Tuijin Jishu/Journal of Propulsion Technology
ISSN:1001-4055
Vol. 44 No.2 (2023)

Evolution of Antioxidant & Cytotoxicity of the Hydro Alcoholic Extract of Dried Root of Digera Muricata Using MTT Assay

Sk.Salma sultana[1,2], J. Banu rekha[3] , B.S. Venkateswarlu[4], M. Kumar[5] , L . Baskar[6]

[1]*Research Scholar, Faculty of Pharmacy, Vinayaka mission's research foundation, Deemed to be University,Salem, Tamilnadu- 636308, India.*

[2]*Department of Pharmacology,Narayana Pharmacy College, Chinthareddypalem, Nellore-524002.*

[3]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Vinayaka mission's research foundation,Deemed to be University,Salem, Tamilnadu- 636308, India.*

[4]*Department of Pharmaceutics, Faculty of Pharmacy, Vinayaka mission's research foundation, Deemed to be University,Salem, Tamilnadu- 636308, India.*

[5]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Vinayaka mission's research foundation, Deemed to be University,Salem, Tamilnadu- 636308, India.*

[6]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Grace College of pharmacy, kerala, tamilnadu.*

***Abstract*:-**Digera Muricata, a medicinal plant with a rich history in traditional medicine, has garnered attention due to its potential health benefits. This study investigates the antioxidant and cytotoxic properties of the hydro-alcoholic extract obtained from the dried root of Digera Muricata using the MTT assay. The dried roots of Digera Muricata were subjected to hydro-alcoholic extraction, and the resulting extract was tested for its antioxidant activity using standard assays, including DPPH and ABTS assays. The extract exhibited significant antioxidant potential, demonstrating its ability to scavenge free radicals. hydro-alcoholic extract of the dried root of Digera Muricata possesses notable antioxidant activity and demonstrates promising cytotoxicity against cancer cells. These findings highlight the therapeutic potential of Digera Muricata as a source of natural antioxidants and cytotoxic agents, which could have implications for the development of novel pharmaceuticals or complementary therapies in the field of cancer research and oxidative stress-related diseases.

Keywords: *Digeria Muricata , DPPH,ABTS,MTT, Antioxidant.*

1. Introduction

The study of natural compounds and their potential health benefits has been a focal point of scientific research for centuries. In recent decades, the exploration of phytochemicals from various plant sources has gained significant attention due to their potential therapeutic properties. Among these phytochemicals, antioxidants have been of particular interest because of their ability to neutralize harmful reactive oxygen species (ROS) and protect cells from oxidative damage(1). This research aims to investigate the antioxidant activity and cytotoxicity of the hydro-alcoholic extract of dried Digera muricata root using the MTT assay(3). The MTT assay is a well-established and widely used method for evaluating the viability of cells and assessing cytotoxicity or cell proliferation. It measures the metabolic activity of cells, primarily through the reduction of MTT, a yellow tetrazolium salt, to purple formazan crystals by mitochondrial enzymes in living cells. This change in color can be quantified spectrophotometrically, allowing for the assessment of cell viability and cytotoxicity.(2)

Plant Profile

Digera muricata, commonly known as "rough-leaved turnsole," is a plant species found in various regions across the world, including Asia and Africa. This plant has been traditionally used in herbal medicine for its potential health-promoting properties(4). The dried root of Digera muricata contains a variety of bioactive compounds,

including flavonoids, phenolics, and other secondary metabolites, which have been associated with antioxidant and cytotoxic effects[5]

Scientific name: Digera muricata (L.) Mart.
Synonym : Achyranthes alternifolia L., Achyranthes muricata L., Digera alternifolia
(L.) Aschers., Digera arvensis Forssk,
Family : Amaranthaceae
Vernacular names
English : False amaranth
Sanskrit : Aranya, Aranyavastuka, kunanjara, kuranjara
Telugu : Chnchali Koora
Tamil : Toya Keeri, kaatu Keerai
Hindi : Latmahuria, Lesua
Kannada : Chenchali soppu, Goraji playa, Kankali soppu
Marathi : Gitana, Getna
Bangali : Lata mouri Ful, Gun gutiya

2. Materials and Method:

Sample Procurement and Identification:

