VOLUME 9 ISSUE 1 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 1, 178-183 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Phytochemical Profiling and Antioxidant Activity of Red Spinach (*Amaranthus dubius*)

Mullai Nila K., Babu M., Ashok K. and Padmapriya V.*

Department of Microbiology and Biotechnology, Faculty of Arts and Science, Bharath Institute of Higher Education and Research (BIHER), Chennai, Tamil Nadu, India

**Corresponding Author*

Received: 4th December, 2022; **Accepted:** 10th January, 2023; **Published online:** 28th January, 2023

https://doi.org/10.33745/ijzi.2023.v09i01.020

Abstract: Plants have long been a significant source of phytomedicines for human health, so it is important to look into the different phytoconstituents that they contain. The existence of secondary metabolites has a number of pharmacological and biochemical effects, according to the phytochemical study of *Amaanthus dubius*. Preliminary analyses of *Amaranthus dubius* with ethanol, methanol, acetone, ethyl acetate, chloroform, and hexane were conducted to identify the following phytocho-chemical extracts: alkaloids, flavonoids, phenols, tannins, saponins, cardiovascular glycosides, steroids, proteins, and carbohydrates. The results of this study indicated that *Amaranthus dubius* methanol extracts with the highest amount of secondary compounds are essential for human health. The methanolic extract of *Amaranthus dubius* had reducing activity, but it was lower than L-ascorbic acid, a reference antioxidant, and the extractives increased the reducing activity as the extract concentration increased. Higher absorbance means further reducing strength. The present study revealed that methanolic extract of *Amaranthus dubius* had significant free radical scavenging potential.

Keywords: Phytochemical analyses, DPPH assay, L-ascorbic acid, Extracts, Alkaloids, Flavonoids, Phenols, Tannins

Citation: Mullai Nila K., Babu M., Ashok K. and Padmapriya V.: Phytochemical profiling and antioxidant activity of red spinach (*Amaranthus dubius*). Intern. J. Zool. Invest. 9(1): 178-183, 2023.

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Introduction

Raw plant extracts have been used to cure a variety of diseases (Chang *et al*., 2020). Extracts from various plants have recently been used to supplement mainstream medicine by using non-traditional diagnostic and medicinal methods in the treatment of a variety of ailments (Behera, 1975, 1982; Adedeji and Fanimokun, 1984). Immune control, antiplatelet function, antioxidant activity, and inhibition of the inflammation-causing leukotriene B4 are some of the pathways involved (Mellem *et al*., 2012; Díaz *et al*., 2013). The growing debate about the efficacy and side effects of prescription drugs versus natural drugs in the treatment of chronic diseases is noteworthy

(Nyaura *et al*., 2014).

The global acceptance of plant products as suitable substitutes has necessitated large-scale clinical trials on the evaluation of natural substances in plants (Wahua and Nwikiri, 2021). In developing nations, the use of herbal medicines for chronic diseases is promoted due to concerns over the side effects of chemical medications, and therapies based on natural medicines tend to be a more gentle way of treating those diseases (Harshiny *et al*., 2015; Molina *et al*., 2015). Information on the conventional use of plants as medicine has played a crucial role in the development of novel plant goods (Sigamoney *et al*., 2016). Various sections of amaranths are processed into various extracts, concoctions, and rice dishes that are served to patients as part of traditional medicine around the world (central and South America, India, and Africa), where the indigenous therapeutic method is predominant (Yong *et al*., 2017).

Extracts from *Amaranthus* species have been used in various ways by residents of the traditional Japanese Peninsula, Nepal, Thailand, and chinese Medicine to treat diseases such as diabetes, cancer, urinary failure, cardiovascular complications, gynaecological disorders, and pulmonary prions (Molina *et al*., 2018). Based on the available data on therapeutic applications of *Amaranthus* species in the pharmaceutical industry, it seems that further effort should be put into the separation and characterization of active compounds with therapeutic potential (Yong *et al.,* 2019). As a result, *Amaranthus dubius* has the potential to be a future crop for a variety of purposes and to alleviate malnutrition, especially in developing countries where the plant is underutilized (House *et al.,* 2020; Sánchez-Urdaneta *et al.,* 2020). In light of the above, we investigated secondary metabolites of *Amaranthus dubius* in different solvent extracts as well as the free radical scavenging properties of the solvent extract with strong secondary metabolite material (Ajayi *et al*., 2021).

Materials and Methods

Plant material and extract preparation:

Amaanthus dubius plants were collected locally from Avadi, Chennai, India. Dr. P. Jayaraman, PARC, Chennai identified the plants. The plants were dried to a fine powder under shade and ground. The powdered plants were extracted in ethanol, methanol, acetone, ethyl acetate, chloroform and hexane. For 24 hours, the powdered plant material was immersed in solvents in order to obtain both polar and non-polar compounds. The extracts were evaporated by a rotating evaporator under decreased heat.

