STUDY ON ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *AMARANTHUS VIRIDIS* L. (HIN NU NWE)

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ABSTRACT

This study aims to investigate the screening of antioxidant and antimicrobial activities of the leaves of the Myanmar indigenous medicinal plant, *Amaranthus viridis* L., which belongs to the family Amaranthaceae. The preliminary phytochemical constituents were investigated by the test tube method, observing the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, tannins, and steroids. The relative abundance of elements present in the leaves of *A. viridis* was determined by an EDXRF spectrometer, observing that K (3.718 %), Ca (1.497 %), Fe (0.043 %), and Zn (0.006 %), respectively. Evaluation of the antioxidant activity of the aqueous and ethanol extracts was conducted by using the DPPH free radical scavenging assay. In *vitro* screening of the antimicrobial activity of the different crude extracts such as pet-ether, ethyl acetate, ethanol, and watery extracts was carried out using six microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* by the agar well diffusion method.

Keywords: *Amaranthus viridis* L., phytochemical constituents, elemental analysis, antioxidant activity, antimicrobial activity,

INTRODUCTION

Plants have been investigated for their medicinal properties throughout the world, mainly due to their potent pharmacological activities, low toxicity, and economic viability. Green leafy vegetable consumption has been associated with a decreased risk of persistent metabolic diseases. In this study, one of the green leafy vegetables, *Amaranthus viridis* L. has been chosen for the study of secondary metabolites present in it and its antioxidant and antimicrobial activities. *A. viridis,* which belongs to the family, Amaranthaceae, is an annual herb which grows from 6 to 100 cm high. It propagates by seeds and flowers all year in subtropical and tropical climates. There are several different spinach varieties. Most are similar in green leaves however; the leaves can be different in shape and texture. *A. viridis* can be eaten as a boiled green or as a vegetable in many parts of the world and contain up to 38 % protein by dry weight. The leaves and seeds contain lysine, an essential amino acid (Adam and Annie, 2012). The leaves are rich in soluble and insoluble fiber, vitamins A, B and K and also rich in iron, potassium, and calcium. This plant can be used in the treatment of many diseases including diuretic, analgesic, antipyretic, vermifuge, antiulcer, antidiabetic, anti-cholesterolemic, laxative, asthma, and veneral diseases. Furthermore, the plant possesses antiproliferative and antifungal properties as well as the production of ribosome-inactivating protein, β-carotene and antiviral activities (Sharma *et al*, 2012).

In Myanmar, the whole parts of the plant *A. viridis* are eaten as a green leafy vegetable and also used in traditional medicine to reduce inflammation in the body and provide an extra boost of nutrition.

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Botanical Aspects of *Amaranthus viridis* L.

Botanical name - *Amaranthus viridis* L.
Family - Amaranthaceae
Kingdom - Plantae
Genus - *Amaranthus*
Species - *viridis*
Synonyms - *Amaranthus gracilis* Desf.
Common name - slender amaranthus, green amaranthus
Myanmar name - Hin-nu-nwe

Plant part used - Leaves

(a) (b)

Figure 1 (a) Plant and (b) leave of Spinach

MATERIALS AND METHODS

Collection and Preparation of Plant Sample

The plant of *A. viridis* was collected from Thu-wa-na Township, Yangon Region. The identification of the botanical name of *A. viridis* was confirmed by an authorized taxonomist at the Botany Department, University of Yangon, Myanmar. The leaves of the collected sample were cleaned by washing thoroughly with water, air-drying at room temperature, and then cut into small pieces. The dried sample was ground into powder by a grinding machine. This powder sample was stored in an airtight container for further investigation.

Preliminary Phytochemical Investigation

The presence or absence of phytoconstituents such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, and tannins were investigated in the leaves of *A. viridis* using the methods described in M-Tin Wa, 1970, and Harbone, 1984.

Elemental Analysis

The relative abundance of elements present in the leaves of *A. viridis* was determined by an EDXRF spectrometer.

Determination of Antioxidant Activity

In the present study, the free radical scavenging effect of the ethanol and watery crude extracts of *A. viridis* was examined by the DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical scavenging assay as mentioned in Marinova and Batchvarov, 2011.

(i) Preparation of 0.002 % (w/v) DPPH solution

In order to obtain a 0.002 % DPPH solution, 2 mg of DPPH powder was dissolved in 100 mL of ethanol in the beaker, which was wrapped with aluminium foil.

(ii) Preparation of test sample solutions and standard solutions

20 mg of crude extract each were mixed with 100 mL of ethanol to obtain a concentration of 200 μg/mL in the stock solution. This stock solution was two-fold serially diluted with ethanol to obtain the concentrations of 200, 100, 50, 25, 12.5, and 6.25 μg/mL.

