

Original Article

## Gallic acid and flavonoids of *Amaranthus retroflexus*

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### ARTICLE INFO

- Received 26 Jul 2018
- Revised 25 Aug 2018
- Accepted 30 Aug 2018

### Keywords:

- *Amaranthus retroflexus*
- Gallic acid
- Total flavonoids
- Polyphenols

### ABSTRACT

*Amaranthus retroflexus* is a medicinal plant. Our aim was to investigate its *in-vitro* antioxidant properties. Therefore, 70% ethanolic extract of *Amaranthus retroflexus* leaves (AREE) and direct exhaustive extracts were taken. The parameter studied were gallic acid and total flavonoidal activities. *In-vitro* models were carried out to evaluate its antioxidant activities. Therefore these results concluded that, the ethanolic and cold water extracts afford significant antioxidant activities which may be attributed due to polyphenols.

## 1. INTRODUCTION

*Amaranthus retroflexus* (AR) is a edible plant which is used as vegetable. It is also used by native practitioner as hepatoprotective in treating different types of jaundice. The leaves of this plant contain polyphenolic compounds like tannins and flavonoids. These polyphenolic compounds have antioxidant property. Therefore antioxidants have been known to possess hepatoprotective activity. Keeping the native knowledge and the above mentioned literature information [1], this plant was selected for present study to screen the leaves of this edible plant for the presence of phytoconstituents and antioxidant activity.

## 2. MATERIALS AND METHOD

### 2.1 Collection and identification of plant

The plant was collected from Kusnoor village (Gulbarga district), in the month of March and was authenticated by Dr. Srinath Rao, chairman, P.G. Department of Studies and Research in Botany, Gulbarga University, Gulbarga, Karnataka. The plant was thoroughly cleaned, leaves were shade dried and made into a coarse powder by rubbing in the palms.

### 2.2 Extraction

80 gms of shade dried leaf powder of *Amaranthus retroflexus* were extracted in Soxhlet's apparatus using petroleum ether for defatting and then it was extracted with 70% ethanol (AREE).

**Direct exhaustive extraction:** In this process, 10 gms of shade dried, leaf powder of *Amaranthus retroflexus* is placed with the water (250 ml) for maceration and whole of the menstrum was kept in a closed vessel for 2 days. During this period shaking is done occasionally. After 2 days, the liquid is strained and marc is pressed. The expressed liquid is mixed with strained liquid. It is then filtered to make a clear liquid. The final volume is not adjusted. These extracts are evaporated on a water-bath at low temperature not exceeding (50°C) and residue was preserved [2].

Plant extracts were prepared using three different extracting solvents :

AR<sub>CW</sub> : *Amaranthus retroflexus* (AR) shade dried leaf powder macerated for 2 days with cold water (distilled water stored at room temperature).

AR<sub>HW</sub> : AR shade dried leaf powder macerated for 2 days with hot water (50°C)

AR<sub>M2</sub> : AR shade dried leaf powder macerated with methanol for 2 days.

### 2.3 Estimation of phenolics and flavonoids in various extracts

Total phenolic and flavonoidal contents of the ethanolic, cold water, hot water and methanolic extracts of *Amaranthus retroflexus* leaves were determined.

### 2.3.1 Total phenolic content (TPC)

Total phenolic content was estimated by using Folin-Ciocalteu reagent according to the method reported by Singleton and Rossi using standard curve generated with Gallic acid [3]. For the preparation of Calibration curve, a series of calibrated 10 ml volumetric flasks were taken and appropriate aliquots of the working standard solution of gallic acid were pipette out. To each flask, 5.0 ml of Folin-Ciocalteu reagent (diluted ten fold) and 4.0 ml of sodium carbonate solution (75 g/l) was mixed with appropriate aliquots of gallic acid. The absorbance was measured after 30 minutes at 765 nm in Shimadzu 1700 UV-visible spectrophotometer.