Plant roots were collected from Narayana hospital, chintareddy palem, Nellore, Andhra Pradesh. An herbarium sheet also prepared and submitted for the identification of plant at Sri Venkateswara University, Tirupati. Vero cells, MCF-7 and HT-29celllines were obtained from National Center for Cell Sciences Pune India[6]. The cell lines were subcultured in Dulbecco's Modified Eagle Media containing 10% Fetal bovine serum & 1% Antibiotic-Antimycotic [15]).The cells were incubated in CO2 incubatorat 37 degree celcius in a humidified environment of 5% CO2. [13]Trypsinization was done to detachthe cells before to MTT Assay.[7]

Extractionofplantextracts:

The root was separated from the whole plant. Cleaned with running tap water and followed bydistilled water. Then the root was cut into small pieces and air dried at room temperature. 20grams ofpowderedrootofthedigeria muricatawasextractedwithhydroalcoholicextractbyusingsoxhlationfor72hours.solventrecovery isdoneby rotaryevaporation.[8]

Chemicals and reagent:

Qualitative phytochemical analysis:

Primary, secondary metabolites of the plants hold responsible for multiple pharmacological actions. Isolation and identification of such compounds always worth to identify the new drug molecules. Preliminary phytochemical qualitative analysis of selected medicinal plants extracts were done with standard procedures (Khandelwal K.R. 2007).[10]) Test is done for Alkaloids, Carbohydrates, Glycosides, Saponins, Phenols, Flavonoids, Steroids Coumarins, Terpenoids, Amino acids, Fatty acids.

Total polyphenols and flavonoids content estimation:

Total polyphenolic content of various extracts of selected medicinal plants were done by Folin–Ciocalteu method and expressed in mg of gallic acid equivalent per gram of the extract. Similarly, total flavonoids content was estimated by aluminum chloride-colorimetric method and expressed as mg of rutin equivalent per gram of the extract.[11]

In Vitro Antioxidant Activity:

1. DPPH radical scavenging assay:

DPPH radical scavenging assay of hydro-alcoholic extracts of selected medicinal plants were done using ascorbic acid as the standard drug and half inhibition concentration (IC50) was calculated.

2.In vitro hydrogen peroxide (H2O2) radical scavenging activity of hydro-alcoholic extract of Digeria muricata:

In vitro hydrogen peroxide radical scavenging activity of hydro-alcoholic extracts of Digeria muricata was done using ascorbic acid as the standard drug and half inhibition concentration (IC50) was calculated**.**

3.In vitro ferric-reducing antioxidant power (FRAP) of hydro-alcoholic extract of Digera muricata:

In vitro ferric-reducing antioxidant power of hydro-alcoholic extracts of selected medicinal plants was done using ascorbic acid as the standard drug and half inhibition concentration (IC50) was calculated.

4. In vitro ABTS+ (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation scavenging assay of hydro-alcoholic extract of Digera muricata:

In vitro ABTS + radical scavenging assay of hydro-alcoholic extracts of selected medicinal plants was done using ascorbic acid as the standard drug and half inhibition concentration (IC50) was calculated.

5.Cytotoxicity potential of hydro-alcoholic extract of Digera muricata on Vero, MCF-7 and HT-29 cell lines:

To determine the cytotoxic potential of the hydro-alcoholic extract of Digera muricata against selected cell lines such Vero cells, MCF-7, and HT-29 under the fastidious experimental conditions through 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay[12]. Before cell viability was determined, all cell lines were exposed to varying doses of hydro-alcoholic extract of Digera muricata for 48 hours. The Vero cells were used in the study to examine cytotoxicity of selected plant extract on normal cell growth was altered after exposure to test compounds[14].

6.Effect of hydro-alcoholic extract of digeria muricata on β-catenin, Cox-2 and TNF-α expression levels:

To assess the anticancer effect of hydro-alcoholic extract of digeria muricata the expression of β-catenin, Cox-2 and TNF-α level were examined by flow cytometer in TH-29 cells at the concentration of 50 and 100 µg/ml. 5-flurouracil has been used as the reference standard.

Statistical analysis:

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Bonferroni multiple comparison test using the computer based fitting program (Prism, Graph pad 5). The significance level was set at $P<0.001$ for all tests.

3. Results

1.Solid-liquid extraction of DIGERIA MURICATA:

Digera muricata was extracted with preferred solvents like n-hexane, chloroform and hydro-alcohol and the total yield was calculated.

