RESEARCH PAPER

Evaluation of Bioactive Potential of the *Digera muricata* Mart

Nalini Tomer[1], Mohammad Irfan Ali[2], Sarmad Moin[1*]

[1]*Applied Sciences, Suresh Gyan Vihar, University, Jaipur, Rajasthan, India*
[2]*Faculty of Life Sciences, Mandsaur University, Mandsaur, Madhya Pradesh, India*

Received: 08th October, 2022; Revised: 15th November, 2022; Accepted: 06th December, 2022; Available Online: 25th December, 2022

ABSTRACT

Digera muricata (L.) mart (Amaranthaceae) is a promising medicinal plant used as a prophylactic, antioxidant, anthelmintic, antimicrobial, allelopathic, and antidiabetic. The present study assesses the quantification of primary and secondary metabolites along with the antimicrobial potential of *D. muricata*. Qualitative and quantitative phytochemical analysis was undertaken to evaluate secondary metabolites extracted from leaf extracts of *D. muricata* by using standard methods. The antibacterial and antifungal potential was studied by using the disc diffusion method. A (gas chromatography-mass spectrometry) (GC–MS) study was carried out to recognize the bioactive molecules in the active fraction. *D. muricata* contained carbohydrates, proteins, steroids, amino acids, cardiac glycosides, coumarin, polyphenol, alkaloids, saponin, tannin, and flavonoids. Results also indicate that ethyl acetate extracts of *D. muricata* showed significant antimicrobial potential against *Streptococcus agalactiae* (ATCC13813)*, Klebsiella pneumoniae* (MTCC432)*, Escherichia coli* (MTCC730)*, Streptococcus pyogenes* (MTCC1924)*, Macrophomina phaseolina*, and *Candida albicans* (MTCC7315). These findings showed that the ethyl acetate extract of *D. muricata* confined bioactive molecules of therapeutic rank with significant antibacterial and antifungal activity.

Keywords: *Digera muricata,* phytochemical analysis, antibacterial, antifungal, Gas chromatography-mass spectrometry

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.4.10

How to cite this article: Tomer N, Ali MI, Moin S. Evaluation of Bioactive Potential of the *Digera muricata* Mart. International Journal of Pharmaceutical Quality Assurance. 2022;13(4):402-407.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Digera muricata (L.) mart family *Amaranthaceae*, the edible and wild plant commonly distributed throughout India, including Rajasthan, Maharashtra, and Andra Pradesh. Its nativity is southwest Asia.[1] *D. muricata,* also known as *Cancali soppu*, Latamouri or Gungutiya, latmahuria, lesua, latmahuria, false amaranth, aranya and kunanjara. *D. muricata* is a herb, growing annually up to 20–70 cm in length.[2] Young shoots and leaves of *D. muricata* are traditionally consumed as a vegetable to release constipation, bowel complaints, diabetes and digestive system disorders. Seeds and flowers are utilised for the treatment of urinary discharges condition. Leaf paste is utilised for the treatment of skin disease to prevent pus. Infusion prepared from boiled root is utilized for induction of lactation after childbirth and as a mild expectorant and astringent.[3-5] In Ayurveda, *D. muricata* is considered astringent, laxative and cooling to the bowels as a folk and traditional system of medicine.

The *D. muricata* leaf extracts were also utilizes in fungal diseases.[6] The petroleum ether extract of *D. muricata* is effective against the *Vibrio cholerae* infection.[7,8] The plant of *D. muricata* supports the defense against toxicity induced by CCl_4, emphasizing therapeutic importance.[9] The *D. muricata* root extracts have phenols and sugar, while the leaves have primary metabolites, including proteins, lipids and carbohydrates and showed antimicrobial potential against *Fusarium oxysporum* and *Escherichia coli*. The leaf extract is utilized to treat kidney stones.[10] *D. muricata* restores the disruptions induced with CCl_4 for various male hormones in rat.[11] *D. muricata* is utilised as an alternative in secondary infertility.[12] The present study was designed to evaluate phytochemical analysis, antimicrobial activity and gas chromatography-mass spectrometry analysis of *D. muricata*.