Same procedure was applied for various extracts of *Amaranthus retroflexus* (1 mg/ml) was mixed with the same reagent as described in the construction of calibration curve. After 30 minutes, the absorbance was measured for the determination of total phenolic compound. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of sample using a standard curve of gallic acid as shown in Graph No. 1. Data of calibration curve for gallic acid are shown in Table No. 1. Total amount of phenolic compounds in *Amaranthus retroflexus* were done in triplicates and concentrations of total phenolic content in various extracts were determined and expressed as Gallic acid in mg equivalents per gram of sample using the standard curve generated with Gallic acid (mean ± SEM). The results obtained were compiled and represented as shown in Table No. 2.

### 2.3.2 Total flavonoidal content (TFC)

Total flavonoidal content was estimated as reported by Woisky and Salatino using standard curve of Quercetin and Naringin [4].

#### 2.3.2.1 Aluminium chloride colorimetric method

The aluminium chloride colorimetric method was modified from the procedure reported by Woisky and Salatino. Quercetin was used to make the calibration curve. 10 milligrams of quercetin was dissolved in 80% ethanol and then diluted to 0.125, 0.25, 0.5, 0.75, 1.00, 1.25 and 1.5 % mg/100 ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm by using Shimadzu UV spectrophotometer (Japan). The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 ml of various extracts of AR (1000 µg/ml) were made to react with aluminium chloride for determination of flavonoid content as described above.

#### 2.3.2.2 2,4-Dinitrophenylhydrazine (2,4-D) colorimetric method

The current method was modified from the procedure described by Nagy and Grancai [5]. Naringin was used as the reference standard. 20 milligrams of Naringin was dissolved in methanol and then diluted to 125, 250, 500, 1000, 2000 ppm. One milliliter of each of the diluted standard solutions was separately reacted with 2 ml of 1% 2,4-dinitrophenylhydrazine reagent and 2 ml of

methanol at 50°C for 50 min. After cooling to room temperature, the reaction mixture was mixed with 5 ml of 1% potassium hydroxide in 70% methanol and incubated at room temperature for 2 min. Then, 1 ml of the mixture was taken, mixed with 5 ml of methanol and centrifuged at 1,000 rpm for 10 min to remove the precipitate. The supernatant was collected and adjusted to 25 ml. The absorbance of the supernatant was measured at 495 nm. Finally, 0.5ml of various extracts of AR (1000 µg/ml) were similarly made to react with 2,4- dinitrophenylhydrazine for determination of flavonoid content as described above. The total flavonoid contents are shown in Table No. 5. The standard curve of Quercetin and Naringin were generated.

### 2.3.3 Standard curve of Quercetin

Quercetin was used to perform the calibration curve (standard solution of 0.1250, 0.2500, 0.500, 0.7500, 1.000, 1.250, 1.500 % mg/100 ml in 80% ethanol (v/v) was prepared). The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-1700 spectrophotometer (Japan). As shown in Table No. 3, Graph No. 2.

### 2.3.4 Standard curve of naringin

The current method was modified from the procedure described by Nagy and Grancai [5]. Naringin was used as the reference standard. 20 mg of naringin was dissolved in methanol and then diluted to 125, 250, 500, 1000, 2000 ppm. One milliliter of each of the diluted standard solutions was separately reacted with 2 ml of 1% 2,4-dinitrophenylhydrazine reagent and 2 ml of methanol at 50°C for 50 min. After cooling to room temperature, the reaction mixture was mixed with 5 ml of 1% potassium hydroxide in 70% methanol and incubated at room temperature for 2 min. Then, 1 ml of the mixture was taken, mixed with 5 ml of methanol and centrifuged at 1,000 rpm for 10 min to remove the precipitate. The supernatant was collected and adjusted to 25 ml. The absorbance of the supernatant was measured at 495 nm. which are represented in Table No. 4, Graph No. 3.

### 2.3.5 Statistical analysis

The data presented in Table No. 1 to 5 (n = 3) were expressed as mean ± SEM.

