Biological Forum – An International Journal **15(4): 1012-1018(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Identification of Bioactive Compounds in Methanol and Chloroform Aerial Part Extract of *Digera muricata* Marl. By GC-MS Analysis and Screening of Anticancer Activity Against Selected Cancer Cell Lines

Deepa K.N. and D. Kotresha*
Department of Studies in Botany, Davangere University,
Shivagangothri, Tholahunase, Davangere (Karnataka), India.

(Corresponding author: D. Kotresha)*
(Received: 21 February 2023; Revised: 14 March 2023; Accepted: 24 March 2023; Published: 20 April 2023)
(Published by Research Trend)

ABSTRACT: Cancer, a life-threatening ailment, ranks as the second leading cause of death worldwide. The increasing threat posed by medication-resistant cancers emphasizes the critical need for advancing the development of more powerful anticancer agents. Plant derived medicine emerges as a highly viable alternative to modern medical approaches in the fight against cancer, providing a cost-effective option. The present study revel phytochemical screening and cytotoxic activity *for chloroform, methanol and aqueous extracts from aerial part Digera muricata and identification of bioactive compounds in plant extract responsible for anticancer activity.* The qualitative phytochemical analysis indicated the presence of a wide range of metabolites in three extracts, including alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, glycosides, carbohydrates, and proteins. All extracts screening for cytotoxic activity against selected HepG2, MDAMB-231, NCIH-460 and K562 cancer cell lines by MTT assay. Three extracts exhibit anticancer activity but the most potent cytotoxic activity in chloroform and methanol extracts of *Digera muricata*. The IC_{50} value showed better in chloroform extract which was 25.85µg/ml in NCIH-460, 48.03 µg/ml in MDAMB-231 cancer cell lines. Where as in methanol extract the IC_{50} value found to be 67.71 µg/ml in NCIH-460 and 42.61 µg/ml in MDAMB-231 and these extracts was utilised for GCMS analysis. GCMS analysis revel various bioactive compounds in both methanol and chloroform extracts.

Keywords: Plants extracts, phytochemical analysis, MTT assay, GC-MS profiling.

INTRODUCTION

Cancer remains a persistent global challenge, marked by significant advancements in treatments and preventive measures. The essence of the disease lies in the continuous and uncontrollable multiplication of cells within the human body. This uncontrolled growth results in formation of malignant tumors, which possess the ability to metastasize (Ochwang *et al.,* 2014). This disease is influenced by various carcinogens and environmental factors, including physical elements like ultraviolet radiation, chemical exposures including tobacco and asbestos, and biological factors like viruses and bacteria (Nelson *et al.,* 2020). Contemporary treatment options encompass chemotherapy, radiotherapy, and pharmaceutical interventions. However, some treatments, notably chemotherapy may exert significant strain on patients and contribute to additional health challenges. Consequently, there is an emphasis on exploring alternative approaches and therapies for combating cancer (Cancer Research UK, 2015). So plant-derived compounds may be a more effective solution for cancer treatment with minimal side effects (Alonso-Castro *et al.,* 2011; Shin *et al.,* 2018). Liver cancer has a significant global mortality rate and presents considerable challenges for treatment. Its prevalence is particularly pronounced in Asia and Africa, with over 75% of reported cases occurring in Asian countries. The incidence of liver cancer is on the rise worldwide, including in nations such as USA and the India (Venook *et al.,* 2010). Globally, lung cancer stands as the predominant form of cancer, affecting males in the age range of 65-70 years, particularly those who are chronic tobacco users. On a global scale, lung cancer surpasses all other cancer types in terms of mortality. As per the latest GLOBOCAN estimates for 2020, a substantial 60% (1,315,136) of newly reported lung cancer cases occurred in Asia alone. Additionally, Asia accounted for 62% (1,112,517) of all lung cancer-related deaths, with an age-standardized incidence rate of 34.4 per 100,000 in East Asia. Breast cancer, one of the most widespread malignancies affecting women globally, is a primary obstacle to increasing life expectancy and remains a leading cause of mortality worldwide. The risk of developing breast cancer rises with age. Women can be diagnosed at any point following puberty. In 2020, the WHO reported that 2.3 million women globally were afflicted with breast cancer resulting in 6,85,000 fatalities (Chavda *et al.,* 2023). Leukaemia constituted roughly 2.5% of the total new cancer cases and contributed to about 3.1% of cancer-related deaths. Globally, the age-standardized incidence and mortality rates for leukaemia were recorded at 5.4 and 3.3 per 100,000 individuals,