The standard BHT (butylated hydroxyl toluene) solutions with concentrations of 200, 100, 50, 25, 12, 5, and 6.25 μg/mL were prepared as the procedure for the test sample solutions.

(iii) Procedure

Firstly, the control solutions (1.5 mL of 0.002 % DPPH solution mixed with 1.5 mL of ethanol), the sample solutions (1.5 mL of 0.002 % DPPH solution mixed with 1.5 mL of test sample solution), and the blank solutions (1.5 mL of test sample solution mixed with 1.5 mL of ethanol) for the different concentrations were prepared in the brown bottles, and then these solutions were incubated at room temperature for 15 minutes and shaken on a shaker for 30 minutes After incubation, the absorbance of different concentrations (200, 100, 50, 25, 12.5, 6.2 μg/mL) of the tested sample was measured at 517 nm. Absorbance measurements were done in triplicate for each solution and they were used to calculate the percentage of radical scavenging activity (% RSA) using the following equation:

$$\% \text{ RSA} = [A_{DPPH} - (A_{Sample} - A_{blank})/ A_{DPPH}] \times 100$$

Where,

% RSA = % radical scavenging activity of test sample
A_{DPPH} = absorbance of DPPH in EtOH solution
A_{Sample} = absorbance of sample + DPPH solution
A_{Blank} = absorbance of sample + EtOH solution

Screening of Antimicrobial Activity

The agar well diffusion method has been chosen for examining the antimicrobial activity of the different crude extracts such as pet-ether, ethyl acetate, ethanol, and watery extracts, using six strains; *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans,* and *Escherichia coli,* which were conducted at the Myanmar Pharmaceutical and Industrial Research Department (MPIRD), Yangon. Fluconazole and tetracycline were taken as positive controls for antibacterial tests.

The organism to be tested (a few colonies) was introduced into the triple sugar iron agar and incubated at 37 ºC for 24 h in an incubator as the procedure described in Mar Mar Nyein *et al*., 1991. After incubation, a few drops of incubated organisms were introduced into the prepared trypticase soy broth and then incubated again for 3 hours at 37 ºC to obtain a bacterial suspension of moderate cloudiness, which contained approximately 10^5 to 10^7 organisms per mL.

A mixture of meat extract (1 g), peptone (1 g), NaCl (0.5 g) and agar powder (1.5 g) was placed in a sterilized 250 mL conical flask and 100 ml of sterile distilled water was added to it to obtain a nutrient agar medium and then heated to dissolve all the contents (Cruickshank, 1960). Using a 0.1M NaOH solution, the pH of the resulting solution was adjusted to 7.2 and again sterilized in an autoclave at 121°C for 15 min. The sterilized medium (20–25 mL) was put into a conical flask, plugged with cotton wool, and autoclaved at 121 °C for 15 min. Then,

the flasks were cooled down to 60 °C and poured into sterilized Petri dishes. The dishes were filled with 0.1 mL of spore suspension and set aside for 30 min. The plate agar well with a diameter of 10 mm was made with the sterilized cork borer. Finally, about 0.1 mL of each extracted sample was added to the prepared agar well and incubated at 37 ℃ for 24 h. If a clear zone appears around the agar well, the crude extract will indicate the efficiency of inhibiting tested microorganisms.

RESULTS AND DISCUSSION

Qualitative Determination of Phytochemical Constituents

After preparation of the dried powder sample, phytochemicals present in the selected sample, *Amaranthus viridis* L., were qualitatively determined by the reported methods. According to the results, alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, tannins, and steroids were found to be present, while α-amino acids and cyanogenic glycosides were not detected in *A. viridis*.

Semi-quantitative Analysis of Elemental Composition

The relative abundance of elements present in the leaves of *A. viridis* was determined by an EDXRF spectrometer, observing that K (3.718 %), Ca (1.497 %), Fe (0.043 %), and Zn (0.006 %), respectively.

Evaluation of Antioxidant Activity

The antioxidant activity of ethanol and watery extracts of the leaves of *A. viridis* was determined by the DPPH free radical scavenging assay. This method is an easy, rapid, and sensitive way to survey the antioxidant activity of plant extracts. The different concentrations (200, 100, 50, 25, 12.5, and 6.25 µg/mL) of the sample solution and standard BHT (butylated hydroxyl toluene) solution were prepared by serial dilution with ethanol. The antioxidant activity was expressed as 50 % inhibition concentration (IC_{50}).

According to the results shown in Table 1 and Figure 2, the IC_{50} values of ethanol extract and watery extract were found to be 40.40 µg/ml, and 124.97 µg/mL, respectively. The ethanol extract has more antioxidant activity than the watery extract but less antioxidant activity than standard BHT (IC_{50} 13.31 µg/mL). However, the selected plant, *A. viridis*, is said to possess mild antioxidant potency. Therefore, the selected plant may be used in the treatment of degenerative diseases such as cancer and aging-related problems.