Name of the plant	**% yield**		
	n-hexane	**Chloroform**	**Hydro-alcoholic**
Digera muricata	5.72	8.16	16.63

2. Phytochemical analysis of solvent extracts of *Digera muricata:*

The phytochemical analysis of dried roots of *Digera muricata* reveals as follows.

Phytochemical Analysis of Dried Roots of *Digera Muricata:*

Phytochemical constituents	***Digera muricata***		
	Hexane	Chloroform	Hydro-alcohol
Alkaloids	+	+	+

Carbohydrates	-	-	-
Glycosides	-	-	+
Saponins	-	-	-
Phenols	-	-	+
Flavanoids	-	+	+
Steroids	+	+	+
Coumarins	-	+	-
Terpenoids	+	+	+
Amino acids	-	-	-
Fatty acids	+	+	-

3.Total polyphenols and total flavonoids content of dried roots of *Digera muricata*

Digera muricata		
Extract type	**Total phenolic content** (mg of GA/g of dry extract)	**Total flavonoids content** (mg of Rutin/g of dry extract)
n-hexane	3.503± 0.794	2.428± 0.541
Chloroform	4.342± 0.644	3.361± 0.531
Hydro-alcohol	12.529±1.511	10.468±0.973

4.*InVitro*AntioxidantActivity:

DPPH radical scavenging assay:

A significant DPPH radical ion scavenging activity in dose dependent inhibition manner. The results were tabulated as follows:

DPPH radical scavenging activity of hydro-alcoholic extract of Digera muricata:

Hydro-alcoholic extract of	**Concentration**	**% inhibition (Mean±sd)**	**IC_{50} value (µg/ml)**
Digera muricata	20	34.78±0.21	**70.85**
	40	39.37±0.12	
	60	47.12±0.30	
	80	53.06±0.36	

5. In vitro hydrogen peroxide (H2O2) radical scavenging activity of hydro-alcoholic extract of Digera muricata

In vitro hydrogen peroxide radical scavenging activity of hydro-alcoholic extracts of digeria muricata root was done using ascorbic acid as the standard drug and half inhibition concentration (IC50) was calculated. The

hydro-alcoholic extract of Digera muricata showing a noteworthy H2O2 radical ion scavenging or inhibition activity was in dose dependent manner. The results were tabulated as follows:

H_2O_2 radical scavenging activity of hydro-alcoholic extract of *Digera muricata* :

Hydro-alcoholic extract of	Concentration	% inhibition (Mean±sd)	IC_{50} value (µg/ml)
Digera muricata	20	20.50±0.10	**74.09**
	40	28.87±0.16	
	60	44.52±0.20	
	80	54.45±0.23	
	100	62.81±0.13	

6. *In vitro* nitric oxide (NO) radical scavenging activity of hydro-alcoholic extract of *Digera muricata* :

In vitro nitric oxide radical scavenging activity of hydro-alcoholic extracts of digeria muricata was done using ascorbic acid as the standard drug and half inhibition concentration (IC_{50}) was calculated. The hydro-alcoholic extract o*f Digera muricata* showing a significant NO radical ion scavenging or inhibition activity was in dose dependent manner. The results were tabulated as follows

NOradical scavenging activity of hydro-alcoholic extract of *Digera muricata*

Hydro-alcoholic extract of	Concentration	% inhibition (Mean±sd)	IC_{50} value (µg/ml)
Digera muricata	20	25.67±0.19	**66.84**
	40	37.49±0.16	
	60	45.98±0.24	
	80	58.80±0.27	
	100	64.97±0.19	

7.In vitro* ferric-reducing antioxidant power (FRAP) of hydro-alcoholic extract of *Digera muricata

In vitro ferric-reducing antioxidant powerof hydro-alcoholic extracts of digeria muricata was done using ascorbic acid as the standard drug and half inhibition concentration (IC_{50}) was calculated. The hydro-alcoholic extract of *Digera muricata* showing a significant FRAP radical ion scavenging or inhibition activity was in dose dependent manner. The results were tabulated as follows

FRAPof hydro-alcoholic extract of *Digera muricata* :

Hydro-alcoholic extract of	Concentration	% inhibition (Mean±sd)	IC_{50} value (µg/ml)
Digera muricata	20	19.76±0.18	**66.06**
	40	34.86±0.15	
	60	46.99±0.18	
	80	58.55±0.15	
	100	70.78±0.12	

***8.In vitro* ABTS$^+$ (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)radical cation scavenging assay of hydro-alcoholic extract of *Digera muricata* :**