Phytochemical Screening:

Various extracts of *Amaranthus dubius* were subjected to the phytochemical testing for alkaloids, flavonoids, phenols, tannins, saponins, cardiac glycosides, steroids, protéins and carbohydrates. The following basic techniques were used in different experimental protocols for the analysis.

Qualitative analysis of phytochemicals:

Phytochemical testing was carried out by the method of Babu *et al*. (2018).

Alkaloids:
Mayer's test (*Potassium mercuric iodide*):

5 g potassium iodide and 1.36 g mercuric chloride were added to 100 ml in distilled water. Few drops of reagent were applied to 1.0 ml of acidic aqueous sample solution. White or pale precipitate formation showed alkaloid involvement.

Flavonoids:

5-10 drops of diluted HCl and a little piece of Zn or Mg were applied to the tube containing 0.5 ml of alcoholic and aquatic extract from the samples then the solution was boiled for a few min. The reddish rose or dirty brown colour was developed showing the presence of flavonoids.

Phenols:
Ferric chloride test:

A few drops of 10 per cent water ferric chloride

solution were applied to a 1.0 ml alcoholic solution of samples of 2.0 ml purified water, and a blue or green colour formation indicated presence of phenols.

Tannins:
Lead acetate test:

Few drops of 1 per cent solution of lead acetate have been added in a test tube containing around 5.0 ml of extract. A precipitation with a yellow or red colour showed that tannins are present.

Saponins:

A few drops of sodium bicarbonate was applied to 5 ml sample. The blend was strongly shaken for 3 min. A sweet peak was shaped like froth which showed the presence of saponins.

Glycosides:

Samples were dissolved in 1 ml of water and sodium hydroxide was applied. A yellow colour formation suggested that glycosides are present.

Steroids:

A condensed H_2SO_4 (1.0 ml) was cautiously applied TO 2.0 ml of samples. Red chloroform colour formation suggested presence of steroid.

Proteins:
Millon's test:

Sample was mixed with 2 volumes of water by 1 portion of mercury, using two sections of HNO_3. 5-6 drops of Millon's reagent were applied. The presence of protein was shown by a precipitate that turned red on heating.

Carbohydrates:
Molish test:

Two drops of a newly formulated 20% alcoholic solution of a naphthol was added and blended in a test tube holding 2.0 ml of the plant extract. In addition to this solution, 2.0 ml concentrated sulphuric acid was applied, so that a coating under the mixture would be formed, a red purple tone was formed at the junction of the solution and excess carbohydration was added to the solution.

Antioxidant activity:

DPPH free radical scavenging ability was used to calculate the extract's antioxidant potential using an updated McCune and Johns method (2002). The DPPH assay data were analyzed using SPSS version 11.

Results and Discussion

Phytochemical Screening:

A comparative analysis was carried out in various extracts of *Amaranthus dubius*. The results were collected. Ethanol extract has shown a good outcome for Alkaloids, Flavonoids, Phenols, Tannins, Saponins, Cardiac Glycosides, Steroids, Proteins, and Carbohydrates,. Methanol extract has shown a promising finding for alkaloids, flavonoids, phenols, tannins, saponins and cardiac glycosides. Acetone extract was positive for alkaloids, flavonoids, phenols, tannins, saponins, steroids, protein and carbohydrates. The results were positive for alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and steroids in the ethyl acetate extract. Chloroform extract has shown promising results for alkaloids, flavonoids, phenols, tannins, saponins, steroids and proteins,. Extraction of hexane was positive for alkaloids, flavonoids, phenols, tannins, saponins and steroids (Table 1).

The secondary metabolites have a variety of pharmacological and biochemical consequences. In order to detect phytocho-chemicals including alkaloids, flavonoids, phenols, tannins, saponins, cardiovascular glycosides, steroids, proteins, and carbohydrates, preliminary tests of *Amaranthus dubius* with ethanol, methanol, acetone, ethyl acetate, chloroform and hexane were undertaken. The finding of this analysis showed that the *Amaranthus dubius* methanol extracts contained the largest volume of secondary compounds (Table 1) which play a significant role in human health. The findings justify the use of this plant in folk medicine for the treatment of different infectious diseases. More secondary metabolites were found in methanol extracts from selected medicinal plants, which make it available for

Table 1: Qualitative analysis of phyto-constituents in different extracts of *Amaranthus dubius*

Phytoconstituents	Different Solvents					
	Ethanol	Methanol	Acetone	Ethyl acetate	Chloroform	Hexane
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phenols	+	+	+	-	+	+
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Glycosides	+	+	-	+	-	-
Steroids	+	+	+	+	+	+
Proteins	+	+	+	-	+	-
Carbohydrates	+	+	+	-	-	-

Table 2: Antioxidant activity of methanol extract of *Amaranthus dubius*

S.NO.	Concentration (mg/ml)	Methanol extract	L-ascorbic acid
1	10	16.25	19.79
2	25	27.47	34.87
3	50	46.08	54.26
4	125	62.25	73.92
5	250	81.66	93.44

further antioxidant assay to identify its free radical scavenging potential.

