Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2013, 3(1):69-74

ISSN : 2249-7412
CODEN (USA): AJPSKY

Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of *Amaranthus spinosus*

V. Y. A. Barku, Y. Opoku-Boahen, E. Owusu-Ansah, and E. F. Mensah

Department of Chemistry, School of Physical Sciences, University of Cape Coast, Ghana

---

ABSTRACT

Amaranthus spinosus (204.84g) roots were extracted successively with petroleum ether (80-100^{0}C), ethyl acetate and methanol by Soxhlet process. All the extracts were subjected to Total phenolic content, Total flavonoid content and antioxidant activity using DPPH assay and Ferric Reducing Antioxidant Power assay (FRAP). Ethyl acetate extract showed the highest antioxidant activity with 61.47 ± 4.8 % inhibition at a higher concentration. The three solvents; Methanol, ethyl acetate and Pet-ether extracts showed moderate activity when compared with the standard. The total phenol content was 48.01 ± 2.0, 135.22 ± 3.7 and 46.01± 1.3 mg g^{-1} in the methanol, ethyl acetate and pet-ether extracts respectively. In all, ethyl acetate extract generally, exhibited the highest values of antioxidants. The potency of radical scavenging effect of A. spinosus extract was about 2 times less than the synthetic antioxidant Ascorbic acid. The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by A. spinosus extract.

Keywords: *Amaranthus*, radical scavenging effect, phenolic compounds, antioxidant activity

---

INTRODUCTION

Oxidation is one of the most important processes, which produce free radicals in food, chemicals and even in living systems. Free radicals have an important role in the processes of food spoilage, chemical materials degradation and also contribute to more human disorders in human beings. Active oxygen and in particular, free radicals are considered to induce oxidative damage in bio molecules and to play an important role in aging, cardiovascular diseases, cancer and inflammatory diseases [1]. They are believed to cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Oxidants are now considered to be involved in a number of aspects of burned injury and tissue repairs. Oxygen free radicals contribute to further tissue damage in the events following skin injury and are known to impair healing process. Antioxidants, on the other hand, significantly prevent tissue damage that stimulates wound healing process [2]. There are available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluenes (BHT), tertiary butylated hydroquinone and gallic acid esters, but have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants.

There has been an increasing interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury since it is the belief that the antioxidant property is the main contributory factor to the therapeutic benefit of many medicinal plants [3]. As a result, many vegetables, fruits and many other plant species have already been exploited commercially either as antioxidant additives or a nutritional supplements

[4] or have been investigated in the search for novel antioxidants [5, 6]. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds [7, 8] such as flavonoids which are a group of polyphenolic compounds with known properties that include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [9]. Some evidences suggest that the biological actions of these compounds are related to their antioxidant activity [10]. Medicinal Plants with antioxidant or free radical-scavenging activity thus can play a significant role in healing of wounds [11] and in the correction of many human disorders.

Amaranthus spinosus Linn. belongs to Amaranthaceae family. It is commonly known as Prickly amaranth or Pig weed and is an annual or perennial herb, native to tropical America and found throughout India as a weed in cultivated as well as fallow lands. It is a common weed of waste places, roadsides and pathsides and near rivers in Ghana [12]. The plant has a long history of usage in traditional medicine against various ailments around the world. The root of the plant is used among the Ewes at Kpando district in Ghana to treat wound infections.

It is known that wound-healing process can be aided by the presence of antioxidants. Literature has revealed that several wound-healing plants also possess considerable antioxidant properties as evident from the results of various *in vitro* and *in vivo* assays. The current study was therefore designed to explore the potential sources of natural antioxidants from the root of *Amaranthus spinosus* plant in order to examine the significant role it plays not only in wound healing but its contribution to health in general.

MATERIALS AND METHODS

2.1. Plant material

The roots of *Amaranthus spinosus* were collected from Cape Coast in the month of May 2012 and were authenticated by the curators Mr. Agyarkwa and Mr. Otoo, Department of Environmental studies, School of Biological Sciences, UCC voucher specimen no. CCG 5165 dated 15-02-1981. The plant sample was air dried at room temperature and powdered.

2.2. Successive solvent extraction

The powdered roots of the plant material (202.84g) were extracted successively with petroleum ether (80-100^{o}C), ethyl acetate and methanol in a Soxhlet's apparatus (two days for each solvent). The solvent was removed from the extract under vacuum yielding the petroleum ether (1.43 g), ethyl acetate (2.16 g) and methanol (16.28 g) extracts.