Table 1 Radical Scavenging Activity (% RSA) and IC_{50} Values of Ethanol and Watery Extracts of *A. viridis* and Standard BHT

Sample	%RSA ± SD at Different Concentrations (µg/mL)						IC_{50} (µg/mL)
	6.25	12.5	25	50	100	200	
Ethanol extract	33.92 ± 0.004	36.41 ± 0.004	42.39 ± 0.001	54.74 ± 0.009	76.19 ± 0.001	91.90 ± 0.00	40.40
Watery extract	28.68 ± 0.007	29.80 ± 0.008	36.04 ± 0.002	38.53 ± 0.001	46.14 ± 0.007	61.6 ± 0.014	124.97
Standard BHT	38.94 ± 0.005	49.32 ± 0.004	59.77 ± 0.007	61.37 ± 0.003	67.30 ± 0.005	69.87 ± 0.002	13.31

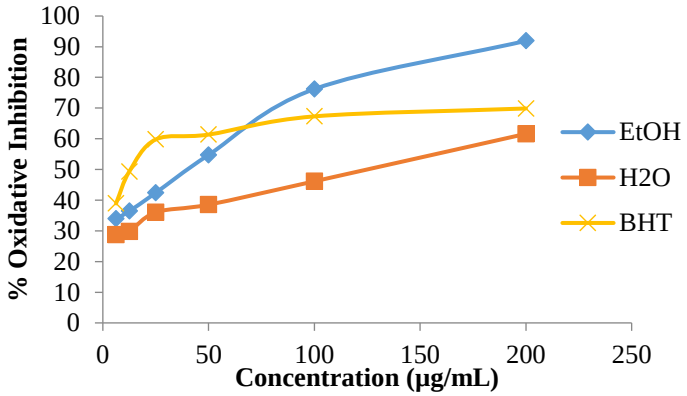

Figure 2 A plot of % RSA of ethanol and watery extracts of the leave of *A. viridis* and standard BHT on antioxidant activity

In *Vitro* Screening of Antimicrobial Activity

In this study, the antimicrobial activity of the different crude extracts of *A. viridis* was screened by the agar well diffusion method. When comparing different antimicrobial agents to a known concentration, the inhibitory zone diameter is taken as a measure of antimicrobial activity. It was discovered that the larger the zone diameter, the greater the activity of the tested organisms.

It was found that the three crude extracts, EtOAc, EtOH, and H_2O extracts, showed antimicrobial activity on all tested microorganisms except PE extract. Among them, ethanol extract exhibited more potent antimicrobial activity than the other extracts, with the inhibition zone diameter ranging from 16–21 mm. According to the experimental results shown in Table 2, the selected plant possessed moderate antimicrobial activity.

Therefore, the selected plant may be used in the treatment of diseases caused by microorganisms such as diarrhea, dysentery, food poisoning, boils, wound sepsis, respiratory tract infections, and skin infections.

Table 2 Antimicrobial Activity of the Leaves of *A. viridis* by Agar Well Diffusion Method

Microorganisms	**Inhibition Zone Diameters of Different Crude Extracts (mm)**			
	PE	**EtOAc**	**EtOH**	**H_2O**
B. Subtilis	11	14	18	12
S. aureus	11	16	19	13
P. aeruginosa	-	11	17	13
B. pumilus	11	13	16	11
C. albicans	11	12	21	12
E. coli	-	14	18	15

Agar well - 10 mm

10 mm ~ 14 mm (low activity) 20 mm above (high activity)

15 mm ~19 mm (moderate activity) (-) No activity

CONCLUSION

This study gives valuable information about the phytochemical constituents associated with the antimicrobial and antioxidant activities of the leaves of *Amaranthus viridis* L. In the screening of antioxidant activity conducted by the DPPH free radical scavenging assay, the ethanol extract (IC_{50} of 40.40 µg/mL) has more antioxidant activity than the watery extract (IC_{50} of 124.97 µg/mL) but less antioxidant activity than standard BHT (IC_{50} of 13.31 µg/mL). However, the selected plant, *A. viridis*, is said to possess mild antioxidant potency. Except for PE extract, the three crude extracts, EtOAc, EtOH, and H_2O extracts, showed antimicrobial activity on all tested microorganisms. Among them, the ethanol extract exhibits moderate antimicrobial activity on all tested microorganisms, which provides the medicinal uses of selected samples concerning the diseases caused by the microorganisms.

According to these observations, the selected medicinal plant, *A. viridis*, may be effectively used in the prevention of oxidative problems associated with age-related diseases and in the treatment of wound infections, and also for the treatment of skin diseases, diarrhea, and other diseases infected by the microorganisms tested.

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