respectively. In 2020, there were a reported total of 474,519 new cases of leukaemia (Huang *et al.,* 2022)**.**
Since ancient times humans have relied on plants as a medicinal resource, and numerous contemporary drugs are derived from these medicinal plants (Zaman *et al.,* 2022; Zaman *et al.,* 2022). Around 80% of the global population in developing countries utilizes plants for the treatment of various ailments (Helmstädter & Staiger 2014). In the conventional treatment of various types of cancers, alternative drugs are commonly employed to mitigate the toxicity and adverse effects associated with chemotherapy. Over the past two decades, there has been a growing focus on researching natural medicinal products, driven by the identification of new molecules with pharmaceutical potential derived from ethnopharmacological knowledge. However, there is still a lack of exploration for over 90% of plant species, as they have not been studied yet (Aravindhan & Rajendran 2014)**.**
Digera muricata is a member of family Amaranthaceae and belong to genus Digera. It is found across various regions in India, spanning Rajasthan, Maharashtra, and Andhra Pradesh, with its origin in southwest Asia (Khan & Ahmed 2009) .The present investigation was taken up for evaluating cytotoxic activity of *Digera muricata,* extracts against various cancer cell lines and *characterization was done using GCMS to identify novel compounds responsible for anticancer activity.* This novel research indicates the possibility of developing an effective herbal medicine for cancer.

MATERIAL AND METHOD

Plant material collection. In the month of August to September, plant sample was collected from the crops fields in the local region in and around Davangere district, Karnataka, India. The plant was identified by the Central Ayurveda Research Institute, Bengaluru, Karnataka. Received the voucher specimen number for *Digera muricata* Marl. (RRCBI-19516). The plant sample washed in running tap water and was then dried in the shade. Subsequently, it was ground into a powder and stored in an airtight container.
Preparation of plant extract: The aerial part of *Digera muricata* was collected and air-dried before being finely powdered using a blender. The resulting powder was stored at room temperature to facilitate subsequent extraction. Soxhelt extraction was carried out in fine powder, employing for three distinct solvents namely methanol, chloroform, and aqueous.
The temperature was maintained according to the respective solvent used, and the filtrates were condensed to achieve the desired consistency of the extracts. The extracts were then evaporated in a desiccator and stored in refrigerator at 4^{o}C for further use.
Qualitative screening phytoconstituents: The stored extracts of methanol, chloroform and aqueous extracts of all the plant samples were screened for preliminary phytoconstituents such as alkaloids, tannins, glycosides, phenols, flavonoids, sterols, saponins, terpenoids, carbohydrates and proteins by using standard procedure with minor modification (Harborne, 1998).
GCMS Analysis. Chemical compounds in the aerial part of chloroform and methanol extracts of *Digera muricata* were identified through GCMS analysis using a Perkin Elmer instrument (GCMS QP2010 SE, Shimadzu instrument, Columbia M.D USA). Aerial part extract was treated with 100ml of derivatization reagent (80µl BFSTA + 20µl TMCS) and incubated at 65^{o}C for 1 hour. Subsequently, each extracts was introduced into a column (Rtx5 MS 30M) with 0.25mm ID and 0.25µM df. The interference temperature was set at 300^{o}C, the ion source temperature at 25^{o}C, and the injection maintained at 300^{o}C with a helium gas flow rate of 1ml per min as the carrier gas. The analysis involved isothermal heating at 100°C for 1 minute, followed by a subsequent heating phase at 300°C for 20 minutes. Mass spectra were recorded at a rate of 2 scans per second, covering a scanning range of 40 to 850m/z. Turbo mass software was utilized to quantify the components based on peak area, and normalization was performed using an internal standard. The chemical composition of the aerial part extract of *Digera muricata* was determined by comparing the identified spectra with those in the NIST/Wiley library and with the compounds obtained during the analysis.
***In vitro* cytotoxic activity.** A 200 µl cell suspension, containing 20,000 cells per cell, was distributed into 96 cell plates and incubated at 37°C with 5% CO_2 for 24 hours, allowing the formation of a partial monolayer. Subsequently, cells were treated with chloroform, methanol and aqueous of aerial part extract at various concentrations (12.5, 25, 50, 100, and 200 µg/ml).
Control wells contained untreated cells with only maintenance medium, while positive controls were exposed to Camptothecin (for MDAMB-231and HepG2 cell lines) and Doxorubicin (for NCIH-460 and K562 cell lines). Following treatment, plates were further incubated at 37°C in a humidified atmosphere with 5% CO_2 and 75% relative humidity for 24 hours (Heal Force, China). Then, 0.5 mg/ml MTT labeling compound was added, and after 3-hour incubation, 100 ml of DMSO was introduced to each well to dissolve formaz an formations. The final step involved measuring the absorbance of the samples using a microplate (ELISA) reader at 570 nm and 630 nm. The sample's absorbance was measured, and the IC_{50} value was determined using a linear regression equation (Y = Mx + C), where Y is 50, and the values of M and C are derived from the viability graph. The effect of plants extracts on cell viability was assessed using the provided formula.

$$\% \text{ Cell Viability} = \frac{\text{Mean Absorbance of treated cells} - \text{Blank}}{\text{Mean absorbance of Untreated cells} - \text{Blank}} \times 100$$

Formula for calculation of the % cytotoxicity = 100 – % cell viability.

