Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(5):661-665

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

HPTLC fingerprinting and antioxidant potential of methanolic extract of *Digera muricata*

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ABSTRACT

Digera muricata (L.), Family Amaranthaceae, wild edible plant commonly known as 'latmahuria'. It is commonly distributed throughout the India. In Ayurveda, the herb is considered as a cooling, astringent to the bowels and also used as laxative. The flowers and seeds are used to treat urinary discharges.Hence, Digera muricata (L.) Mart. is used in both folk and traditional system of medicine. In the present study, H.P.T.L.C analysis and antioxidant [2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) assay] activity of methanolic extract of whole plant of Digera muricata were investigated.The methanolic extract was obtained by soxhlation for 72 hrs. The methanolic extract showed elevated DPPH scavenging activity, and had a promising activity which increased with concentration. A positive correlation was established between the level of phytoconstituents and antioxidant activities.

INTRODUCTION

The WHO Assembly in number of resolutions emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards [1, 2].

The active ingredients of medicinal and aromatic plants can be found either in the roots, leaves, stems, flowers or bark which can be extracted using an appropriate extraction method [3].

Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations.[4,5] HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [6,7].

The *Digera muricata* (L.) is a wild edible herb used by village people. It is popularly known for herbal remedy for various ailments. In Ayurveda, this herb is considered as cooling, astringent of bowels and also used as a laxative. The leaves are used for treatment of diabetic. The flower and seeds are used to treat urinary discharges. Ethyl alcohol extract of plant is diuretic. The whole plant is used in digestive system disorders. The leaves and young shoots of this plant are locally used as a vegetable and given to relieve constipation. The whole plant is used in urinary disorders. The decoction of leaves was given once in a day for kidney stone treatment. The extract of this plant used in biliousness and in urinary discharges [8,9,10].

Collection and authentication of plant material
The fresh plants of *Digera muricata* were collected from the field behind Faculty of Pharmacy, Integral University, Lucknow.The plant specimens were authenticated by Dr. A.K.S Rawat, Head, Pharmacognosy and Ethnopharmacology division, National Botanical Research Institute, Lucknow. A voucher specimen has been deposited with reference no.N.B.R.I/CIF/263/2011 at National Botanical Research Institute, Lucknow.

Preparation of extract
The plant was air-dried and powdered. The powdered material was packed in muslin cloth and subjected to soxhlet extraction by Methanol after defatting. Thereafter, the extract of the plant was filtered through Whatman paper no.42 and the resultant filtrate was concentrated under reduced pressure and finally vacuum dried.

High Performance Thin Layer Chromatography [11]
HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively.

High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition it is a reliable method for the quantitation of nanograms level of samples.Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials.

Basic steps involved in HPTLC
Extracts used: Methanol extract of *Digera muricata*.
Application mode: CAMAG Linomet IV.
Development mode: CAMAG Twin Trough chamber.

Sample application
The samples were dissolved in same solvent and 10 µl quantity of sample was applied on the HPTLC silica merk 60F 254 graded plate sized 6cm x 10 cm as narrow bands using CAMAG Linomat 5 injector.84

Chromatogram Development
It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

Plates were scanned under UV at 365nm. The data's obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in the extract and Rf values were tabulated.

Fractionation of Methanolic extract
The powder was extracted with methanol in a Soxhlet apparatus for 6 hours. The extract was concentrated under reduced pressure by a rotary vacuum evaporator. This extract was then fractionated by solvent-solvent extraction into three flavonoid rich fractions namely chloroform, ethylacetate and residual water.

