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Determination of infochemicals, phytochemical screening and evaluation of antioxidant potential of *Digera muricata*

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ABSTRACT

In this work, we reported the results of the phytochemical studies and the assessment of the potential antioxidant activity with DPPH that we realized on the whole plant of Digera muricata.The plant treatment augments the antioxidants defense mechanism against carbon tetrachloride induced toxicity and provides evidence that it may have a therapeutic role in free radical mediated diseases setting in evidence of polyphenols, flavonoids, tannins, coumarins, sterols, terpenoids,alkaloids and saponins. The studied aqueous fraction of the methanolic extract showed an antioxidant/antiradical activity opposite the DPPH.

Keywords: Medicinal plant; phytochemistry; DPPH; TLC; antioxidant activity

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INTRODUCTION

An antioxidant is a molecule that decreases or inhibits the oxidation of other substances. As can define it as being a trapper of free radicals (FR), that means all composed susceptible to capture a free electron [1]. The FR play an important role in stress oxidizing (SO). The SO appears when the antioxidant/pro-oxidizing balance is broken; it follows an overproduction of RL then it is recognized to be responsible for numerous pathologies: the Parkinsonism, Alzheimer's illness,cardiovascular illnesses, cancers, mongolism, atherosclerosis, arthritis, diabetes, asthma, neurodegeneration, rheumatisms, precocious ageing … [2,3].The interest carried these last decades to the antioxidant molecules, don't stop growing. Indeed, many works among others [4], return the antioxidant activity of the natural substances more and more (NS) of plant origin. The NS are studied to find new structures models to the ends of the conception of a new generation of medicines.

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 [5].Boiled root infusion given to mother after child birth for lactation purpose [6].

Digera muricata is used in renal disorders in folk medicine. Generation of reactive radicals has been implicated in carbon tetrachloride-induced nephrotoxicity, which are involved in lipid peroxidation, accumulation of dysfunctional proteins, leading to injuries in kidneys. [7]

D. muricata treatment augments the antioxidants defense mechanism against carbon tetrachloride induced toxicity and provides evidence that it may have a therapeutic role in free radical mediated diseases [8].

Hence, *Digera muricata* (L.)Mart. is used in both folk and traditional system of medicine.

MATERIALS AND METHODS

T.L.C. with different visualizing agents.
T.L.C of various extracts of *Digera muricata*. has been performed by U.V. (366nm), Aluminium chloride and Anisaldehyde reagent.

With regard to the flavonoids, characterization has been done with a specific reagent, $AlCl_3$. Several different fluorescence stains were observed on the TLC, after revelation then visualization under UV 366 nm. For every specific spot of color with Rf, an assignment was made with a type of compound, using method described by Markham and used by Mohammedi.(9,10)

Determination of total phenolic contents
Total soluble phenolics in the different extractives of *D.muricata* were determined with Folin Ciocalteu reagent using gallic acid as a standard [13]. An aliquot (1 ml containing 10 mg) of extract in a 25 ml volumetric flask was diluted with 9 ml of distilled water. About 1 ml of Folin Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 5 min, 3 ml of sodium carbonate was added and made up to the mark. A reagent blank using distilled water was prepared. The mixture was allowed to stand in dark for 1 h with intermittent shaking and absorbance was measured at 760 nm. All determinations were performed in triplicate. The phenolic content was calculated from the calibration curve prepared by repeating the operation using 1ml of gallic acid solutions at concentrations (25, 50, 100, 200, 300 and 500µg/ml) in distilled water

Determination of total flavonoids
Total flavonoid content was estimated by aluminum chloride colorimetric method [14]. An aliquot (1 ml containing 10mg/ml) of the extract was added in 10 ml volumetric flask containing 4 ml distilled water and mixed with 0.3 ml of 5% sodium nitrite. After 5 min. 0.3 ml of 10% aluminum chloride was added. At 6^{th} minute 2 ml of 1M-NaOH was added and made up the volume to 10 ml with distilled water and the content of the flask was mixed thoroughly. A reagent blank using distilled water was prepared and the absorbance of the reaction mixture was measured against blank mixture at 510 nm. The calibration curve was prepared by measuring the absorbance of rutin solutions at concentrations (25, 50, 100, 200, 300 and 500 µg/ml) in distilled water.

Weight (Mean values in g/g ± SD) of Phytoconstituents.
Standard Pharmacological procedures were used to screen for the infochemicals (qualitatively and quantitatively). These phytochemical tests were carried out using aqueous specimens.