RESULT

Preliminary qualitative phytochemical analysis: In the preliminary studies we have carried qualitative analysis of phytochemicals of *Digera muricata* with the three different solvents such as aqueous, methanol and chloroform. In the phytochemicals estimation we have included both secondary metabolites like alkaloids,

flavonoids, saponins, tannins, terpenes and phenolics similarly primary metabolites includes glycosides, carbohydrates and proteins. The results showed the presence metabolites in the *Digera muricata* (Table 1).

Table 1: Qualitative analysis of different solvent extracts of *Digera muricata*.

Metabolites	Three different solvents of *Digera muricata*		
	Methanol	Chloroform	Aqueous
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	-
Tannins	+	+	+
Phenols	+	+	+
Terpenoids	+	+	+
Glycosides	+	-	+
Carbohydrates	+	+	+
Protein	+	-	+

Note: M- Methanol, C-Chloroform, A-Aqueous. Symbol sign '+' indicates presence of the test and '-' indicates absence of the test.

GCMS Analysis. The GC–MS chromatograms obtained for two extracts of chloroform and methanol suggest that *Digera muricata* is notably rich in bioactive compounds in aerial parts of the plant, Each chloroform and methanolic extract spectrum displays the retention time on the column, and the identified peaks represent the relative abundance of bioactive compounds present in that extract are displayed in Fig. 1 & 2. A total of 33 major phytochemical compounds were identified in the *Digera muricata* extracted using both chloroform and methanol. The name, molecular weight, molecular formula, retention time and percentage of area of these bioactive compounds are detailed in Table 2 & 3.

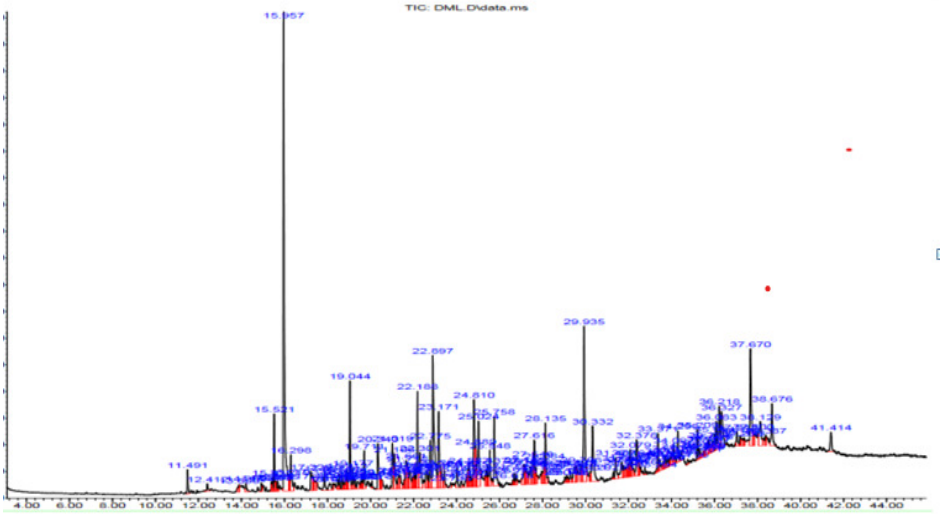

Fig. 1. Chromatogram showing different bioactive compounds retention time of *Digera muricata* chloroform extract.

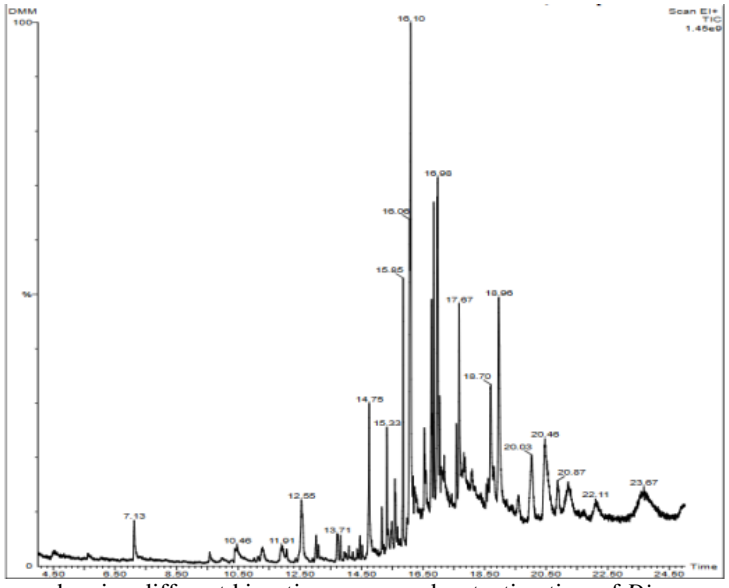

Fig. 2. Chromatogram showing different bioactive compounds retention time of *Digera muricata* chloroform extract.