2.3. Determination of flavonoid contents

The aluminum chloride colorimetric method was used to measure the flavonoid content of all plant extracts [13]. Extract solution (0.25ml, 1mg/ml) of each plant extract was added to 1.25 ml of distilled water. Sodium nitrite solution (0.075ml, 5%) was then added to the mixture followed by incubation for 5 minutes after which 0.15ml of 10% aluminium chloride was added. The mixture was allowed to stand for 6min at room temperature before 0.5ml of 1 M sodium hydroxide was finally added and the mixture diluted with 0.275 ml distilled water. The absorbance of the reaction mixture was measured at 510 nm with a UV/VIS spectrophotometer immediately. Quercetin was used as the standard for the calibration curve. Flavonoid contents were expressed as mg quercetin equivalent (QE)/g dry weight (D.W.).

2.4. Determination of total phenolic content

Total phenol content was estimated using Folin-Ciocalteau reagent based assay as previously described [14] with little modification. To one ml of each extract (100µg/ml) in methanol, 5ml of Folin -Ciocalteau reagent (diluted ten-fold) and 4 ml (75 g/l) of Na_2CO_3 were added. The mixture was allowed to stand at 20^{o}C for 30 min and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 ml aliquots of 20, 40, 60, 80, 100µg/ml methanolic gallic acid solutions were used as standard for calibration curve. All determinations were performed in triplicate. Total phenol value was obtained from the regression equation: $y = 0.00048x \pm 0.0055$ and expressed as mg/g gallic acid equivalent using the formula, C = cV/M; where C = total content of phenolic compounds in mg/g GAE, c = the concentration of gallic acid (mg/ml) established from the calibration curve, V = volume of extract (0.5ml) and m = the weight of pure plant methanolic extract (0.052g).

2.5. Ferric reducing antioxidant power assay

The reducing antioxidant power of plant methanolic extracts was determined by the method of Oyaizu (1986) as described by [15]. Different concentrations of plant extracts (250 – 1000 ppm) in 1 ml of distilled water were mixed

with phosphate buffer (3.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer (T 70 UV-VIS Spectrometer, PG Instruments Ltd). Increased absorbance of the reaction mixture indicates increase in reducing power. Ascorbic acid was used as standard.

2.6. Scavenging activity against 1,1-diphenyl-2-picryl hydrazyl radical (DPPH)

The crude extracts of different solvents (petroleum-ether, ethyl acetate and methanol) of the plant were screened for DPPH radical Scavenging activity. DPPH radical scavenging activity was measured according to the method of [16, 17, 18]. Extract solutions were prepared by dissolving 0.05g of dry extract in 50ml of methanol. An aliquot of 2ml of 0.004% DPPH solution in methanol and 1ml of plant extract in methanol at various concentrations (200, 400 and 800 ppm) were mixed and incubated at 25°C for 30 min. and absorbance of the test mixture was read at 517nm using a spectrophotometer (T 70 UV-VIS Spectrometer, PG Instruments Ltd.) against a DPPH control containing only 1 ml of methanol in place of the extract. The DPPH solution in methanol was prepared daily before the absorbance measurements. DPPH is a purple coloured stable free radical. When reduced it becomes the yellow colored Diphenyl picryl hydrazine. All experiments were performed thrice and the results were averaged. Ascorbic acid was used as a standard [19, 20]. Percent inhibition was calculated using the following expression % Inhibition = $(A_{blank} - A_{sample} / A_{blank}) \times 100$ Where A_{blank} and A_{sample} stand for absorption of the blank sample and absorption of tested extract solution respectively.

3.7. Statiscal Analysis

All analyses were run in triplicate and the results expressed as mean ± standard deviation (SD), was analyzed using Microsoft office excel 2007. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the significance of the difference among samples, with a significance level of 0.05.

RESULTS AND DISCUSSION

The biological properties, including cytotoxic and antioxidant properties, of plants are considered in an evaluation of the medicinal and nutritional values of medicinal plants [21, 22].

The therapeutic benefit of medicinal plants is often attributed to their antioxidant property [3]. Antioxidants play very important roles in coetaneous tissue repair as they significantly prevent tissue damage that stimulates wound healing process. The human body frequently produces reactive oxygen species (ROS) which are beneficial in small amounts. However, large amounts of these ROS are produced during increased oxidative stress encountered in the body due to either environmental hazard, or impairment in the body metabolism due to varying disease conditions including drugs or having insufficient amount of dietary antioxidants. This situation may be dangerous and has to be curbed by exogenous supply of antioxidants as a choice of therapy or preventive measure [23]. Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. The natural sources are much safer to use due to less toxicity and side effects [24]. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds.

DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. In vitro antioxidant studies of the three extracts, the extent of DPPH radical scavenging at different concentrations (200-800 ppm) of *A. spinosus* extracts was measured, with ascorbic acid as the standard. The radical scavenging effect was found to increase generally with increasing concentrations. The DPPH radical scavenging activities of extracts therefore increased gradually in a dose dependent manner.

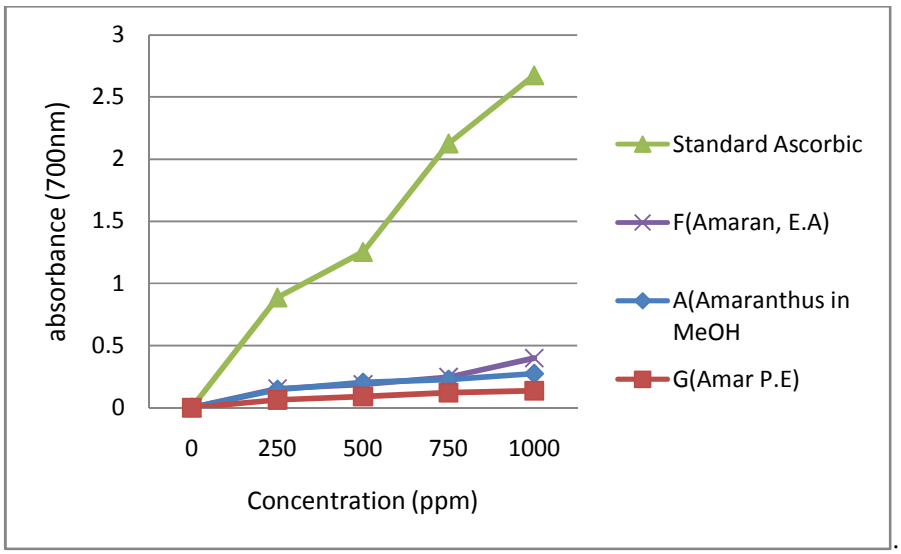
The control (standard ascorbic acid) and the plant extracts showed their maximum activity at: control (96.78 $\pm$ 1.9%), methanol (46.57 $\pm$ 1.9%), ethyl acetate (61.47 $\pm$ 4.8 %) and petroleum ether (53.05 $\pm$ 25.2 %).

DPPH radical scavenging activity assay assesses the capacity of the extract to donate hydrogen or to scavenge free radicals. The results revealed that the ethyl acetate extract of the plant exhibited the highest radical scavenging activity with 61.47 $\pm$ 4.8 % followed by the petroleum ether extract with 53.05 $\pm$ 25.2 % and methanol extract 46.57 $\pm$ 1.9% (Table 1).

Table 1: DPPH free radical scavenging activity of the extracts

Test Component	Concentration(ppm)	Inhibition (%)
Pet-ether	200	30.80 ± 1.2
	400	53.05 ± 25.2
	800	45.15 ± 17.6
Ethyl acetate	200	43.96 ± 2.6
	400	48.03 ± 3.6
	800	61.47 ± 4.8
Methanol	200	40.87 ± 2.5
	400	41.71 ± 0.5
	800	46.57 ± 1.9
Ascorbic acid	200	92.75 ± 0.23
	400	93.61 ± 9.4
	800	96.78 ± 1.9

Figure 1: Ferric reducing power of various solvent extracts of *A. spinosus* compared with ascorbic acid as standard.



The flavonoid content of the extracts in terms of quercetin equivalent (the standard curve equation: y = 0.0092x + 0.0249, r^2 = 0.985; Figure 2) were between 63.16 ± 10.7, 121.69 ± 7.0 and 204.26 ± 3.4 mg g^{-1} for methanol, ethyl acetate and pet ether extracts respectively (Table 2). Table 2 also shows the contents of total phenols that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 0.00048x ± 0.0055, r^2= 0.9873; Figure 3). The total phenol content was 48.01 ± 2.0, 135.22 ± 3.7 and 46.01± 1.3 mg g^{-1} in the methanol, ethyl acetate and pet-ether extracts respectively. In all, ethyl acetate extract generally, exhibited the higher values of antioxidants. The result clearly shows that the solvent influences the extractability of the phenolic compounds. The antioxidant activity has a positive correlation (R = 0.49) with phenolic content of all the solvent extracts of the plant. This confirms the assertion that phenolic content of plants contribute directly to their antioxidant properties. The DPPH scavenging capacity of the plant extracts may therefore be related to the phenolic compounds present. The values recorded for all extracts of the plant, even though lower than the standard antioxidant (ascorbic acid), showed that *A. spinosus* is a relatively good source of antioxidant activity. From the FRAP assay, absorbance increased with increasing concentration of plant extracts. All the samples increased their reducing ability when the concentration of extracts was increased. This signified the consistent reduction of Fe^{3+} to Fe^{2+} indicating the reduction potential of the plant. However, the ability of reducing power of all the solvent extracts of the root of *A. spinosus* was significantly lower than the synthetic antioxidant, ascorbic acid.