Quantitatively, alkaloid was determined using the procedure put forward by Harborne (15) as described by Edeoga et al.(16).Briefly, five grammes (5 g) of the powdered sample were weighed into 250 ml beaker. 100 ml of 10% acetic acid in ethanol was then added. The mixture was covered and allowed to stand for 4 h. This was then filtered and the extract concentrated on a water bath to ¼ of the original volume. Thereafter, concentrated ammonium hydroxide added drop wise until precipitation was completed. The solution was then allowed to settle and the precipitate collected, washed with diluted ammonium hydroxide and filtered. The residue that was dried and weighed was alkaloid.

Phenols were quantified according to the methods described by Edeoga et al(16). Briefly, one gramme (1 g) of plant sample was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic components for 15 min.

Then, 5 ml of the extract pipette into a 50 ml flask, 10 ml of distilled water, 2 ml ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were added. The sample was then made up to mark and left to react for 30 min for color development. This mixture was then measured at 505 nm using a spectrophotometer.

Flavonoids were determined by the methods developed by Boham and Kocipaiabyazan (17). Briefly, 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered using Whatman No. 42 (125 mm) filter paper. The filtrate was later transferred into crucible and evaporated to dryness over a water bath and weighed to a constant weight. The weight was flavonoids.

Saponins were determined according to the method described by Obadoni and Ochuko(18). According to this method, 10 g of the powdered sample for each plant species was transferred into a conical flask, and 50 ml of 20% aqueous ethanol was added. This was heated over a hot water bath for 4 h while stirring continuously at 55°C. Thereafter, the mixture was filtered and the residue re-extracted with another 100 ml of 20% ethanol.

The combined extracts were reduced to 40 ml over water bath at about 90°C. Then, the concentrate was transferred into a 250 ml separatory funnel. 10 ml Diethyl ether was added to the funnel and the mixture shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

In addition, 30 ml of n–butanol was added. The combined n butanol extract was washed twice with 5 ml of 5% aqueous sodium chloride. The remaining solution was then heated in a water bath. After evaporation, the samples were dried in a hot air oven at 70°C to a constant weight. The weight of samples recorded was the saponin.

The radical scavenging activity was determined by the 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) method [19]. The DPPH molecule is a stable-free radical by virtue of the delocalization of the spare electron over the molecule; this delocalization produces a deep violet color, characterized by an absorption band in ethanol or methanol solution centered at about 517 nm.

When a solution of DPPH is mixed with that of a substance, that can donate a hydrogen atom, this gives rise to the reduced form (diphenylpicrylhydrazine), with the loss of the violet color. An aliquot (0.5 mL) of ethanol solution containing aqueous fraction of methanolic extract obtained from whole plant of *Digera muricata* (25–250 µg/mL) was added to 1.5 mL of daily prepared ethanol 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. Ascorbic acid and butylated hydroxytoluene (BHT) were used as reference drugs, at the same concentrations (25–250 µg/mL) as was used for the sample. Results were expressed as mean inhibiting concentration (IC_{50}). IC50 parameter 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:

$$IC_{50}\ (\%) = 100 \times (A_0 - As)/A0,$$

where A_0 and As are the values for the absorbance of the negative control and the absorbance of the sample, respectively.

RESULTS AND DISCUSSION

TLC of *DIGERA MURICATA*

Extract	Solvent system	U.V.(nm)	Aluminium Chloride	Anisaldehyde/ H2so4	Conclusion
Methanol	Chloroform: Methanol: diethylamine (6:4:1)	0.93(red), 0.78(blue), 0.37(yellow)	0.78(yellow), 0.72(yellow) 0.65(yellow)	1.2,1.0, 0.66,0.60	Phenolics, flavones, flavonols
Chloroform	Toluene: Ethylacetate (93:7)	0.90(red), 0.72(blue), 0.34,0.74(blue) 0.60.	0.91(yellow), 0.75(yellow), 0.72(orange)	0.33,0.66, 1.16,1.5,1.8	Flavonols, Flavonones, Isoflavones
Petroleum ether	Petroleum ether: Benzene(6:4)	0.90(red), 0.84(green)	0.60(orange), 0.35(yellow)	1.0,0.8, 0.66,0.33	Phenolics, flavones.

Total Flavonoid content in *Digera* was found to be-1.56±0.21 and total Phenolic content in *Digera* was found to be-2.89±0.33.

Weight (Mean values in g/g ± SD) of Phytoconstituents. (Edeoga et al. ,2005).