Table 2: Active compounds identified in the chloroform extract of *Digera muricata* by chromatography/mass spectrometry (GC/MS) analysis.

Sr. No.	Identified Compound Names	Molecular Weight	Molecular Formula	RT	Area%
1.	Octacosane	394.7601	$C_{28}H_{58}$	11.49	0.49
2.	Tetracosane	338.6538	$C_{24}H_{50}$	12.41	0.22
3.	Cetene	224.4253	$C_{16}H_{32}$	13.89	0.2
4.	Nonadecane	268.5209	$C_{19}H_{40}$	14.2	0.27
5.	Eicosane	282.5475	$C_{20}H_{42}$	14.92	0.22
6.	Heptadecane	240.4677	$C_{17}H_{36}$	15.38	0.14
7.	Hentriacontane	436.83	$C_{31}H_{64}$	15.43	0.3
8.	Docosane	310.6006	$C_{22}H_{46}$	15.52	1.26
9.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	366.707	$C_{26}H_{54}$	15.58	0.22
10.	10-Methylnonadecane	282.5475	$C_{20}H_{42}$	15.68	0.23
11.	Hexacosane	366.707	$C_{26}H_{54}$	18.25	0.11
12.	Hexadecane	226.4412	$C_{16}H_{34}$	18.67	0.35
13.	10-Methylnonadecane	282.5475	$C_{20}H_{42}$	18.85	0.31
14.	Triacontane	422.8133	$C_{30}H_{62}$	19.4	0.31
15.	Phytol	296.531	$C_{20}H_{40}O$	21.01	1.18
16.	Pentacosane	352.6804	$C_{25}H_{52}$	21.31	0.49
17.	Oxirane, tetradecyl-	240.4247	$C_{16}H_{32}O$	21.38	0.58
18.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276.3707	$C_{17}H_{24}O_3$	22.3	1.37
19.	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl este	355.17835	$C_{21}H_{25}NO_4$	35.73	0.47
20.	1,4-Bis (trimethylsilyl) benzen	222.4741	$C_{12}H_{22}Si_2$	35.81	0.17
21.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	366.707	$C_{26}H_{54}$	36.08	0.8
22.	(.+/-.)-.alpha.-Tocopherol acetate	472.7428	$C_{31}H_{52}O_3$	36.21	1.01
23.	Stigmasterol	412.7	$C_{29}H_{48}O$	37.67	3.08

Table 3: Active metabolites from methanol extract of *Digera muricata* by chromatography/mass spectrometry analysis.

Sr. No.	Identified Compound Names	Molecular Weight	Molecular Formula	RT	Area%
1.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.1253	$C_6H_8O_4$	7.135	94.9
2.	Dodecanoic acid	200.1776	$C_{12}H_{24}O_2$	12.557	39
3.	Pentadecanoic acid	242.2245	$C_{15}H_{30}O_2$	14.758	3.91
4.	Neophytadiene	278.2973	$C_{20}H_{38}$	15.333	48.8
5.	Hexadecanoic acid, methyl ester	270.2558	$C_{17}H_{34}O_2$	15.858	71.3
6.	Tetradecanoic acid	228.2089	$C_{14}H_{28}O_2$	16.104	3.85
7.	9-Octadecenoic acid	282.2558	$C_{18}H_{34}O_2$	16.774	3.79
8.	Eicosanoic acid	312.3028	$C_{20}H_{40}O_2$	17.674	0.91
9.	9-Octadecenoic acid (Z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester	440.3137	$C_{25}H_{44}O_6$	18.705	0.8
10.	16-Hexadecanoyl hydrazide	270.2671	$C_{16}H_{34}N_2O$	18.96	0.7
11.	Glycidyl (Z)-9-Heptadecenoate	324.2664	$C_{20}H_{36}O_3$	20.46	5

***In-vitro* cytotoxic assay.** Cytotoxicity assays were conducted on the aerial part of *Digera muricata* to explore their anticancer activity in four different cancer cell lines, namely MDAMB-231, HePG2, K562, and NCIH-460 were subjected to the assays. Using Camptothecin as a positive control for HepG2 and MDAMB-231 cell lines and Doxorubin for K562 and NCIH-460 cell lines using different concentrations of plant extracts (12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml) at 48-hour incubation period. Among all four cancer cell lines methanolic, chloroform extracts shows good cytotoxic activity in NCIH-460, MDAMB-321 cancer cell line with good

IC_{50} values (Fig. 3 & 4) and aqueous extract showed moderate cytotoxic activity.