In vitro ABTS $^+$ radical scavenging assayof hydro-alcoholic extracts of digeria muricata was done using ascorbic acid as the standard drug and half inhibition concentration (IC_{50}) was calculated. The hydro-alcoholic extract of *Digera muricata* showing a significant ABTS radical cation scavenging or inhibition activity was in dose dependent manner. The results were tabulated as follows:

ABTSradical cation scavenging assay of hydro-alcoholic extract of *Digera muricata :*

Hydro-alcoholic extract of	Concentration	% inhibition (Mean±sd)	IC_{50} value (µg/ml)
Digera muricata	20	20.24±0.12	**63.95**
	40	37.10±0.10	
	60	51.35±0.05	
	80	60.23±0.10	
	100	69.12±0.03	

9.Cytotoxicity potential of hydro-alcoholic extract of *Digera muricata* on Vero, MCF-7 and HT-29 cell lines:

HT-29 cells were showing more sensitive than breast cancer cells (MCF-7) and normal cells (Vero cell) and the cytotoxicity was in a dose-dependent manner. The percent cell viability for TH-29 was estimated to be 29.16% at 1000 ug/ml, whereas to MC-7 and Vero cells were 35. 08% and 54. 84%, respectively at similar concentration. The results showed that TH-29 cells were more sensitivity to hydro-alcoholic extract of *Digera muricata* than Vero and MCF-7 cells. Hydro-alcoholic extract of *Digera muricata* showed a significant anti-proliferative effect on TH-29 cell lines.

Cytotoxicity effect of hydro-alcoholic extract of *Digera muricata* on Vero, MCF-7 and HT-29 cell lines:

Concentration (µg/ml	Absorbance (O.D)			Cell viability (%)		
	Digera muricata			*Digera muricata*		
	Vero cells	MCF-7	HT-29	Vero cells	MCF-7	HT-29
1000	0.3705	0.237	0.197	54.84	35.08	29.16
500	0.3405	0.2565	0.2365	50.40	37.97	35.01
250	0.333	0.3465	0.328	49.29	51.29	48.55
125	0.4195	0.4295	0.429	62.10	63.58	63.50
62.5	0.518	0.53	0.5385	76.68	78.46	79.71
31.2	0.666	0.667	0.665	98.59	98.74	98.44
Cell control	0.6755	0.6755	0.6755	100	100	100

Cytotoxicity potential of 5-flurouracil on Vero, MCF-7 and HT-29 cell lines :

In the present study, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay used to assess the cytotoxicity potential of 5-flurouracilon different cell lines like Vero cells, MCF-7 and HT-29 under the specific experimental conditions. All cell lines were treated at various concentrations of plants extract for 48 hrs before cell viability was assessed. Vero cells were utilized in the study to determine whether either cancer cell

growth was impacted following treatment with 5-flurouracil. The 5-flurouracil exhibited a significant cytotoxicity towards HT-29 cells in dose dependent manner as compared to breast cancer cell lines MCF-7 while the normal Vero cells showed significant cytotoxicity. The % cell viability for TH-29 was calculated to be 19.25% at 100 µg/ml, whereas for MC-7 and Vero cell was 21.69% and 28.05% at 100 µg/ml, respectively. The results were displayed in the Table 15 and Figure 9.

Cytotoxicity potential of 5-flurouracil on Vero, MCF-7 and HT-29 cell lines:

Concentration (µg/ml)	Absorbance (O.D)			Cell viability (%)		
	5-flurouracil			5-flurouracil		
	Vero cells	MCF-7	HT-29	Vero cells	MCF-7	HT-29
20	0.66	0.68	0.65	95.48	83.86	80.75
40	0.63	0.54	0.52	88.75	66.32	63.73
60	0.61	0.43	0.42	67.73	50.41	49.07
80	0.59	0.35	0.33	43.97	44.19	34.94
100	0.57	0.25	0.19	28.05	21.69	19.25
Cell control	0.68	0.69	0.68	100	100	100

10.Effect of hydro-alcoholic extract of *Digera muricata* on β-catenin, Cox-2 and TNF-α expression levels:

To assess the anticancer effect of hydro-alcoholic extract of *Digera muricata*the expression of β-catenin, Cox-2 and TNF-α level were examined by flow cytometer in TH-29 cells at the concentration of 50 and 100 µg/ml. The expression of β-catenin, Cox-2 and TNF-α level were significantly ($p \leq 0.05$) decreased compared to control. 5-flurouracil has been used as the reference standard.