MATERIAL AND METHODS

Plant Collection and Authentication

The leaves of *D. muricata* were brought from Chittorgarh, Rajasthan. The collected plants were authenticated and identified by Dr. Vinod Maina, Scientist D, Botanical Survey of India, Jodhpur, Rajasthan.

Preparation of Extract

The leaves of *D. muricata* were shade dried under and then made into a coarse powder, and the extracts were prepared with

**Author for Correspondence: moinsarmad@gmail.com*

Table 1: Qualitative Phytochemical Screening of *D. muricata* leaf extracts

S. No.	*Qualitative tests*	*Petroleum ether*	*Benzene*	*Ethyl acetate*	*Ethanol*	*Water*
1	Carbohydrates	+	+	+	+	+
2	Proteins	+	+	+	+	+
3	Steroids	+	+	+	+	+
4	Amino acids	+	+	+	+	+
5	Cardiac glycosides	+	+	+	+	+
6	Glycosides	+	+	+	-	-
7	Anthocyanin	-	-	-	-	-
8	Phlobatannin	-	-	+	-	-
9	Coumarin	+	+	+	+	-
10	Polyphenol	+	+	+	+	+
11	Alkaloids	+	+	+	+	+
12	Saponin	+	+	+	+	+
13	Tannin	+	+	+	+	+
14	Flavonoids	+	+	+	+	+

petroleum ether, benzene, ethyl acetate, ethanol, and water by soxhlet apparatus for 48 hours. The various extracts were concentrated to dryness by using a vacuum rotatory evaporator. It gives a thick greenish semisolid residue. The extracts were placed in an air-tight vessel at 4°C for further use.

Phytochemical Screening

The qualitative preliminary phytochemical analysis was carried out on individual extracts using standard procedures.[13] Quantitative analysis of alkaloid carried out by using standard methods.[14]

The modified anthrone method, as adopted by Beck and Bibby (1961) was used to estimate the carbohydrate, and the protein measurement was conducted by using the modified Lowry method described by Hartree.[15]

Microorganisms and Growth conditions

Streptococcus agalactiae (ATCC13813)*, Klebsiella pneumoniae* (MTCC432)*, E. coli* (MTCC730)*, and Streptococcus pyogenes* (MTCC1924) used for antimicrobial potential study were revived in nutrient broth (NB) and incubated at 37 ± 2°C for 24 hour. The cell suspension was diluted with NB to obtain a dilution (1.5×108 CFU/mL) equal to McFarland 0.5 standard. *Macrophomina phaseolina*, and *Candida albicans* (MCCC7315) fungus were utilized for antifungal study and were maintained on potato dextrose agar (PDA) at 4°C.

Antibacterial Susceptibility Assay

The antibacterial assay of the leaf extracts of *D. muricata* was carried out by using the standard disc diffusion method.[16] DMSO without extract was used as a control and ciprofloxacin.

Antifungal Susceptibility Assay

The antifungal activity was carried out by using standard disc diffusion assay.[17] Ketoconazole was utilized as the positive control, and dimethyl sulfoxide (DMSO) was utilized as a negative control.

GC-MS analysis of extract

GC-MS analysis was carried out by using Schimadzu GCMS-QP2010 consisting of a gas chromatograph interfaced to a mass spectrometer (GC-MS).

