



Qualitative and Quantitative Phytochemical profiling of *Digera muricata* (L.) Mart Leaves

Tamilarasan S¹, Vidya R^{2*}, Amudha P², Rajna V¹

¹Research scholar, Department of Biochemistry, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Chennai, India

²Assistant Professor, Department of Biochemistry, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Chennai, India

Email: vidyabiochem@gmail.com¹

*Corresponding author's E-mail: vidyabiochem@gmail.com

Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 27 Nov 2023	<i>Plants possess an extensive variety of therapeutic compounds that exhibit significant potential for application within the pharmaceutical industry. In order to identify the diverse phytochemical composition present in the plant, range of solvents including alcoholic, aqueous, hexane, petroleum ether and hydroalcoholic were employed these extracts were used and the preliminary qualitative analysis reveals the presence of phytochemicals in higher concentrations in the hydro ethanolic extract compared to other solvent. The powdered plant sample was further investigated for the quantification of phytochemicals which revealed the presence of high total phenolic content, total flavonoid content, saponin content, total tannin content and steroids content of 246.82 ± 17.27 GAE equivalents per gram, 193.18 ± 13.52 milligrams of quercetin equivalents per gram, 134.76 ± 9.43 milligrams of saponin equivalents per gram, of 61.80 ± 4.32 milligrams of Tannic acid equivalents per gram and 117.66 ± 8.23 milligrams of Cholesterol equivalents per gram respectively. The present study elucidates the potential for isolating specific compounds possessing economically valuable bioactive properties through the utilization of suitable plants and careful selection of an appropriate solvent for extraction.</i>
CC License CC-BY-NC-SA 4.0	Keywords: Phytochemicals, Antioxidants, Medicinal plants, Bioactive compounds

1. Introduction

The utilization of natural products derived from medicinal plants has played a significant role in enhancing therapeutic interventions from the management of diverse pharmaceutical conditions. The utilisation of medicinal plants as a valuable source of medicine has been well-documented since ancient times. Throughout history, various cultures and civilizations have recognised the therapeutic properties of plants and have relied on them to treat a wide range of ailments. This article aims to explore the enduring significance of medicinal plants as a reliable source of medicine, highlighting their historical importance and the continued relevance of their usage in contemporary healthcare practices. Historical Significance The historical significance of medicinal plants cannot be overstated. Ancient civilizations the investigation of therapeutic compounds derived from plants has garnered significant attention from experts worldwide, owing to their minimal side effects and remarkable efficacy in promoting human health. Plants that have been extensively utilised in ethnomedicine throughout history possess significant potential as a rich reservoir of bioactive compounds for the development of pharmaceutical interventions targeting various illnesses and infectious diseases. Medicinal plants have long been recognised as a valuable source of diverse bioactive compounds with a wide range of therapeutic properties (1). *Digera muricata* L. belongs to the family Amaranthaceae and used in folk medicine to treat renal disorders (2). It is a wild edible plant commonly known as "Cancali soppu" and has been widely distributed throughout India. The plant has traditionally been used as laxative and astringent to the bowels in ayurvedic system of medicine. The plant root has been traditionally given to lactating mothers to increase the lactation after the child birth and the flowers and seeds are used to treat urinary discharges(3). The plant has a good antimicrobial property that was

evaluated by Mathad et al., compared to the standard antifungal agent Nystatin, the plant extract showed good inhibitory concentration against *C. albicans* (4). In another study conducted by Rida et al., revealed that the gold nanoparticle activity of *Digera muricata* extracts showed good antibacterial property against *Klebsiella*, *Staphylococcus pyrogen*, *Enterobacter* and *Citrobacter* (5). The leaves were found to poses anticancer activity against prostate cancer and antiproliferative activity of crude extracts was determined on human cervical cancer and lung cancer (6,7). The plant also possess antioxidant property with increasing concentrations (8). Though the plant has been used to treat various ailments the phytochemicals that are responsible for the development of novel drugs in treating pathological conditions are unrevealed.