**Table 1.** Standard curve for Gallic acid

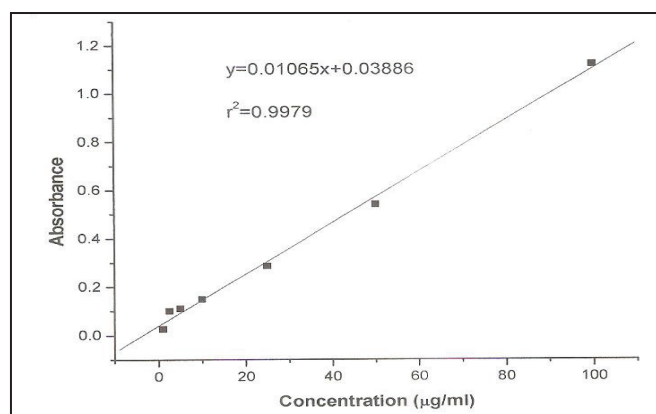
S. No.	Conc. (µg/mL)	Absorbances at (765 nm)*
1	1.0	0.026 ± 0.0005
2	2.5	0.100 ± 0.0015
3.	5.0	0.110 ± 0.0015
4.	10	0.148 ± 0.0012
5	25	0.286 ± 0.0015
6	50	0.540 ± 0.0005
7	100	1.122 ± 0.0017

\*Mean ± SEM of three replicates

**Table 2.** Total phenol content in AR leaf extract

Sl. No.	Extract of <i>Amaranthus retroflexus</i>	Total phenol content mg/gms (in gallic acid equivalent)
1.	AREE	220.14 ± 2.45
2.	AR <sub>CW</sub>	237.123 ± 3.48
3.	AR <sub>HW</sub>	28.474 ± 1.98
4.	AR <sub>M2</sub>	39.636 ± 2.24

\*Mean ± SEM of three replicates



**Fig. 1** Standard curve for Gallic acid

**Table 3.** Standard curve for Quercetin

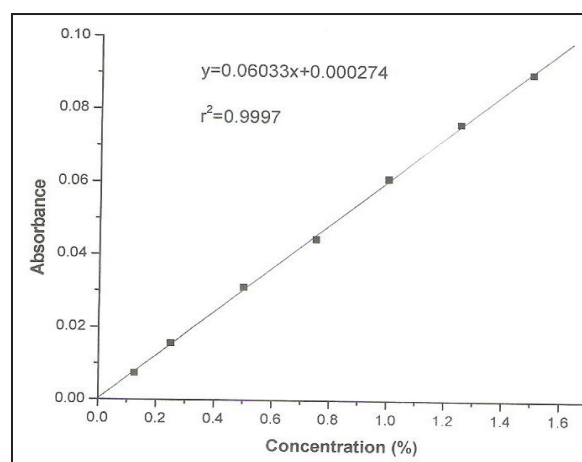
S. No.	Conc. (% mg/100 mL)	Absorbances at (415 nm)*
1.	0.1250	0.0074 ± 0.0002
2.	0.2500	0.0156 ± 0.0001
3.	0.5000	0.0311 ± 0.0002
4.	0.7500	0.0444 ± 0.0002
5.	1.0000	0.0612 ± 0.0001
6.	1.2500	0.0763 ± 0.0001
7.	1.5000	0.0902 ± 0.0001

\*Mean ± SEM of three replicates

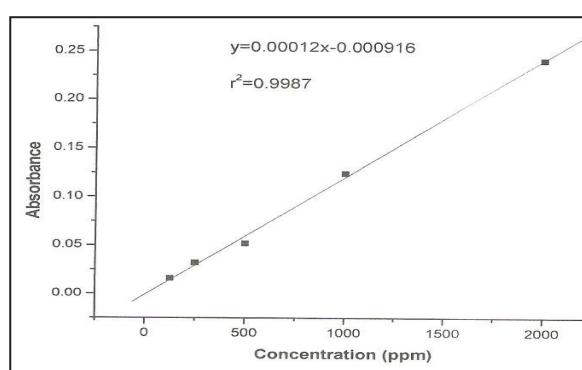
**Table 4.** Standard curve for Naringin