RESULTS

Qualitative Phytochemical Screening of *D. muricata* leaf extracts

The qualitative analysis of petroleum ether extracts of *D. muricata* revealed the existence of carbohydrates, steroids, cardiac glycosides, amino acids, glycosides, coumarin, alkaloids, tannin polyphenol, proteins, and flavonoids. Anthocyanin and phlobatannin were absent. Benzene extract exhibits the presence of carbohydrates, cardiac glycosides, steroids, glycosides, coumarin, alkaloids, flavonoids, polyphenol, proteins, amino acids, and tannin. Anthocyanin and phlobatannin were absent. Ethyl acetate extract revealed the existence of carbohydrates, proteins, steroids, cardiac glycosides, phlobatannin, coumarin, polyphenol, alkaloids, tannin, saponin, amino acids and flavonoids. Anthocyanin was absent. Phytochemical found in ethanol extracts was carbohydrates, proteins, steroids, cardiac glycosides, coumarin, polyphenol, alkaloids, amino acids, saponin, flavonoids and tannin, while glycosides, anthocyanin, and phlobatannin were absent. Water extracts discovered the existence of carbohydrates, steroids, cardiac glycosides, alkaloids, tannin, saponin, proteins, polyphenol, amino acids, and flavonoids, while glycosides, coumarin, anthocyanin, and phlobatannin were absent (Table 1).

Quantitative Analysis of Extracts of *D. muricata* Leaf Extract

Quantitative analysis of petroleum ether extracts revealed the presence of highest amount of carbohydrates (2.22 ± 0.02 mg/mL), followed by tannins (1.740 ± 0.03 mg/mL), flavonoids

Table 2: Quantitative analysis of extracts of *D. muricata*

Sr. No.	*Phytochemicals*	*Petroleum ether (mg/mL, Mean ± SD)*	*Benzene (mg/mL, Mean ± SD)*	*Ethyl acetate (mg/mL, Mean ± SD)*	*Ethanol (mg/mL, Mean ± SD)*	*Water (mg/mL, Mean ± SD)*
1	Alkaloids	0.44 ± 0.009	0.33 ± 0.025	0.46 ± 0.05	0.42 ± 0.08	1.18 ± 0.01
2	Carbohydrates	2.22 ± 0.02	0.14 ± 0.02	2.76 ± 0.11	2.45 ± 0.12	1.23 ± 0.06
3	Proteins	0.007 ± 0.002	0.077 ± 0.002	0.036 ± 0.005	0.17 ± 0.015	0.30 ± 0.03
4	Steroids	1.060 ± 0.01	0.123 ± 0.025	1.07 ± 0.015	1.08 ± 0.009	0.94 ± 0.045
5	Flavonoids	0.98 ± 0.009	0.88 ± 0.02	1.36 ± 0.11	1.06 ± 0.02	1.89 ± 0.065
6	Saponins	0.22 ± 0.02	0.95 ± 0.035	0.85 ± 0.055	0.86 ± 0.02	0.84 ± 0.04
7	Tannins	1.740 ± 0.03	0.67 ± 0.015	1.60 ± 0.05	2.66 ± 0.025	6.30 ± 0.45
8	Phenols	0.45 ± 0.01	0.30 ± 0.015	0.16 ± 0.015	0.26 ± 0.02	0.67 ± 0.025

Each value given as mean ± SD (n=3)

Table 3: Antimicrobial activity of extract of *D. muricata*

Microorganism	*Petroleum ether ZOI (mm) ± SD)*	*Benzene ZOI (mm) ± SD)*	*Ethyl acetate ZOI (mm) ± SD)*	*Ethanol ZOI (mm) ± SD)*	*Water ZOI (mm) ± SD)*	*Control (mm) ± SD)*
*S. agalactia*e	11.33 ± 0.2	10.66 ± 0.57	13.56 ± 0.11	10.76 ± 0.15	11.4 ± 0.2	14.3 ± 0.2
K.pneumoniae	11.23 ± 0.28	10.73 ± 0.25	13.33 ± 0.57	11.40 ± 0.2	11.63 ± 0.25	15.37 ± 0.21
E. coli	10.63 ± 0.15	10.66 ± 0.32	11.50 ± 0.1	10.86 ± 0.55	11.40 ± 1.04	17.6 ± 0.44
S. pyogenes	10.76 ± 0.05	11.2 ± 0.6	14.0 ± 0.1	10.46 ± 0.57	10.50 ± 0.1	14.7 ± 0.2
M. phaseolina	11.03 ± 0.41	10.33 ± 0.57	11.66 ± 0.56	10.63 ± 0.25	11.03 ± 0.2	15.7 ± 0.27
C. albicans	10.70 ± 0.26	10.26 ± 0.15	12.20 ± 0.26	11.06 ± 0.23	10.43 ± 0.2	16.3 ± 0.25