2. Materials And Methods

Collection of Plant Sample

The leaves of *Digera muricata* was collected from the cultivator and was authenticated by Dr. N.Mathivanan, Director and Head, Centre for Advanced Studies in Botany, University of Madras, Chennai. The voucher number of the specimen is MUBL1030.

Preparation of Plant Extracts

The leaves of *Digera muricata* was collected, and shade dried. A 10g of the plant in powdered form underwent a cold extraction process, undergoing maceration using various solvents, including hexane, petroleum ether, 70% hydro-ethanolic, aqueous and ethanol over a 24-hour period with periodic agitation. The resulting crude extracts were then filtered through Whatman filter No. 1, and the resulting filtrate was subsequently employed for phytochemical analysis.

Qualitative Phytochemical Screening Of *Digera Muricata* Leaf Extracts

Preliminary phytochemical screening was carried out by Harborne (9), Sofowara (10), Trease and Evans. The following test were performed based on Dubale et al. (11)

Test for Tannins

1ml of different extracts of *D. muricata* was taken in a test tube and 2ml of distilled water was added and filtered. To the filtrate 0.1% of ferric chloride was added and observed for the formation of blue-black colour.

Test for Saponins

2ml of different extracts of *D. muricata* was boiled with 20ml of distilled water. Then the solution was filtered and to 10 ml of filtrate 5ml of distilled water was added and vigorously shaken until the froth was persistent. To the froth 3 drops of olive oil was mixed and shaken. Then the formation of emulsion was observed.

Test for Flavonoids

To 1 ml of different extracts of *D. muricata* 5 ml of dilute ammonia was added in different test tubes and concentrated sulphuric acid was added. In the presence of flavonoids yellow colour is observed and the colour disappeared on standing.

Test for Terpenoids

To 5 ml of plant extracts 2 ml of chloroform and concentrated sulphuric acid was added and observed for a layer formation and an interface with reddish brown colour was found for the presence of terpenoids.

Test for Steroids

To 1 ml of different extracts of *D. muricata* 2 ml of acetic anhydride was added followed by the addition of sulphuric acid and the colour change from violet to blue-green indicated the presence of steroids.

Test for Alkaloids

The test was performed with Mayer's reagent. To 1 ml of plant extracts a drop of Mayers reagent was added and a creamy white precipitate was observed indicated the presence of alkaloids in the plant extract.

Test for Triterpenoids

To 1 ml of different extracts of *D. muricata* 1ml of chloroform was added followed by the addition of acetic anhydride and concentrated sulphuric acid. The formation of reddish violet colour indicated the presence of triterpenoids in the extracts.

Test for Polyphenols

To 1 ml of different extracts of *D. muricata* 5 ml of ethanol was added in different test tubes and warmed in water bath for 15 minutes. To these extracts freshly prepared ferric cyanide solution was added and observed for the formation of blue green colour which indicated the presence of polyphenols.

Test for Cardiac Glycosides

The test is also known as Keller Killiani test where to 5 ml of different extracts of *D. muricata* 2 ml of glacial acetic acid was added followed by a drop of ferric chloride solution and 1 ml of concentrated sulphuric acid. The formation of brown ring at the interface indicates the presence of cardenolides (deoxy sugar) and violet ring below the brown ring with a layer of acetic acid and a greenish ring formation throughout the thin layer.

Test for Anthraquinones

5 ml of different extracts of *D. muricata* was hydrolysed with diluted sulphuric acid and benzene, followed by the addition of diluted ammonia and the formation of pink coloration indicated the presence of Anthraquinones.

Test for Anthocyanins

To 2 ml of different extracts of *D. muricata* 2 ml of 2N hydrochloric acid and ammonia was added and the colour change from pinkish-red to blue-violet indicated the presence of Anthocyanins.

Test for Coumarins

2ml of various *D. muricata* extracts were combined with 3 ml of 10% NaOH and the development of a yellow colour signified the existence of coumarins.