DPPH Assay
About 0.5 ml of ethanol solution containing methanolic extract obtained from the extract and fractions of methanol of the plant (25-250 µg/mL) was added to 1.5 mL of freshly prepared ethanolic DPPH solution (0.05 mM). The optical density change at 517 nm was measured 30 min later by a spectrophotometer. A blank was used to remove the influence of the color of the sample. An ethanolic solution of DPPH was used as negative control. [12, 13]

Ascorbic acid was used as reference drug, at the same concentrations (25-250 µg/mL) as was used for the sample. Results were expressed as mean inhibiting concentration. The parameter IC_{50} is defined as the concentration (µg/mL) of substrate that causes 50% loss of DPPH activity (color) and it was calculated by using the following equation:

IC_{50} **(%) = 100 × (A_o - A_s)/A_o,**

where A_o and A_s are the values for the absorbance of the negative control and the absorbance of the sample, respectively. Tests were carried out in triplicate. [14, 15]

RESULTS AND DISCUSSION

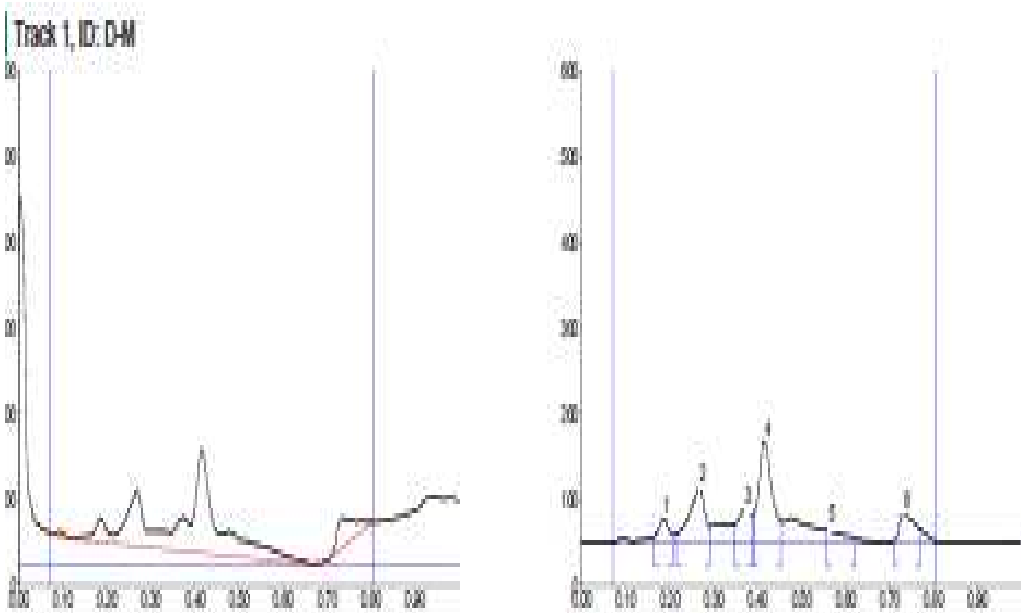

Fig.1 HPTLC chromatograms of methanolic extract of *Digera muricata* showing different peaks of phytoconstituents

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.17	6.9	0.19	28.6	9.39	0.21	11.6	628.2	7.00	unknown *
2	0.22	9.9	0.27	65.1	21.34	0.29	18.2	2123.4	23.67	unknown *
3	0.35	20.6	0.37	39.0	12.78	0.39	31.7	1122.9	12.52	unknown *
4	0.39	31.8	0.42	120.2	39.41	0.46	24.4	3407.2	37.99	unknown *
5	0.56	14.6	0.56	16.5	5.41	0.62	3.7	563.8	6.29	unknown *
6	0.71	0.8	0.73	35.6	11.66	0.77	15.1	1123.9	12.53	unknown *