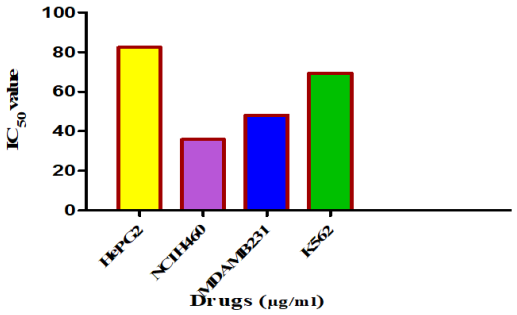

Fig. 3. Cytotoxicity activity of *Digera muricata* of chloroform extract on HePG2, NCIH-460, and MDAMB-231 and K562 treatment at 48hr (MTT assay; mean IC_{50} values).

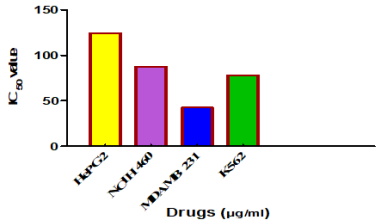

Fig. 4. Cytotoxicity activity of *Digera muricata* of Methanol extract on HePG2, NCIH-460, MDAMB-231 and K562 treatment at 48hr (MTT assay; mean IC_{50} values).

DISCUSSION

The metabolites in phytoplankton are species specific and also geographical conditions. Therefore, it is of interested to investigate on primary metabolites (Proteins, carbohydrates and organic acids etc). To complex secondary metabolites (polyphenols, flavonoids, alkaloids and terpenoids etc.) for various biotechnological applications. In the present study we have selected aerial part of *Digera muricata* aiming for the anticancer studies. From the plant, based on the polarity we have extracted bioactive compounds with aqueous, methanol and chloroform solvents. In the preliminary screening for potent anticancer source, we have estimated phytochemicals qualitatively. The study depicted that *Digera muricata* aerial part extract showed phytochemicals with the methanol, chloroform and aqueous extracts. In the study examining the cytotoxic activity of extracts against various cancer cell lines, it was observed that all extracts exhibited anticancer activity in the selected cell lines. Previous research work explored that cytotoxic activity of *Digera muricata* extracts, particularly focusing on its leaf extracts reported that significant antiproliferative effects of an ethanol-based leaf extract on MG-63 cell lines, accompanied by the ability to induce apoptosis (Vidyashri *et al.,* 2021)**.** Similarly notable cytotoxicity and apoptotic activity of an ethanolic leaf extract against the B16-F10 skin cancer cell line (Dhivyadharshini *et al.,* 2021). One more research work demonstrated promising cytotoxic effects of both methanolic and aqueous fractions of *Digera muricata* extract against Hela and A549 cancer cell lines (Usmani *et al.,* 2014). One more work reported that significant cytotoxic activity of an ethanolic leaf extract against the A549 lung cancer cell line, as evidenced by DAPI staining revealing fragmented nuclei and condensed chromosomes, indicative of inhibited cell proliferation lung cancer cell line by Notably (Deepthi *et al.,* 2021). Our studies on aerial part work on *Digera muricata* displayed promising cytotoxic activity in both methanol and chloroform extracts against the NCIH-460 and MDAMB231 cancer cell lines. In chloroform extracts, the IC_{50} values was found to be 35.85µg/ml in NCIH460 and 48.03 µg/ml in MDAMB231, whereas in methanol extract, the IC_{50} values was 67.71 µg/ml in NCIH460 and 42.61 µg/ml in MDAMB231 (Fig. 1 & 2).

In present investigation, methanol and chloroform extracts of *Digera muricata* to identify volatile compounds by GCMS analysis. Identification of the major peaks in the chromatogram was achieved by comparing mass fragmentation patterns with those in the NIST library. Drawing upon existing literature, we selected bioactive compounds and utilized Chem Draw Ultra 8.0.3 software to draw their structures. Many of the reported volatile compounds are derivatives or degradation products of fatty acids. Here, we discuss selected potential bioactive volatile compounds from the crude extract, with a focus on those identified in the chloroform extract of *Digera muricata*. Among the compounds identified, tetracosane, found in the chloroform extract, has demonstrated anticancer properties in various plants, such as *Acrostichum aureum* (Uddin *et al.,* 2012) and has also shown potential for Alzheimer's prevention (Lomarat *et al.,* 2015). Heptadecane, another compound identified in the chloroform extract, has been reported in *Spirulina platensis* (Kim *et al.,* 2013)**,** a blue-green alga, and is known for its anti-inflammatory properties. Hentriacontane, commonly found in leaf wax, exhibits anti-inflammatory properties and has demonstrated anticancer activity through the suppression of caspase-1 activation in plants like *Oldenlandia diffusa* (Kim *et al.,* 2011). Additionally, hexacosane, known for its antimicrobial properties, has been isolated from plants like *Sansevieria liberica* (Rukaiyat *et al.,* 2015)**.**