Test for Emodins

To 1 ml of different extracts of *D. muricata* 2 ml of ammonium hydroxide and 3 ml of Benedict's reagent was added the formation of red colour indicated the presence of Emodins.

Quantitative Phytochemical Analysis of *Digera muricata* Leaf Extracts

Determination of Total Phenols

The total phenolic contents of *Digera muricata* were determined by modified Kim et al., method spectrophotometrically. To 1 g of plant powder 10ml of ethanol was added and filtered after 15 minutes. Gallic acid was used as a standard for calibration. 1 ml of diluted filtrate was added to a 25 ml volumetric flask and 9 ml of distilled water was added. To the mixture, 1 ml of Folin's-Ciocalteu's phenol reagent and vortexed, followed by the addition of 7% sodium carbonate solution. The mixture was kept in dark for 90 minutes at room temperature and the absorbance was read at 750 nm. The total phenolic content was determined by the calibration curve of gallic acid at concentration ranging from 10 to 100 µg/ml and the total phenolic content was expressed as milligrams of Gallic acid (GAE equivalents per gram of dried sample)(12).

Determination of Total Flavonoids

The total flavonoid contents were determined according to Katasani method by using aluminium chloride colorimetric method. To 1 g of powdered plant sample 1 ml of water was added and filtered after 15 minutes. The mixture was prepared by adding 0.5 ml of filtered plant extract, 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The absorbance of the reaction mixture was read at 510 nm using UV-Vis spectrophotometer(13).

Determination of Total Saponins

Total saponin contents in *Digera muricata* were determined calorimetrically. 2 g of powdered *Digera muricata* plant sample was added in 100 ml of conical flask and to it 25 ml of methanol was added and incubated at 25°C at 120 rpm for 24 hrs and the extract was centrifuged at 3500 rpm for 20 minutes. The extract was filtered using Whatman filter paper No.1 and the methanol was evaporated using water bath and later dissolved in 10 ml of distilled water. The contents were transferred to a separating funnel and thrice the volume of n-butanol was added. After the extraction the solvent was evaporated using water bath. The dried saponin content was dissolved in 5 to 10 ml of distilled water and transferred the solution into a container that was weighed previously and then freeze dried. 10 mg of the saponin extract was dissolved in 5ml of 80% aqueous methanol and 50 µl of plant extract were taken and 0.25 ml of 8% vanillin reagent was added. The test tubes were placed on ice cold water bath and 2 ml of 75% sulphuric acid was added slowly and the contents were mixed and the tubes were left for 3 minutes. These tubes were placed on water bath at 60°C for 10 minutes and placed on ice. The

absorbance was measured at 544 nm with standard saponin and the contents were expressed as saponin equivalents (mg/gm of extract)(14).

Determination of Steroids

Steroid contents of *Digera muricata* plant sample was determined by Attarde Daksha et al., method, 10 g of plant sample was dissolved in 10 ml of chloroform and further diluted to 10 times. To 3 ml of diluted plant sample, 2 ml of Liberman-Burchard reagent and 2 ml of chloroform was added. The test tubes were covered with black carbon paper and kept on ice in dark for 15 minutes. The reagent reacts with the sterol to produce green color and the absorbance was read at 640 nm and cholesterol was used as a standard and the values were expressed as milligrams of cholesterol equivalents per gram of dried sample(15).

Determination of Tannin

Five grams of powdered plant sample were combined with 400 ml of distilled water, boiled for one hour and then adjusted to a total volume of 500 ml in a standard flask. For the analysis, 0.2 ml of the plant extract was mixed with 7.5 ml of water, followed by the addition of 0.5 ml of Folin-Denis reagent and 1.0 ml of sodium carbonate solution. The resulting sample was further diluted with distilled water. After a 30-minute incubation, the absorbance was measured at 760 nm using tannic acid as a standard. The total tannin content was quantified and expressed as milligrams of tannic acid equivalents per gram of dried sample (16).

