoid content



### Total Phenolics content (TPC) in Methanolic extract of *Digera muricata*

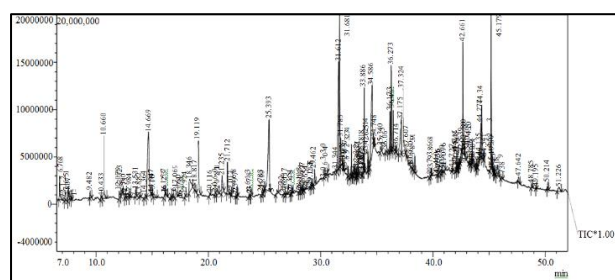
The calibration curve of standard gallic acid has been shown in Fig.4. The results showed that the sample i.e. methanolic extract of *Digera muricata*, was found to have total phenolic content as  $19.50 \pm 0.06$  mgGAE/ gm of sample.



**Fig.4** Calibration curve of gallic acid for the estimation of total phenol content

### GC-MS Analysis of methanolic extract of *Digera muricata*

A number of compounds, including neophytadiene, dodecanamide octodecanoic acids, and hexadecanoic acid, were detected in the DMME by GC-MS analysis. Additionally, several esters were discovered, as shown in table no. 5. The majority of the chemicals found fall into the fatty acid category, which could lead to the supposition that these substances as efficient anticancer agents.



**Fig.5** Chromatogram of GC-MS Analysis of methanolic extract of *Digera muricata*

**Table:6** Identified compounds from GC-MS Analysis of methanolic extract of *Digera muricata*

Peak	R. Time	Area	Area %	Name
1	15.023	532614	8.62	1,2-Benzenedicarboxylic Acid, Diethyl Ester
2	15.526	15642	0.25	Succinic acid, 2-methylpent-3-yl hept-1,6-dien-4-yl ester

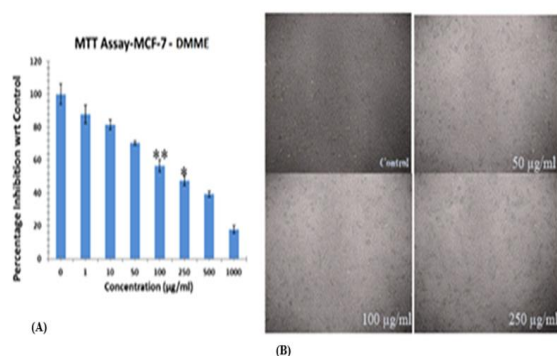
3	15.735	63104	1.02	Methyl (3-Oxo-2-Pentylcyclopentyl)Acetate
4	15.911	119258	1.93	1-(4-Isopropylphenyl)-2-Methylpropyl Acetate
5	16.005	13858	0.22	.Alpha.-Cis-1-Hydroxymethyl-2,2,6-Trimethyl ester
6	17.066	453754	7.34	Dodecanamide
7	17.682	123382	2.00	Neophytadiene
8	17.764	34349	0.56	Threo-Pentonic Acid, 2,3-Dideoxy-2-Methyl-ester
9	17.808	33314	0.54	1,3-Butanediamine, N,N,N',N'-Tetramethyl-ester
10	17.880	15611	0.25	4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8-Hexahydrocyl
11	17.934	20337	0.33	Cyclohexaneethanol
12	18.129	39123	0.63	4-Hexen-1-ol, 2-Isopropenyl-5-Methyl-, Acetate
13	18.504	46923	0.76	7,9-Ditert-Butyl-1-Oxaspiro[4.5]Deca-6,9-Diene
14	18.601	136558	2.21	Hexadecanoic acid, methyl ester
15	19.171	116365	1.88	Dodecanamide
16	19.619	17936	0.29	2-Butenal
17	20.523	68041	1.10	Octadecanoic Acid
18	21.104	136382	2.21	Octadecanamide
19	21.810	481891	7.80	N-(4-Methyl-1-Piperazinyl)-1-Naphthamide
20	22.042	32855	0.53	Glycidyl palmitate
21	22.677	37648	0.61	9-Octadecenamide, (Z)-
22	22.875	35990	0.58	Dodecanamide
23	23.482	175123	28.33	octadecanoic acid, 3-oxo-, ethyl ester
24	23.569	80267	1.30	Diethyl-di(prop-2-



				enyl)-silane
25	23.634	339823	5.50	Pregnane, Silane Deriv.
26	23.977	73152	1.18	Bis(2-Ethylhexyl) Phthalate
27	24.445	119822	1.94	URS-12-EN-28-AL
28	25.027	516676	8.36	Glycerol monostearate, 2TMS derivative