Table 1. HPTLC profile of the methanolic extract of *Digera muricata*

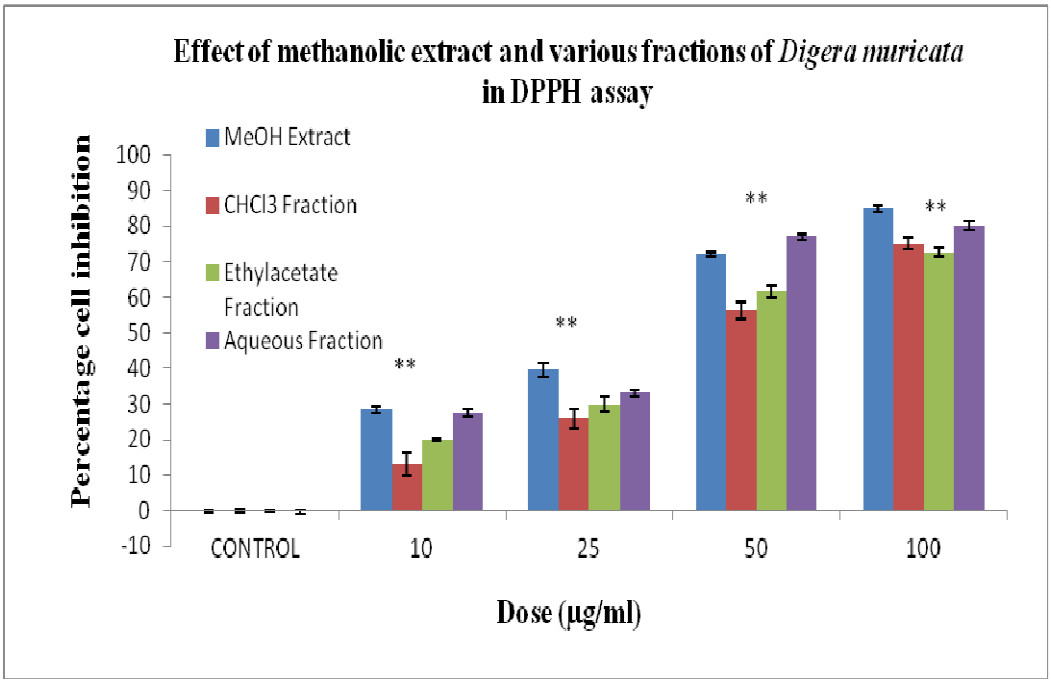

Fig.2. DPPH Assay of methanolic extract and its fractions of *Digera muricata*

Determination of IC_{50}

Table 2. Determination of IC_{50}

Digera muricata	**IC_{50} (µg/ml)**
Methanol Extract	30.24
Chloroform Fraction	43.73
Ethylacetate Fraction	38.90
Aqueous Fraction	33.90
Ascorbic acid	12.41

From the above data, it was found that the IC_{50} values of the methanolic extract was lowest followed by aqueous fraction while it was almost double when compared to the Ascorbic acid which indicates towards the importance of the studies on isolation from the most active extract.

H.P.T.L.C analysis

Methanolic extract of whole plant of *Digera muricata* showed that there are six polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.17 to 0.70 in which highest concentration of the phytoconstituents was found to be 39.41% and its corresponding R_f value was found to be 0.39 respectively and was recorded in Table.1.

Antioxidant activity of Crude extract and fractions of *Digera muricata*

Crude extract of *Digera muricata* showed inhibition at all concentrations in a dose dependent manner with a nominal amount of 28.5 percentage inhibition at a concentration of 25µg/ml and a marked 85 percentage inhibition at a concentration of 250µg/ml. The Chloroform fraction of *Digera muricata* showed inhibition at all concentrations in a dose dependent manner with a nominal amount of 13.1 percentage inhibition at a concentration of 25µg/ml and a marked 75.21 percentage inhibition at a concentration of 250µg/ml.

The Ethylacetate fraction of *Digera muricata* showed inhibition at all concentrations in a dose dependent manner with a nominal amount of 20 percentage inhibition at a concentration of 25µg/ml and a marked 72.7 percentage inhibition at a concentration of 250µg/ml. The Aqueous fraction of *Digera muricata* showed inhibition at all concentrations in a dose dependent manner with a nominal amount of 27.4 percentage inhibition at a concentration

of 25µg/ml and a marked 80 percentage inhibition at a concentration of 250µg/ml. A marked decrease in percentage inhibition was found at higher concentrations (between 100 and 250µg/ml)

CONCLUSION

The present H.P.T.L.C fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. It is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. H.P.T.L.C analysis can be used further in authentication and characterization of the medicinally important plant. The developed H.P.T.L.C fingerprints will help the manufacturer for quality control and standardization of herbal formulations.

Since, the antioxidant activity can be attributed to the significant presence of phytoconstituents like flavonoids, phenolics as well as alkaloids. Their quantitative evaluation shows the significant amount of these constituents in the plant which has been previously reported. The overall results indicate the promising baseline information for the potential uses of the methanol extract as an anti-oxidant agent.

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