3. Results and Discussion

Qualitative Phytochemical Analysis

The findings validated the existence of steroids, terpenoids, polyphenols, and glycosides in aqueous, ethanol, hexane, petroleum ether, and hydro-ethanolic extracts of *Digera muricata*. The aqueous extract of *Digera muricata* revealed the presence of tannins, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthraquinone, polyphenols, glycosides, coumarins, emodins, anthocyanins, and the absence of alkaloids. The ethanolic extract of *Digera muricata* exhibited the presence of tannins, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthraquinone, polyphenols, glycosides, coumarins, alkaloids, and the absence of emodins and anthocyanins. The hexane extract of *Digera muricata* demonstrated the presence of tannin, steroids, terpenoids, anthroquinone, polyphenols, and glycosides. Petroleum ether extracts showed the presence of steroids, terpenoids, polyphenols, and glycosides, with the absence of tannins, saponins, flavonoids, triterpenoids, alkaloids, anthroquinone, coumarins, emodins, and anthocyanins (refer to Table 1). In the hydro-ethanolic extract of *Digera muricata*, all tested phytochemicals were present, indicating a higher concentration and efficacy compared to other extracts. Consequently, this extract was used for further analysis.

Table 1: Qualitative phytochemical analysis of *Digera muricata* Leaf extracts

Phytochemicals	Aqueous	Ethanol	Extracts Hydro-ethanolic	Hexane	Petroleum ether
Tannin	++	++	++	+	--
Saponin	++	+	++	-	--
Flavonoids	++	++	++	-	--
Steroids	+	+	++	+	+
Terpenoids	++	+	+	+	+
Triterpenoids	+	++	++	-	--
Alkaloids	--	+	++	-	--
Anthraquinone	+	+	++	+	--
Polyphenol	++	++	++	+	+
Glycoside	+	+	+	+	+
Coumarins	++	+	+	-	--
Emodins	++	--	+	--	--
Anthocyanins	+	--	++	--	--

(-) Absent, (+) Present and (++) High concentration

Quantitative Phytochemical Analysis

Preliminary quantitative phytochemical analysis of *Digera muricata* revealed that the plant possessed high amount of total phenolic content of 246.82 ± 0.27 GAE equivalents per gram, total flavonoid content of 193.18 ± 0.52 milligrams of quercetin equivalents per gram, saponin content of 134.76 ± 0.43 milligrams of saponin equivalents per gram, total tannin content of 61.80 ± 0.32 milligrams of Tannic acid equivalents per gram and 117.66 ± 0.23 milligrams of Cholesterol equivalents per gram steroids content in the plant (Table 2). Thus, the results obtained suggest that the hydro-alcoholic extract of *Digera muricata* has good phytochemical contents that play a vital role in its medicinal applications.

Table 2: Total quantitative phytochemicals of *Digera muricata*

Name of Plant Extract	Total phenols (mg/g)	Flavonoids (mg/g)	Saponin (mg/g)	Tannin (mg/g)	Steroids (mg/g)
Hydro-ethanolic extract of <i>Digera muricata</i> Leaves	246.82 ± 0.27	193.18 ± 0.52	134.76 ± 0.43	61.80 ± 0.32	117.66 ± 0.23