### Cytotoxicity Assay

Results revealed that IC<sub>50</sub> dose of Methanolic extract of *Digera muricata* (DMME) was observed as 185.6 µg/ml and 121.3 µg/ml for human breast cancer cell lines i.e. MCF-7 and MDA-MB 231 respectively with respect to control. It is therefore assumed that the plant extract produces minimal effect on normal cells at its IC<sub>50</sub>.



**Fig:6** Percent cell viability of breast cancer cell lines (A) MCF-7 and (B) Morphological alterations treated MCF-7 cell lines at different concentrations vs control, \*\* $p < 0.01$  and \* $p < 0.05$

### 5. Discussion

The phytochemical ingredients were investigated in our current investigation, and further, effective extracts were used to study the *in vitro* anticancer study at varying concentrations and compare the outcomes. According to the findings, the aerial parts of *Digera muricata* were extracted in aqueous, methanol, ethanol, and chloroform. These extracts contained alkaloids, flavonoids, glycosides, amino acids, fatty acids, proteins, phenolic compounds, tannins, sterols, terpenoids, anthocyanins, leucoanthocyanins, coumarins, and emodins. Comparing the current work to the other four solvents, we were able to determine that methanol was one of the best solvents because it detected a large number of chemicals. At a concentration of 185.6 mg/ml of plant extract, *Digera muricata* exhibited the highest level of enzyme inhibition, with the highest value of 50% observed.

The World Health Organization (WHO) predicts that diverse kinds of cancer would be the cause of more than 11 million deaths worldwide by 2030 [26]. The second-leading factor in fatalities in the modern world, after heart disease, is cancer, which has a high prevalence with no cure after metastasis. For the development of medicines for the treatment of neoplasms, critical comprehend the critical [27]. On neoplastic tumor cells, chemotherapy medications should ideally have a particular lethal impact; but, in practice, this treatment causes some fundamental changes in the patient [28]. The best form of planned cell death is involved in regulating the number of normal cells as well as their multiplication during gradual development [29]. Most notably in underdeveloped nations, 75–80 percent of the people use herbal therapies. They do so because they think natural remedies are less likely to have adverse reactions than chemical medications, in addition to being readily available and affordable [30]. A sensitive, accurate, and trustworthy colorimetric test for determining cell viability is the MTT assay. In some kind of variety of cell lines, the percentage is exactly relative [31, 32, 33].

To ensure the quality control of pharmaceuticals through various physicochemical criteria, standardization is a crucial step [34, 35]. The number of active ingredients, which can contribute to a variety of pharmacological activities, is determined using the extractive values. Plant-derived MeOH extract contains a noticeably greater concentration of flavonoids, alkaloids, and polyphenolic contents, which may be associated with its anti-cancer activities, according to the early phytochemical analysis of extracts [36]. Most oxidizing substances and several other free radicals linked to numerous disorders are typically significantly scavenged by flavonoids [37,38]. In the current investigation, extract was examined and found to have significant quantities of polyphenolic chemicals in comparison to information for other plants that were previously published in the literature [39]. According to observations on phytochemicals, these phytochemicals are now prominent in the MeOH extract. The most widely used method for fully standardizing herbal medicines and their metabolites is an HPTLC chromatographic fingerprinting approach [40]. This technology scans the indicated phytochemicals with the use of marker molecules.

Since ancient times, the nation has used indigenous herbal remedies to cure a variety of illnesses. According to certain reports, some spiritual leaders can successfully treat cancer with medicinal herbs. The abundance of biomolecules such as flavonoids including quercetin and rutin is the cause of the above. Consequently, The DMME might contain novel agents for the treatment of carcinoma. Because the cytotoxic capacity of the DMME *in-vitro* investigation was established by the data discussed herein. Additionally, a precise methodology will be used to understand the molecular processes that underlie the phytochemicals' remarkable cytotoxic activity and that might



serve as a model for the creation of medicines with potential clinical applications. The *in-vivo* MeOH must be investigated further, as must the molecular reasons underlying such potential.

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