4. Discussion

Phytochemical evaluation of the hydroalcoholic extract of Digera muricata showed the presence of saponins, phenols and flavonoids. Moreover, total phenolic and flavonoid content is higher in hydroalcoholic fraction than in n-hexane and chloroform solvents**.** Digera muricata displayed intense antioxidant activity and dose-dependently in lower-produced H2O2 radical ions. Nitric oxide radical, FRAP radical ion and ABTS radical cation levels are considerably reduced following Digera muricata treatment. Vero cells were used to study the effect of plant extracts on normal cells. We chose HT-29 cells for further studies since the study indicated that HT-29 cells were more sensitive to plant extracts than MCF-7 cells. Digera muricata was cytotoxic towards HT-29 cells. Notably, the plant extract was identified as significantly nontoxic on normal cells. Digera muricata showed reduced levels of β -catenin, Cox-2 and TNF- α significantly compared to the disease group ($p< 0.05$).

5. Conclusion

Study provides a foundation for understanding the pharmacological properties of Digera muricata's dried root extract and underscores its potential as a source of natural compounds with antioxidant and cytotoxic properties that warrant further investigation in the field of medicine and drug development.

6. References

[1] May JM. How does ascorbic acid prevent endothelial dysfunction?. Free radical biology andmedicine. 2000 May 1;28(9):1421-9.
[2] Diaz MN, Frei B, Vita JA, Keaney Jr JF. Antioxidants and atherosclerotic heart disease. NewEngland Journal of Medicine. 1997 Aug 7;337(6):408-16.
[3] Doba T, Burton GW, Ingold KU. Antioxidant and co-antioxidant activity of vitamin C. The effect ofvitamin C, either alone or in the presence of vitamin E or a water-soluble vitamin E analogue, uponthe

peroxidation of aqueous multilamellar phospholipid liposomes. Biochimica et Biophysica Acta(BBA)-Lipids and Lipid Metabolism. 1985 Jul 9;835(2):298-303.
[4] Guo Q, Packer L. Ascorbate-dependent recycling of the vitamin E homologue Trolox bydihydrolipoate and glutathione in murine skin homogenates. Free Radical Biology and Medicine.2000 Aug 1;29(3-4):368-74.
[5] Thiele JJ, Schroeter C, Hsieh SN, Podda M, Packer L. The antioxidant network of the stratum corneum. CURRENT PROBLEMS IN DERMATOLOGY-BASEL-. 2001 Jan 1;29:26-42.
[6] Manfredini S, Pavan B, Vertuani S, Scaglianti M, Compagnone D, Biondi C, Scatturin A, TanganelliS, Ferraro L, Prasad P, Dalpiaz A. Design, synthesis and activity of ascorbic acid prodrugs ofnipecotic, kynurenic and diclophenamic acids, liable to increase neurotropic activity. Journal ofmedicinal chemistry. 2002 Jan 31;45(3):559-62
[7] Diplock AT, Charuleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, VinaRibes J. Functional food science and defence against reactive oxidative species. British journal ofnutrition. 1998 Aug;80(S1):S77-112.s
[8] Hou YC, Janczuk A, Wang PG. Current trends in the development of nitric oxide donors. Currentpharmaceutical design. 1999 Jun 1;5(6):417-42.
[9] Packer L. Nitric Oxide Part A: Sources and Detection of NO; NO Synthase. Shock. 1997 Jan1;7(1):77.
[10] Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease.Physiological reviews. 2007 Jan;87(1):315-424.
[11] Reaven PD, Witztum JL. Oxidized low density lipoproteins in atherogenesis: role of dietarymodification. Annual review of nutrition. 1996 Jul;16(1):51-71.
[12] Halliwell B, Gutteridge JM. Free radicals in biology and medicine, Clarendon.
[13] Shivakumar A, Yogendra Kumar MS. Critical review on the analytical mechanistic steps in theevaluation of antioxidant activity. Critical reviews in analytical chemistry. 2018 May 4;48(3):214-
[14] Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. The FASEB journal.1996 May;10(7):709-20.
[15] Homma T, Kobayashi S, Fujii J. Induction of ferroptosis by singlet oxygen generated fromnaphthalene endoperoxide. Biochemical and Biophysical Research Communications. 2019 Oct20;518(3):519-25.