Plant	Alkaloid	Flavonoid	Saponins	Phenols
Digera	0.08 ± 0.04	0.16± 0.01	0.12± 0.01	0.165± 0.01
muricata				

The Aqueous fraction of methanolic extract showed a high antioxidant activity, with an IC_{50} value of 50µg/mL. Ascorbic acid and butylated hydroxytoluene (BHT) produced IC50 values of 2.50µg/mLand7.58µg/mL, respectively. Based on previous data, it is possible that the powerful antioxidant activity of polar extracts is due to the presence of substances with free hydroxyls [21]. In this context, flavonoids possess an ideal structure for the scavenging of free radicals, since they present a number of hydroxyls acting as hydrogen-donators which makes them important antioxidant agents [22].

The key role of phenolic compounds as free radical scavengers is emphasized in two important reports [23,24]. Antioxidative properties of essential oils and various extracts from many plants are of great interest in both academia and the food industry, since their possible use as natural additives has emerged from a growing trend to replace synthetic antioxidants by natural ones. Regarding this trend, the study of medicinal plant species has became of great importance, to find and test their bioactive compounds. The results indicate that Aqueous fraction of methanolic extract obtained from whole plant of *Digera muricata* showed the capacity to donate hydrogen; therefore they present DPPH scavenging activity. This activity might be due to the presence of phenolic and flavonic constituents detected in the samples.

REFERENCES

[1] Caï Y. Z., Mei S., Jie X., Luo Q. and Corke H., *Life Sci*,**2006**,78(25),2872-2888.
[2] Jones D. P, *Antioxid Redox Signal* **2006**,8,1865-1879.
[3] Hermann D., *News perspectives*,**1992**,5,461-466.
[4] Mates J. M. and Sanchez-Jimenez F. M. (**2000**), *Int J Biochem Cell Biol* 32: 157-170.
[5] Parrotta J A, Healing Plants of Peninsular India. CABI Publishing CAB International, NewYork, USA,**2001**,56.
[6] Kirtikar and Basu B.D.,Indian medicinal plants, M/S Bishen Singh, Mahendra Pal Singh, Dehradun, India,**1975**,3,2055.
[7] Khan M.R, Rizvi W, Khan RA, *J Ethnopharmacol*,**2009**,122(1),91-9.
[8] Khan M.R, Rizvi W., Khan RA, *Food and ChemicalToxic*ology,**2009**,47(6),1393-1399.
[9] Markham K.R.,Techniques of flavonoid indentification. Biological techniques Series.Ed Treherne J. E. et Rubery P. H. Academic press,**1982**,113.
[10] Mohammedi Zohra Thèse de magistère, Université Abou Bakr Belkaïd Tlemcen(Algérie),**2006**,155.
[11] Taga MS, Miller EE, Pratt DE.. *Journal of the American Oil Chemists' Society*.**1984**,61**,**928–931.
[12] Chang C, Yang M, Wen H, Chern J. *J. Food Drug Anal.* . **2002**,10**,**178-182.
[13] Harborne J.B.Phytochemical Methods, London. Chapman and Hall, Ltd. **1973**,49-188.
[14] Edeoga HO, Okwu DE, Mbaebie BO *Afr. J. Biotechnol*. ,**2005**, 4 :(7),685-688.
[15] Boham BA, Kocipai-Abyazan R *Pac. Sci*. **1994**,48,458-463.
[16] Obdoni BO, Ochuko PO. *Glob. J. Pure Appl. Sci.*,**2001**, 8,203-208.
[17] Sreejayan, N.; Rao, M.N.A. *Drug Res*. **1997**, *46*,169-171.
[18] Mensor, L.L.; Menezes, F.S.; Leitão, G.G.; Reis, A.S.; Santos, T.C.; Coube, C.S. *Phytother. Res*. **2001**, *15*, 27-30.
[19] Cao, G.; Sofic, E.; Prior, R.L. *Free Rad. Biol. Med*. **1997**, *22*, 749-760.
[20] Madsen, H.L.; Nielsen, B.R.; Bertelsen, G.; Skibsted, L.H. *Food Chem.* **1996**, *57*, 331-337.
[21] Moller, J.K.S.; Madsen, H.L.; Altonen, T.; Skibsted, L.H. *Food Chem.* **1999**, *64*, 215-219.
[22] Bhavna Marya, Keyur H. Dattani, *Der Pharma Chemica*, **2011**, 3(3): 282-287