Each value given as mean ± SD (n=5)

(0.98 ± 0.009 mg/mL), steroids (1.060 ± 0.01 mg/mL), phenols (0.45 ± 0.01 mg/mL) alkaloids (0.44 ± 0.009 mg/mL), saponins (0.22 ± 0.02 mg/mL), while least amount of proteins (0.007 ± 0.002 mg/mL). Benzene extracts shows the presence of highest amount of steroids (0.123 ± 0.025 mg/mL), followed by saponins (0.95 ± 0.035 mg/mL), tannins (0.67 ± 0.015 mg/mL), flavonoids (0.88 ± 0.02 mg/mL), alkaloids (0.33 ± 0.025 mg/mL), phenols (0.30 ± 0.015 mg/mL) carbohydrates (0.14 ± 0.02 mg/mL), while least amount of proteins (0.077 ± 0.002 mg/mL). Ethyl acetate extracts shows the presence of highest amount of carbohydrates (2.76 ± 0.11 mg/mL), followed by tannins (1.60 ± 0.05 mg/mL), flavonoids (1.36 ± 0.11 mg/mL), steroids (1.07 ± 0.015 mg/mL), saponins (0.85 ± 0.055 mg/mL), alkaloids (0.46 ± 0.05 mg/mL), and phenols (0.16 ± 0.015 mg/mL) while least amount of proteins (0.036 ± 0.005 mg/mL). Ethanol extracts exhibits the presence of highest amount of tannins (2.66 ± 0.025 mg/mL), followed by carbohydrates (2.45 ± 0.12 mg/mL), steroids (1.08 ± 0.009 mg/mL), flavonoids (1.06 ± 0.02 mg/mL), saponins (0.86 ± 0.02 mg/mL), alkaloids (0.42 ± 0.08 mg/mL), and phenols (0.26 ± 0.02 mg/mL) while least amount of proteins (0.17 ± 0.015 mg/mL). Water extracts revealed the presence of highest quantity of tannins (6.30 ± 0.45 mg/mL), followed by flavonoids (1.89 ± 0.065 mg/mL), carbohydrates (1.23 ± 0.06 mg/mL), alkaloids (1.18 ± 0.01 mg/mL), steroids (0.94 ± 0.045 mg/mL), saponins (0.84 ± 0.04 mg/mL), and phenols (0.67 ± 0.025 mg/mL) while least amount of proteins (0.30 ± 0.03 mg/mL). Manwatkar, (2021) revealed the lower amount of protein (9.47%) found in *D. muricata* (Table 2).