Values represent mean $\pm$ standard deviation of triplicate sample

Cardiac glycosides have predominantly been used in the treatment of congestive heart failure as they are found to increase the force of myocardial contraction and they directly act on the smooth muscles. *Digera muricata* in this study was found to contain cardiac glycosides which are utilized for their pharmacological applications. In a study, the water extracts of *N. oleander* leaf and flower extracts showed good amount of cardiac glycoside content of 169.89 ± 0.21 and 123.44 ± 0.10 mg SE/g, respectively(17). Flavonoids are large class of phytochemicals that play a vital role in the pigments that are present in the flowers, seeds, fruits, bark and leaves. They are natural aromatic phytochemicals that are mostly reported to be phenolics. The amount of flavonoid contents was found to 193.18 ± 13.52 milligrams of quercetin equivalents per gram in the plant extract of *Digera muricata*. In some green leafy vegetables the highest amount of flavonoids were found in parsley of 14.35mg quercetin acid equivalent per gram dry weight which indicated that the plant has antioxidant potential thus the free radical scavenging activity is high comparatively(18). The solubility of polyphenols in the solvent depends on the degree of polymerization of the phenols, the interactions of the phenols with other plant molecules and the insolubility of complexes from the plant material and their recovery. Polyphenols such as epicatechin, rutin, tannic acid, ferulic acid and quercetin when polymerized enhances the antioxidant capacity of them and establishes the inhibition of XO, anticancer, antibacterial and oxidation of LDL(19). The plant possessed high amount of total phenolic content of 246.82 ± 17.27 GAE equivalents per gram, similarly in a study conducted revealed that high concentrations of 7.6%, 5.2% and 4.9% GAE, w/w polyphenols were found in *S. cumini*, *H. lanceolatum* and *P. mauritianum* which could be compared with other dietary sources like thyme, star anise or cocoa(20). Previous literatures suggest that extracts of *A. theiformis* contain 95% of total phenolic content which of which mangiferin identified in the plant possessed anti diabetic potential and also lowered the cholesterol levels(21). Steroids are one of the broad class of chemical compounds that are distinguished by their carbon structure, they are found to reduce swelling, redness and inflammations (22) the plant powder showed the presence of steroids in all the extracts and the highest concentration of steroid was obtained from the hydro ethanolic extract of the plant. Studies suggest that among all species, *E. arborea* has been identified as prominent source of steroids and triterpenoids that aerial parts of the plant has high amount of ursolic acid and alpha amyryn thus the plant has great antioxidant potential, antidiabetic (23). The plant extract contained 117.66 ± 8.23 Milligrams of Cholesterol equivalents per gram of steroid in the hydro alcoholic extract. Saponins are steroid or triterpene glycosides that are extensively found in plants and exhibit hemolytic and toxic effects (23).

4. Conclusion

The *Digera muricata* plant has been utilized in the traditional Indian medical system for its pharmacological properties, demonstrated through the preparation of decoctions or powders. The research concluded that the plant displays significant pharmacological potential, attributed to the presence of diverse class of phytochemicals including alkaloids, tannins, steroids, polyphenols, cardiac glycosides, terpenoids, coumarins, emodins, flavonoids, anthraquinone and anthocyanin. Consequently, further studies are necessary to identify new sources of phytochemicals aiding in the development of potential Phyto therapeutics for treating chronic degenerative and infectious diseases. Ultimately, to study the highest level of clinical evidence, it is essential to conduct thorough

investigations into the most promising secondary metabolites of the plant through well- designed randomized clinical trials.

References:

1. Aye MM, Aung HT, Sein MM, Armijos C. A Review on the Phytochemistry, Medicinal Properties and Pharmacological Activities of 15 Selected Myanmar Medicinal Plants. *Molecules*. 2019 Jan;24(2):293.
2. Khan MR, Rizvi W, Khan GN, Khan RA, Shaheen S. Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of *Digera muricata*. *J Ethnopharmacol*. 2009 Feb 25;122(1):91–9.
3. Parrotta JA. Healing plants of peninsular India. *Heal Plants Penins India* [Internet]. 2001 [cited 2023 Jul 8]; Available from: <https://www.cabdirect.org/cabdirect/abstract/20036793673>
4. Mathad P, Mety SS. Phytochemical and Antimicrobial Activity of *Digera muricata* (L.) Mart. *J Chem. NaN/NaN/NaN*;7:275–80.
5. Shah1 R, Shah1 SA, Shah2 S, Ullah3 SF and F. Green Synthesis and Antibacterial Activity of Gold Nanoparticles of *Digera muricata*. *Indian J Pharm Sci*. 2020 Apr 30;82(2):374–8.
6. Vidyashri S, Elumalai P, Raghunandhakumar S, Lakshmi T, Roy A. Anticancer effect of *Digera muricata* Leaf Extract against Prostate Cancer Cell Lines. *J Pharm Res Int*. 2021 Dec 29;431–8.
7. Usmani S, Hussain A, Farooqui AHA, Arshad M, Siddiqui S, Ahmad M, et al. 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. *Pharmacogn J*. 2014;6(6):32–8.
8. Usmani S, Hussain A, Farooqui AHA, Khatoon S. HPTLC fingerprinting and antioxidant potential of methanolic extract of *Digera muricata*. *J Chem Pharm Res* [Internet]. 2015 May 31 [cited 2023 Jul 8];7(5). Available from: <https://www.jocpr.com/abstract/hptlc-fingerprinting-and-antioxidant-potential-of-methanolic-extract-of-digera-muricata-7795.html>
9. Harborne JB. *Phytochemical Methods* [Internet]. Dordrecht: Springer Netherlands; 1984 [cited 2023 Jul 8]. Available from: <http://link.springer.com/10.1007/978-94-009-5570-7>
10. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *J Exp Pharmacol*. 2023 Feb 8;15:51–62.
11. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nat Protoc*. 2007 Apr;2(4):875–7.
12. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J Food Drug Anal*. 2014 Sep 1;22(3):296–302.
13. Yu M, Gouvinhas I, Rocha J, Barros AIRNA. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Sci Rep*. 2021 May 11;11(1):10041.
14. Shahzad MN, Ahmad S, Tousif MI, Ahmad I, Rao H, Ahmad B, et al. Profiling of phytochemicals from aerial parts of *Terminalia neotaliala* using LC-ESI-MS2 and determination of antioxidant and enzyme inhibition activities. *PLOS ONE*. 2022 Mar 31;17(3):e0266094.
15. Otari SS, Patel SB, Lekhak MM, Ghane SG. Phytochemical Studies on Two Unexplored Endemic Medicinal Plants of India, *Barleria terminalis* and *Calacanthus grandiflorus*. *Front Pharmacol* [Internet]. 2022 [cited 2023 Mar 6];12. Available from: <https://www.frontiersin.org/articles/10.3389/fphar.2021.817885>
16. Bakir Çilesizoglu N, Yalçin E, Çavuşoğlu K, Sipahi Kuloğlu S. Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. *Sci Rep*. 2022 Dec 11;12(1):21421.
17. Chandra S, Khan S, Avula B, Lata H, Yang MH, ElSohly MA, et al. Assessment of Total Phenolic and Flavonoid Content, Antioxidant Properties, and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. *Evid Based Complement Alternat Med*. 2014 Mar 23;2014: e253875.
18. Basak S, Raja ASM, Saxena S, Patil PG. Tannin based polyphenolic bio-macromolecules: Creating a new era towards sustainable flame retardancy of polymers. *Polym Degrad Stab*. 2021 Jul 1; 189:109603.
19. Checkouri E, Reignier F, Robert-Da Silva C, Meilhac O. Evaluation of Polyphenol Content and Antioxidant Capacity of Aqueous Extracts from Eight Medicinal Plants from Reunion Island: Protection against Oxidative Stress in Red Blood Cells and Preadipocytes. *Antioxidants*. 2020 Oct;9(10):959.
20. Kulkarni VM, Rathod VK. Exploring the potential of *Mangifera indica* leaves extract versus mangiferin for therapeutic application. *Agric Nat Resour*. 2018 Apr 1;52(2):155–61.
21. Agidew MG. Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bull Natl Res Cent*. 2022 Apr 1;46(1):87.
22. Gadouche L, Alsoufi ASM, Pacholska D, Skotarek A, Pączkowski C, Szakiel A. Triterpenoid and Steroid Content of Lipophilic Extracts of Selected Medicinal Plants of the Mediterranean Region. *Molecules*. 2023 Jan;28(2):697.
23. Ur Rahman S, Ismail M, Khurram M, Ullah I, Rabbi F, Iriti M. Bioactive Steroids and Saponins of the Genus *trillium*. *Molecules*. 2017 Dec;22(12):2156.