Phytol is a diterpene and its derivatives exert a wide range of biological activities including anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory properties (Islam *et al.,* 2018). (.+/-.)-.alpha. Tocopherol acetate was identified and it is the derivative of Vitamin E, the literature revealed it has anticancer activity (Ju *et al*., 2010). Stigmasterol has been identified it is a phytosterol found in many herbaceous plants and this has more attention for its various pharmacological effects including anti-inflammation (Morgan *et al*., 2021). And it has significant anti-tumour bioactivity in a variety of malignancies (Zhang *et al.,* 2022).

In this section we have discussed about compounds detected in the methanolic extract of *Digera muricata* Among the identified compounds the bioactive related

to cytotoxicity or anticancer` compounds were discussed. Particularly the identified compound 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl- has potential antioxidant activity (Cechovska *et al.,* 2011) and it has identified previously in the *Capparis cartilaginea* fruit extracts for their anticancer activity. Bioactive Dodecanoic acid is identified, it is also called Lauric Acid which is predominantly present in breast milk and coconut oil and some plants. It is nontoxic and recent studies revealed that it has several bioactivities like antibacterial (Matsue *et al*., 2019) antifungal (Akula *et al*., 2021) and anticancer properties (Sheela *et al.,* 2019)**.** Identified Pentadecanoic acid and previously reported it as exerted selective cytotoxic effects on human breast carcinoma MCF-7/stem-like cells (To *et al.,* 2020).

Another compound Neophytadiene was identified and literature revealed that it has anti-inflammatory, antioxidant and cardioprotective properties (Bhardwaj *et al*., 2020)**.** Hexadecanoic acid methyl ester, commonly known as methyl palmitate, was identified in our study. This compound has been previously reported in *Hibiscus sabdariffa* (Ajoku *et al.,* 2015) and *Juglans regia* (Wang *et al.,* 2009). Past research has demonstrated its potential therapeutic effects, including anti-phagocytic activity, modulation of immune response to prevent Kupffer cell activation in liver-transplanted rats, and anti-inflammatory and antifibrotic properties (El-Demerdash, 2011; Mantawy, 2012).

In our study, we have, for the first time, investigated the cytotoxic effects of *Digera muricata* aerial parts on selected cancer cell lines. Particularly, our examination of NCIH-460 and MDAMB-231 cancer cell lines has unveiled significant anticancer activity. This discovery represents a significant contribution to the field, opening up new avenues for exploration in cancer research. The notable efficacy against NCIH-460 and MDAMB-231 cell lines indicates a promising pathway for further research into the therapeutic potential of *Digera muricata* aerial parts in cancer treatment.

CONCLUSIONS

In summary, our study highlights the anticancer potential of the aerial part of *Digera muricata* against selected cancer cell lines, notably demonstrating significant cytotoxicity against NCIH-460 and MDAMB-231 cancer cell lines. The existence of active secondary metabolites, as indicated by phytochemical analysis, may contribute to this observed cytotoxic activity. Furthermore, GCMS analysis supports these findings by identifying active anticancer compounds within both chloroform and methanolic extracts. These findings suggest a promising avenue for future research aimed at isolating potent anticancer compounds from this plant extracts. Ultimately, such efforts hold the potential to yield novel herbal medicines for cancer treatment with minimal side effect.

FUTURE SCOPE

Future research aims to isolate and characterize specific compounds and their action on cancer cell lines, with in vitro and pathway studies potentially leading to the development of effective herbal medicine for cancer treatment.

Acknowledgement. The authors express their gratitude to the department of Studies in Botany, Davangere University, and Davangere for facilitating the execution of this research.
Conflict of Interest. None.

REFERENCES

Ajoku, G. A., Okwute, S. K., & Okogun, J. I. (2015). Isolation of hexadecanoic acid methyl ester and 1, 1, 2-ethanetricarboxylic acid-1-hydroxy-1, 1-dimethyl ester from the calyx of green Hibiscus Sabdariffa (Linn). *Nat Prod Chem Res*, *3*(2), 169-174.

Akula, S. T., Nagaraja, A., Ravikanth, M., Kumar, N. G. R., Kalyan, Y., & Divya, D. (2021). Antifungal efficacy of lauric acid and caprylic acid–Derivatives of virgin coconut oil against Candida albicans. *Biomedical and Biotechnology Research Journal (BBRJ)*, *5*(2), 229-234.