Antimicrobial Activity of Extract of *D. muricata* Leaf Extract

In an antimicrobial susceptibility test of *D. muricata* against *Streptococcus agalactiae*, the maximum zone of inhibition (ZOI), i.e. 13.56 ± 0.11 mm by ethyl acetate extract followed by the water extract (ZOI- 11.4 ± 0.2 mm), petroleum ether extracts (ZOI- 11.33 ± 0.2 mm) and ethanol extract (ZOI- 10.76 ± 0.15 mm), while lowest ZOI against benzene extract, i.e., 10.66 ± 0.57 mm as compared to control (ZOI- 14.3 ± 0.2 mm). The ethyl acetate extract revealed maximum antimicrobial potential with ZOI- 13.33 ± 0.57 mm against *Klebsiella pneumoniae* followed by water extract (ZOI- 11.63 ± 0.25 mm), ethanol extract (ZOI- 11.40 ± 0.2 mm), and petroleum ether extract (ZOI- 11.23 ± 0.28 mm), while lowest antimicrobial potential observes in benzene extract with ZOI- 10.66 ± 0.32 mm as compared to control (ZOI- 15.37 ± 0.21 mm). In the case of *E. coli, t*he maximum antimicrobial potential found in ethyl acetate extract (ZOI- 11.50 ± 0.1 mm) followed by water extract (ZOI- 11.40 ± 1.04 mm), ethanol (ZOI-10.86 ± 0.55 mm) and benzene extract (ZOI- 10.66 ± 0.32 mm), while minimum antimicrobial potential exhibits by benzene extract (ZOI- 10.66 ± 0.32 mm) as compared to control (ZOI- 17.6 ± 0.44 mm). The maximum ZOI observed in ethyl acetate extract against *S. pyogenes* with ZOI- 14.0 ± 0.1 mm followed by benzene extract (ZOI- 11.2 ± 0.6 mm), petroleum ether extract (ZOI-10.76 ± 0.05 mm), and water extract (ZOI- 10.50 ± 0.1 mm) while the ethanol extract shows minimum antimicrobial potential with

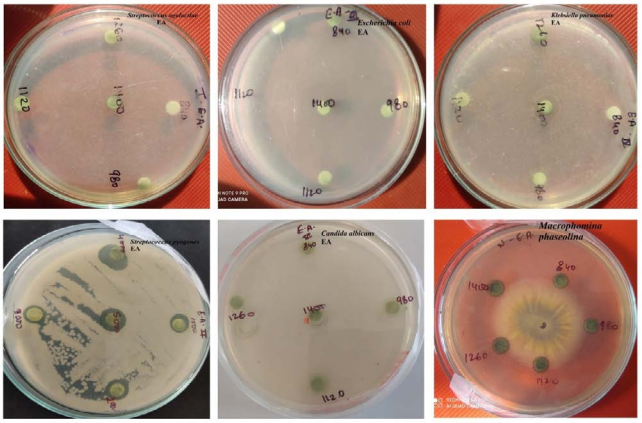

Figure 1: Antimicrobial activity of ethyl acetate extract of *D. muricata*

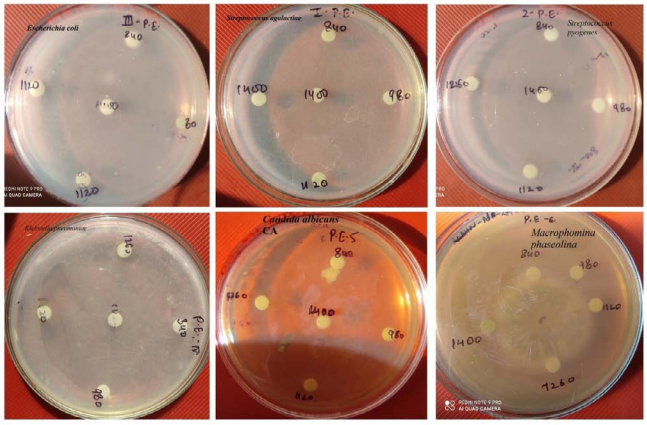

Figure 2: Antimicrobial activity of petroleum ether extract of *D. muricata*

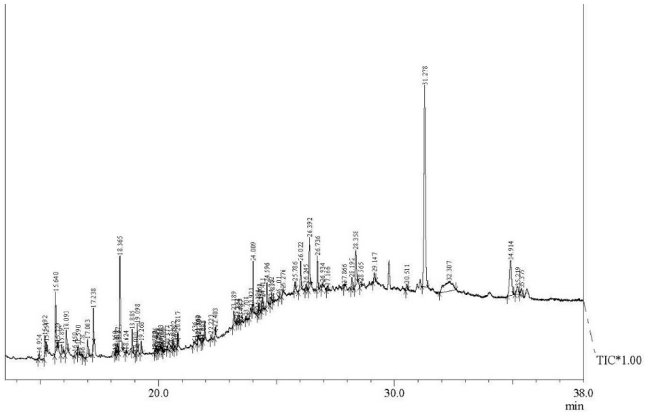

Figure 3: GC-MS Analysis of ethyl acetate extract of D. muricata

ZOI- 10.46 ± 0.57 mm as compared to control (ZOI- 14.7 ± 0.2 mm) (Table 3 & Figure 1).