Table 2: Phenolic and flavonoid contents of the extracts

Extract	Phenolic Content (mg of GAE/g of extract)	Flavonoid content (mg of QE/g of extract).
Methanol	48.01±2.0	63.16 ± 10.7
Ethyl acetate	135.22 ± 3.7	121.69 ± 7.0
Pet-ether	46.01 ± 1.3	204.26 ± 3.4

Figure 2: Standard calibration curve for quantification of Flavonoid content

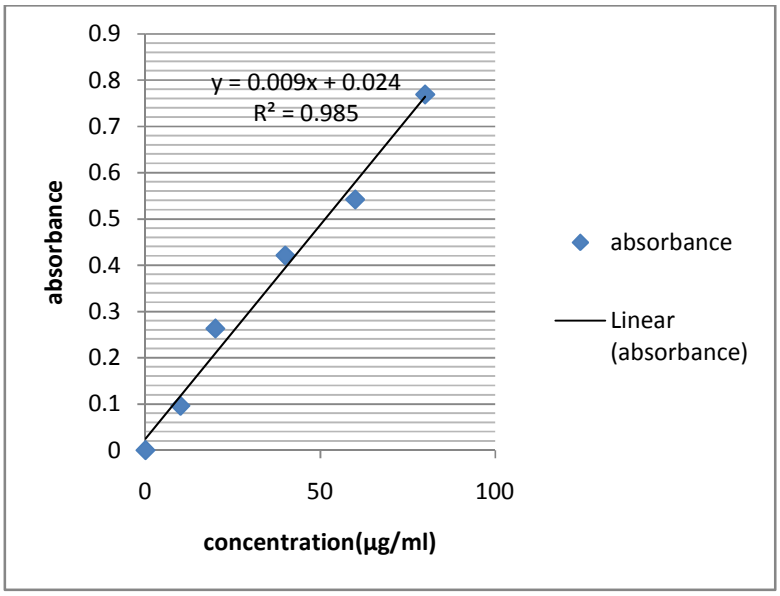
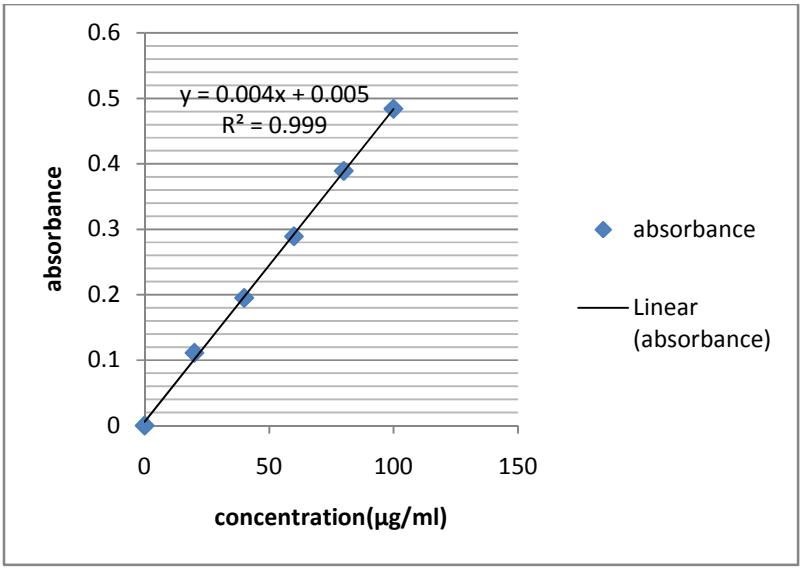


Figure 3: Standard calibration curve for the quantification of Total phenolic content



CONCLUSION

In conclusion, among all the solvent extracts of the plant analysed, ethyl acetate showed the highest yield of total phenolic content (135.22 ± 3.7 mg of GAE/g of extract) and antioxidant activity (61.47 ± 4.8 % at 800 ppm) using DPPH assay. From the correlation analysis, it had been shown that total phenolic content correlated positively with the antioxidant capacity (R = 0.49). This shows that the roots of *A. spinosus* plant may be potent sources of natural antioxidants. Hence may be used to heal wounds and prevent deleterious consequences of oxidative stress.