Alonso-Castro, A. J., Villarreal, M. L., Salazar-Olivo, L. A., Gomez-Sanchez, M., Dominguez, F., & Garcia-Carranca, A. (2011). Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. *Journal of ethnopharmacology*, *133*(3), 945-972.

Aravindhan, V., & Rajendran, A. (2014). Diversity of invasive plant species in boluvampatti forest range, the southern Western Ghats, India. *American-Eurasian Journal of Agricultural & Environmental Sciences*, *14*(8), 724-731.

Bhardwaj, M., Sali, V. K., Mani, S., & Vasanthi, H. R. (2020). Neophytadiene from Turbinaria ornata suppresses LPS-induced inflammatory response in RAW 264.7 macrophages and Sprague Dawley rats. *Inflammation*, *43*, 937-950.

Cancer Research UK [Accessed 23 January 2015]; What is cancer 2014 Available at: http://www.cancerresearchuk.org/about-cancer/what-is-cancer.

Čechovská, L., Cejpek, K., Konečný, M., & Velíšek, J. (2011). On the role of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-(4 H)-pyran-4-one in antioxidant capacity of prunes. *European Food Research and Technology*, *233*, 367-376.

Chavda, V. P., Nalla, L. V., Balar, P., Bezbaruah, R., Apostolopoulos, V., Singla, R. K., & Uversky, V. N. (2023). Advanced phytochemical-based nanocarrier systems for the treatment of breast cancer. *Cancers*, *15*(4), 1023.

Deepthi, S., Elumalai, P., Raghunandhakumar, S., Lakshmi, T., & Roy, A. (2021). Evaluation of cytotoxic potential of Digera muricata leaf extract on lung cancer cell line. *J Pharm Res Int*, *29*, 285-293.

Dhivyadharshini, J., Elumalai, P., Raghunandhakumar, S., Lakshmi, T., & Roy, A. (2021). Investigation of Proapoptotic Effect of Digera muricata Leaf Extract against the Skin Cancer Cell Line (B16-F10). *Journal of Pharmaceutical Research International*, *33*(63A), 274-284.

El-Demerdash, E. (2011). Anti-inflammatory and antifibrotic effects of methyl palmitate. *Toxicology and applied pharmacology*, *254*(3), 238-244.

Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media.

Helmstädter, A., & Staiger, C. (2014). Traditional use of medicinal agents: a valid source of evidence. *Drug Discovery Today*, *19*(1), 4-7.

Huang, J., Chan, S. C., Ngai, C. H., Lok, V., Zhang, L., Lucero-Prisno III, D. E., & Wong, M. C. (2022). Disease burden, risk factors, and trends of leukaemia: A global analysis. *Frontiers in oncology*, *12*, 904292.

Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., & Atanasov, A. G. (2018). Phytol: A review of biomedical activities. *Food and chemical toxicology*, *121*, 82-94.

Ju, J., Picinich, S. C., Yang, Z., Zhao, Y., Suh, N., Kong, A. N., & Yang, C. S. (2010). Cancer-preventive activities of tocopherols and tocotrienols. *Carcinogenesis*, *31*(4), 533-542.

Khan, M. R., & Ahmed, D. (2009). Protective effects of Digera muricata (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. *Food and Chemical Toxicology*, *47*(6), 1393-1399.

Kim, D. H., Park, M. H., Choi, Y. J., Chung, K. W., Park, C. H., Jang, E. J., & Chung, H. Y. (2013). Molecular study of dietary heptadecane for the anti-inflammatory modulation of NF-kB in the aged kidney. *PloS one*, *8*(3), e59316.

Kim, S. J., Chung, W. S., Kim, S. S., Ko, S. G., & Um, J. Y. (2011). Antiinflammatory effect of Oldenlandia diffusa and its constituent, hentriacontane, through suppression of caspase-1 activation in mouse peritoneal macrophages. *Phytotherapy Research*, *25*(10), 1537-1546.

Lomarat, P., Chancharunee, S., Anantachoke, N., Kitphati, W., Sripha, K., & Bunyapraphatsara, N. (2015). Bioactivity-guided separation of the active compounds in Acacia pennata responsible for the prevention of Alzheimer's disease. *Natural product communications*, *10*(8), 1934578X1501000830.

Mantawy, E. M., Tadros, M. G., Awad, A. S., Hassan, D. A., & El-Demerdash, E. (2012). Insights antifibrotic mechanism of methyl palmitate: impact on nuclear factor kappa B and proinflammatory cytokines. *Toxicology and applied pharmacology*, *258*(1), 134-144.