Antioxidant activity:

Table 2 and Figure 1 show the per cent inhibition of DPPH by methanol extract in comparison to L-ascorbic acid (standard). The findings showed a dose-dependent inhibition of DPPH activity in both the methanol extract and the standard; at 250 mg/ml, the percentage of free radical scavenging was higher in the methanol extract. At 250 mg/ml, however, L-ascorbic acid displayed more activity than methanol extract, which may be attributable to the antioxidative existence of the norm (L-ascorbic acid). These findings showed that the methanolic extract of *Amaranthus dubius* had a high potential for free radical scavenging.

Amaranthus dubius is a plant that has been used to cure a number of ailments. Amaranth contains much more lysine than other grains, an essential amino acid that the body cannot produce. Lysine aids in the conversion of fatty acids to energy, the absorption of calcium, and also the will maintenance of hair. To add to the above, eating it help with hair loss. Fiber in amaranth seeds and oil (found in the seed) helps to lower cholesterol and reduces the chance of constipation. It is also high in phytosterols, which are thought to help lower cholesterol. Amaranth is usually offered to people who are healing from sickness or fasting. It is the combination of amino acids that makes digestion so easy. The methanolic extract of *Amaranthus dubius* had heavy DPPH radical scavenging operations, according to our findings. Furthermore, the high content of polyphenolic compounds in methanol extract can contribute to its potent antioxidative activity. As a result, the methanolic extract from the *Amaranthus dubius* plant could be used in the pharmaceutical industry and as a health-care food supplement. The findings revealed that the extractives have proton-donating properties, indicating that they may be used as free radical inhibitors or scavengers, as well as key antioxidants. The work sufficiently demonstrated that there is a connection between the polyphenolic content of extractives and their

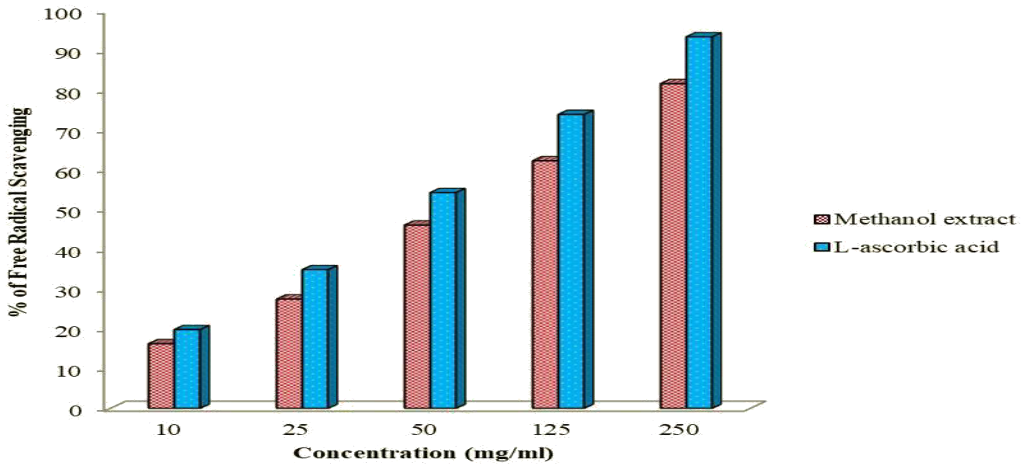

Fig. 1: Antioxidant activity of methanol extract of *Amaranthus dubius.*

antioxidant properties. As a result, this may be used as a health supplement (Mellem *et al*., 2009; Rodríguez *et al*., 2011; Vargas-Ortiz *et al.,* 2021).

Conclusion

In terms of DPPH radical scavenging activity, the methanol extract of *Amaranthus dubius* showed superior and potent antioxidant properties. *Amaranthus dubius* methanol extract has a high phenolic content and antioxidant capacity, which may help to maintain antioxidant status and protect against free radical disruption. *Amaranthus dubius* can be used as an antioxidant in future studies of experimental animal models against free radicals produced in response to oxidative stress.

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