Acknowledgement
The financial support provided by the Management of University of Cape Coast is highly acknowledged.

REFERENCES

[1] V. K. Gupta, S. K. Sharma, *Int J. Biol Chem.* **2010,** 4, 134-140.
[2] S. D. Fitzmaurice, R. K. Sivamani, R. R. Isseroff, *A clinical guide to currently commercially available products*. **2011**, 24(3), 113-126.
[3] B. S. Nayak, P. Pereira, M. Lexley, *BMC Comp. Altern. Med*. **2006**, 6, 41-41.
[4] P. Schuler, In: Hudson BJF Food Antioxidants (Ed.), Natural antioxidants exploited commercially (Elsevier, London **1990)** 99-170.
[5] Y. Chu, *J. Sci Food Agr*. **2000**, 80, 561-566.
[6] I. I.Koleva, T. A. Van Beek, J. P. H. Linssen, A. de Groot, L. N. Evstatieva, *Phytochemi Analysis*. **2002**, 13, 8-17.
[7] J. Pokornynds, *Trends in Food Science and Technology*. **1991,** 9, 223-227**.**
[8] N. Subhangkar, P. S. Himadri, R. B. Nishith, C. Bodhisattwa, *Asian Journal of Plant Science and Research*, **2012**, 2 (3):254-262
[9] S. Shetty, S. Udupa, L. Udupa, *Evidence-Based Complementary and Alternative Medicine*. **2008**, 5(1), 95–101.
[10] S. B. Nayak, J. Kanhai, D. M. Milne, L. P. Pereira, W. H. Swanston, *Evidence-Based Complementary and Alternative Medicine*. **2011**, 6.
[11] J. V. Kamath, A. C. Rana, A. R. Chowdhury, *Phytotheraphy Research*. **2003**, 17, 970–972.
[12] O. B. Dokosi; Herbs of Ghana, Ghana Universities Press, Accra, Ghana, **1998.**
[13] Q. Nguyen, J. Eun, *Journal of Medicinal Plants Research*. **2011**, 5(13): 2798-2811.
[14] S. McDonald, P. D. Prenzler, M. Antolovich, K. Robards, *Food Chem*. **2001**, 73, 73-84.
[15] M. R. Saha, S. M. R. Hasana, R. Aktera, M. M. Hossaina, M. S. Alamb, M. A. Alam, M. E. H. Mazumderc, *J. Vet. Med*. **2008**, 6, 2, 197–202.
[16] A. Braca, N. D. Tommasi, L. D. Bari, C. Pizza, M. Politi, I. Morelli, *Journal of Natural Products.* **2001**, 64, 892-895.
[17] R. P. Rajeswara, R. E. Sambasiva, B. Yasodhara, V. S. Praneeth Dasari, R. T. Mallikarjuna, *Journal of Pharmacy Research.* **2012**,5(2),1051-1053
[18] I, D. Coutinho, R. G. Coelho, V. M. F. Kataoka, N. K. Honda, J. R. M. W. Silva, W. Vilegas, C. A. L. Cardoso, *Eclemitica Quimica*. **2008**, 33, 4, 53-60.
[19] O. James, O. P. Nnacheta, *African J. Biotech*. **2008**, 7(17), 3129-33.
[20] . Ramnik, S. Narinder, B. S. Saini, S. R. Harwinder, *Indian J. Pharmacol*. **2008**, 40(4), 147-151.
[21] M. R. Kumbhare, V. Guleha, T. Sivakumar, *Asian Pacific Journal of Tropical Disease.* **2012**, 144-150.
[22] J. B. Harborne, C. A. Williams, *Phytochemistry*. **2000**, 55(6), 481-504.
[23] M. Tosun, S. Ercisli, M. Sengul, H. Oezr, T. Polat, E. Ozturk, *Biol Res*. **2009**, 42, 175-181.
[24] B. K. Kumawat, M. Gupta, Tarachand Y. Singh, *Asian J. of Plant Science and Research*, **2012**, 2 (3):323-329