Matsue, M., Mori, Y., Nagase, S., Sugiyama, Y., Hirano, R., Ogai, K., & Okamoto, S. (2019). Measuring the antimicrobial activity of lauric acid against various bacteria in human gut microbiota using a new method. *Cell transplantation*, *28*(12), 1528-1541.

Morgan, L. V., Petry, F., Scatolin, M., de Oliveira, P. V., Alves, B. O., Zilli, G. A. L., ... & Müller, L. G. (2021). Investigation of the anti-inflammatory effects of stigmasterol in mice: insight into its mechanism of action. *Behavioural pharmacology*, *32*(8), 640-651.

Nelson, V. K., Sahoo, N. K., Sahu, M., Sudhan, H. H., Pullaiah, C. P., & Muralikrishna, K. S. (2020). In vitro anticancer activity of Eclipta alba whole plant extract on colon cancer cell HCT-116. *BMC complementary medicine and therapies*, *20*, 1-8.

Ochwang'i, D. O., Kimwele, C. N., Oduma, J. A., Gathumbi, P. K., Mbaria, J. M., & Kiama, S. G. (2014). Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. *Journal of ethnopharmacology*, *151*(3), 1040-1055.

Rukaiyat, M., Garba, S., & Labaran, S. (2015). Antimicrobial activities of hexacosane isolated from Sanseveria liberica (Gerome and Labroy) plant. *Adv Med Plant Res*, *3*(3), 120-125.

Shazia Usmani, S. U., Farooqui, A. H. A., Mohd. Arshad, M. A., Sahabjada Siddiqui, S. S., Mohd. Ahmad, M. A., & Shadma Wahab, S. W. (2014). Antiproliferative activity of crude extract and fractions obtained from Digera muricata on Hela cell lines of human cervix and A549 cell lines of human lung, 32-38.

Sheela, D. L., Narayanankutty, A., Nazeem, P. A., Raghavamenon, A. C., & Muthangaparambil, S. R. (2019). Lauric acid induce cell death in colon cancer cells mediated by the epidermal growth factor receptor downregulation: An in silico and in vitro study. *Human & experimental toxicology*, *38*(7), 753-761.

Shin, S. A., Moon, S. Y., Kim, W. Y., Paek, S. M., Park, H. H., & Lee, C. S. (2018). Structure-based classification and anti-cancer effects of plant metabolites. *International journal of molecular sciences*, *19*(9), 2651.

To, N. B., Nguyen, Y. T. K., Moon, J. Y., Ediriweera, M. K., & Cho, S. K. (2020). Pentadecanoic acid, an odd-chain fatty acid, suppresses the stemness of MCF-7/SC human breast cancer stem-like cells through JAK2/STAT3 signaling. *Nutrients*, *12*(6), 1663.

Uddin, S. J., Grice, D., & Tiralongo, E. (2012). Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichum aureum*. *Pharmaceutical Biology*, *50*(10), 1276-1280.

Venook, A. P., Papandreou, C., Furuse, J., & Ladrón de Guevara, L. (2010). The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *The oncologist*, *15*(S4), 5-13.

Vidyashri, S., Elumalai, P., Raghunandhakumar, S., Lakshmi, T., & Roy, A. (2021). Anticancer effect of Digera muricata Leaf Extract against Prostate Cancer Cell Lines [Internet]. *Journal of Pharmaceutical Research International*, 431-438.

Wang, Y. N., Wang, H. X., Shen, Z. J., Zhao, L. L., Clarke, S. R., Sun, J. H., & Shi, G. L. (2009). Methyl palmitate, an acaricidal compound occurring in green walnut husks. *Journal of economic entomology*, *102*(1), 196-202.

Zaman, W., Ye, J., Ahmad, M., Saqib, S., Shinwari, Z. K., & Chen, Z. (2022). Phylogenetic exploration of traditional Chinese medicinal plants: A case study on Lamiaceae. *Pak. J. Bot*, *54*(3), 1033-1040.

Zaman, W., Ahmad, M., Zafar, M., Amina, H., Saqib, S., Ullah, F., & Park, S. (2022). Diversity of medicinal plants used as male contraceptives: An initiative towards herbal contraceptives. *21*(3), 616-624

Zhang, X., Wang, J., Zhu, L., Wang, X., Meng, F., Xia, L., & Zhang, H. (2022). Advances in Stigmasterol on its anti-tumor effect and mechanism of action. *Frontiers in oncology*, *12*, 1101289.

How to cite this article: Deepa K.N. and D. Kotresha (2023). Identification of Bioactive Compounds in Methanol and Chloroform Aerial Part Extract of *Digera muricata* Marl. By GC-MS Analysis and Screening of Anticancer Activity Against Selected Cancer Cell Lines. *Biological Forum – An International Journal, 15*(4): 1012-1018.