The antimicrobial potential of *D. muricata* against *Macrophomina phaseolina* revealed the highest potential with ethyl acetate extract (ZOI-11.66 ± 0.56 mm) followed by water extract (ZOI-11.03 ± 0.2 mm) petroleum ether (ZOI- 11.03 ± 0.41 mm) and ethanol extract (ZOI- 10.63 ± 0.25 mm) while benzene extract exhibits minimum antimicrobial potential

Table 4: GC-MS Analysis of ethyl acetate extract of *D. muricata*

S.No.	*Rt (min)*	*Area (Ab*s)*	*Area%*	*Name of compounds*
1	15.192	330831	1.09	Cyclohexadecane
2	15.640	1279396	4.22	Neophytadiene
3	17.238	669366	1.56	1-Hexadecanol
4	18.365	2369113	7.81	Phytol
5	18.885	354016	1.17	2-Methyltetracosane
6	19.098	361418	1.19	1-Nonadecene
7	23.189	348567	1.15	Tetratetracontane
8	24.009	761285	2.51	Squalene
9	24.411	332436	1.10	alpha-Tocospiro B
10	24.596	546017	1.80	Tetracontane
11	26.022	696208	2.30	Tetratriacontane
12	26.392	1084731	3.58	Vitamin E
13	26.736	741330	2.45	alpha-Tocopherol acetate
14	28.192	308133	1.02	2-Nonadecanone
15	28.358	1137882	3.75	Chondrillasterol
16	31.278	7882740	26.00	Phytyl tetradecanoate
17	34.914	1968989	6.49	1-Eicosanol
18	35.219	392251	1.29	Isopropyl 9,12,15-octadecatrienoate
19	35.375	328382	1.08	10-(2-hexylcyclopropyl) decanoic acid

with ZOI- 10.33 ± 0.57 mm as compared to control (ZOI- 15.7 ± 0.27 mm). The ethyl acetate extract revealed the maximum antimicrobial extract against *C. albicans* (ZOI- 12.20 ± 0.26 mm), followed by ethanol extract (ZOI- 11.06 ± 0.23 mm), petroleum ether extract (ZOI- 10.70 ± 0.26 mm), water extract (ZOI- 10.43 ± 0.2 mm) while benzene extract showed minimum antimicrobial potential (ZOI- 10.26 ± 0.15 mm) as compared to control (ZOI- 16.3 ± 0.25 mm) (Table 3, Figure 1 and 2).

GC-MS Analysis of ethyl acetate extract of *D. muricata*

GC-MS analysis of ethyl acetate extract of *D. muricata* is shown in Figure 3 and Table 4. GC-MS study of ethyl acetate extract of *D. muricata* showed 20 constituents in abundant quantity. The highest abundance of phytyl tetradecanoate followed by phytol has been observed, as shown in Figure 3.

DISCUSSION

In recent times, the demand of plants that have medicinal and therapeutic value has dramatically increased. The qualitative estimation of petroleum ether extracts of *D. muricata* revealed the existence of carbohydrates, steroids, cardiac glycosides, amino acids, glycosides, coumarin, alkaloids, tannin polyphenol, proteins, and flavonoids. Usmani *et al.* discovered the existence of flavonoids and phenolics in *D. muricata*.[9] Another phytochemical analysis study by Elgailani discovered the presence of alkaloids, saponin and flavonoids content in leaves and branches, while tannin was devoid in stems and present in the leaves.[18] Sharma *et al.* revealed the occurrence of soluble sugars in roots, starch, lipids and protein in leaves while phenols in roots of *D. muricata*.[10]