S. No.	Conc. (ppm)	Absorbances at (495 nm)*
1.	125	0.015 ± 0.0003
2.	250	0.032 ± 0.0020
3.	500	0.052 ± 0.0010
4.	1000	0.124 ± 0.0015
5.	2000	0.241 ± 0.0005

\*Mean ± SEM of three replicates



**Fig. 2** Standard curve for Quercetin



**Fig. 3** Standard curve for Naringin

**Table 5.** Total flavonoid contents of AR determined by aluminium chloride and 2,4- dinitro phenylhydrazine colorimetric methods

Sl. No.	Name of the sample	Total flavonoid contents (%) <sup>a</sup>		Total (µg/ml)
		AlCl <sub>3</sub> <sup>b</sup>	2-4-D <sup>c</sup>	
<i>Amaranthus retroflexus</i>				
1.	AREE	69.34 ± 0.62	29.85 ± 2.78	99.19 ± 2.88
2.	AR <sub>CW</sub>	63.31 ± 0.1	27.08 ± 2.78	90.39 ± 2.69
3.	AR <sub>HW</sub>	5.96 ± 0.1	16.52 ± 0.55	22.48 ± 0.47
4.	AR <sub>M2</sub>	9.06 ± 0.06	16.24 ± 0.28	25.3 ± 0.31

a : Results are presented as mean ± SEM (n = 3)

b: Levels calculated as quercetin equivalents

c : Levels calculated as naringin equivalents

### 3. RESULTS AND DISCUSSION

#### 3.1 Estimation of total phenolic content (TPC)

Various chemical constituents like phenolics, flavonoids, sterols, alkaloids, tannins, proteins and gums were present in AREE upon preliminary phytochemical investigations. Water, methanol and 70% ethanol can extract some amounts of antioxidants from

herbs and these antioxidants are responsible for organ protective and antioxidant activities which are reported in few recent publications. Hence, the shade dried powder of *Amaranthus retroflexus* was subjected to direct extraction with cold distilled water, hot distilled water and methanol. Successive solvent extraction was done separately for shade dried leaf powder of AR. Finally, all these extracts were subjected to quantitative estimation of antioxidant-phytoconstituents so that the extracts with maximum antioxidants were selected.

The quantitative estimation for phenolic content (TPC) showed the presence of high quantities of phenolic constituents in AR<sub>CW</sub> was  $237.123 \pm 3.48$  mg/gms where as AREE showed moderate TPC of  $220.14 \pm 2.45$  mg/gms but AR<sub>M2</sub> showed least TPC of  $39.636 \pm 2.24$  mg/gms of gallic acid equivalent as presented in Table No. 2.

### 3.2 Estimation of total flavonoidal content (TFC)

Flavonoids which are very important antioxidant-phytoconstituents were also estimated. Quercetin and Naringin equivalent flavonoidal content was found to be highest in AREE which exhibited  $99.19 \pm 2.88$  µg/ml, AR<sub>CW</sub> showed moderate  $90.39 \pm 2.69$  µg/ml. But AR<sub>HW</sub> exhibited least TFC  $22.48 \pm 0.47$  µg/ml as shown in Table No. 5.

The *in-vitro* antioxidation action offered by AREE and AR<sub>CW</sub> may be due to the presence of antioxidant phytoconstituents like flavonoids, phytosterols and other polyphenolic constituents. Therefore, these extract show very good antioxidant activity.

## 4. CONCLUSION

The good *in-vitro* antioxidant property of AREE and AR<sub>CW</sub> is attributed to the presence of antioxidant phytoconstituents. Therefore, the above findings reveals that the use of *Amaranthus retroflexus* leaves in our food, protects our vital organs, from different types of diseases.

**Scope for future study:** As it is a medicinal plant, hence isolation of its phytoconstituents are needed to screen various organ protective potentials.

### Acknowledgements

We are thankful to the Management and Principal of Luqman college of Pharmacy, Gulbarga for providing all the necessary facilities to carry out this research work.

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