Mathad and Mety revealed the antibacterial potential of chloroform, petroleum ether, ethanol and aqueous extracts of the *D. muricata* against *K. pneumonia, S. aureus, E. coli, V. cholerae* and *S. typhi*.[7] Jain *et al.* also observes similar results and found 0.781 mg/mL MIC in *D. muricata* extracts against *P. aeruginosa* represents the presence of antimicrobial activity in the *D. muricata* plants.[19] In another study the leaf extract showed maximum antimicrobial potential against *F. oxysporum* and *E. coli*.[10] Petroleum ether, chloroform, ethanol and purified water extract of *D. muricata* have exhibited the substantial ZOI against the tested pathogen and methanol extract of *D. muricata* shown the maximum antimicrobial potential against pathogenic bacteria and fungi.[20]

The antimicrobial activities of ethyl acetate extract of *D. muricata* is due to the phytochemical present in it. *D. muricata* shows antioxidant and anticancer activity due to cyclohexadecane.[21, 22] Neophytadiene is reported to possess antibacterial activity.[23] Recent study exhibit anxiolytic, cytotoxic, metabolism-modulating, antioxidant, apoptosis-inducing, autophagy, antinociceptive, immune-modulating, anti-inflammatory, and antimicrobial effects with phytol.[24] 2-methyltetracosane is a free radical scavenger and exhibits antioxidant potential.[25]

CONCLUSION

The present study showed that *D. muricata* leaf extract contained chemical compounds of therapeutic importance. The ethyl acetate extract of *D. muricata* leaf exhibited good antifungal and antimicrobial potential, which may arise due to promising antimicrobial compounds. The GC-MS analysis of *D. muricata* leaf ethyl acetate fraction shows the presence of bioactive compounds that show pharmacological potential. The bioactive compounds can be isolated from the ethyl acetate of *D. muricata* leaf.

ACKNOWLEDGMENT

Authors thank the Board of Management and Academic Council of Suresh Gyan Vihar University for providing facilities throughout the research.

REFERENCES

1. Aravindhan V, Rajendran A. Diversity of invasive plant species in boluvampatti forest range, the southern Western Ghats, India. American-Eurasian Journal of Agricultural & Environmental Sciences. 2014;14(8):724-31.
2. Ali T, Ishtiaq A, Mushtaq I, Ayaz N, Jan MI, Khan W, Khan U, Murtaza I. Mentha longifolia Alleviates Exogenous Serotonin-Induced Diabetic Hypoglycemia and Relieves Renal Toxicity via ROS Regulation. Plant Foods for Human Nutrition. 2021 Dec;76(4):501-6. doi: 10.1007/s11130-021-00932-5
3. Miah MM, Das P, Mridha SA, Kuddus MR, Rashid MA. Bioactivities of Digera muricata (L.) Mart. Available in Bangladesh. Dhaka University Journal of Pharmaceutical Sciences. 2017;16(2):251-4. https://doi.org/10.3329/dujps.v16i2.35264
4. Shah A, Marwat SK, Gohar F, Khan A, Bhatti KH, Amin M, Din NU, Ahmad M, Zafar M. Ethnobotanical study of medicinal plants of semi-tribal area of Makerwal & Gulla Khel (lying between Khyber Pakhtunkhwa and Punjab Provinces), Pakistan. American Journal of Plant Sciences. 2013;4(1): 98-116. doi: 10.4236/ajps.2013.41015
5. Austin DF. Healing plants of peninsular India. Economic Botany. 2002;56(3):292. https://doi.org/10.1663/0013-0001(2002)056[0292:HPOPI]2.0.CO;2
6. Chandra M, Mahesh NM. Antifungal activity of medicinal plant extracts against seed-borne pathogenic fungi. Acta Biologica Indica. 2013;2(2):481-483.
7. Mathad P, Mety SS. Phytochemical and Antimicrobial Activity of Digera Muricata (L.) Mart. E-Journal of Chemistry. 2010;7(1):275-280. https://doi.org/10.1155/2010/509254
8. Palozza P, Krinsky NI. β-Carotene and α-tocopherol are synergistic antioxidants. Archives of Biochemistry and Biophysics. 1992;297(1):184-187. doi: 10.1016/0003-9861(92)90658-j
9. Usmani S, Hussain A, Farooqui AH. Pharmacognostical and phytochemical analysis of Digera muricata Linn. growing as a weed in fields of uttar Pradesh region of India. Int J. Pharm Pharm Sci. 2013;5(1):142-145.
10. Sharma N, Tanwer BS, Vijayvergia R. Study of medicinal plants in Aravali regions of Rajasthan for treatment of kidney stone and urinary tract troubles. International Journal of PharmTech Research. 2011;3(1):110-113.
11. Khan MR, Ahmed D. Protective effects of Digera muricata (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. Food and Chemical Toxicology. 2009;47(6):1393-1399. doi:10.1016/j.fct.2009.03.020
12. Chettleborough J, Lumeta J, Magesa S. Community use of non timber forest products: A case study from the Kilombero Valley. Frontier Tanzania. Kilombero Valley Integrated Environmental Management Programme. The Society for Environmental Exploration, UK & The University of Dar es Salaam. 2000:16. doi: 10.4172/2161-1009.1000144
13. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochemistry and Analytical Biochemistry. 2013;2(4):1-4.
14. Shamsa F, Monsef H, Ghamooshi R, Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai Journal of Pharmaceutical Sciences. 2008;32:17-20.
15. Hartree EF. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Analytical biochemistry. 1972;48(2):422-427. doi: 10.1016/0003-2697(72)90094-2
16. Perez C. Antibiotic assay by agar-well diffusion method. Acta biologiae et medicinae experimentalis. 1990;15:113-115.
17. Bauer AW. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 1966;45:149-158.
18. Elgailani IE. Spectrophotometric analysis of some metals and phytochemical screening of Digera muricata (Leaves and stems). Pakistan Journal of Pharmaceutical Sciences. 2018;31(5):1923-1926.
19. Jain P, Kumari S, Malik A. Inhibition of pyocyanin production in pseudomonas aeruginosa by natural antimicrobial compounds from herbal extracts. Asian Journal of Pharmaceutical and Clinical Research. 2017:389-392.
20. Muanza DN, Kim BW, Euler KL, Williams L. Antibacterial

and antifungal activities of nine medicinal plants from Zaire. International Journal of Pharmacognosy. 1994;32(4):337-345. https://doi.org/10.3109/13880209409083012

21. Habib MR, Karim MR. Chemical characterization and insecticidal activity of Calotropis gigantea L. flower extract against Tribolium castaneum (Herbst). Asian Pacific Journal of Tropical Disease. 2016;6(12):996-999. https://doi.org/10.1016/S2222-1808(16)61171-4
22. Kumar DR, George VC, Suresh PK, Kumar RA. Cancer-specific chemoprevention and anti-metastatic potentials of Rheum emodi rhizome ethyl acetate extracts and identification of active principles through HPLC and GC-MS analysis. Pakistan Journal of Pharmaceutical Sciences. 2015;28(1):83-93.
23. Lalitharani S, Mohan VR, Regini GS. GC-MS analysis of ethanolic extract of Zanthoxylum rhetsa (roxb.) dc spines. Journal of Herbal Medicine and Toxicology. 2010;4:191-192.
24. Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, Shill MC, Karmakar UK, Yarla NS, Khan IN, Billah MM. Phytol: A review of biomedical activities. Food and chemical toxicology. 2018;121:82-94. doi: 10.1016/j.fct.2018.08.032
25. Ramya B, Malarvili T, Velavan S. GC-MS analysis of bioactive compounds in Bryonopsis laciniosa fruit extract. International Journal of Pharmaceutical Sciences and Research. 2015;6(8):3375. 10.13040/IJPSR.0975-8232.6(8).3375-79