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Antioxidant capacities of *Amaranthus tristis* and *Alternanthera sessilis*: A comparative study

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The aim of the present study was to compare the phytochemical and radical scavenging activities in the stem and leaf fractions of two species in Amaranthaceae family *Amaranthus tristis* and *Alternanthera sessilis*. Total flavonoids and phenolics were estimated using aluminium chloride and Folin-Ciocalteau methods, respectively; radical scavenging activities of the extracts were determined by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay. Our results showed that *A. sessilis* had higher levels of flavonoids (70.42 mg/100 g) and phenolics (103.75 mg/100 g), when compared with *A. tristis* that had relatively lower levels of flavonoids (62.87 mg/100 g) and phenolics (96.89 mg/100 g) in leaf fractions. The stem and leaf fractions of *A. sessilis* showed more DPPH and FRAP values indicating the higher radical scavenging activity of the extract, when compared to *A. tristis*. It was also found that the flavonoids and phenolics content are directly proportional to the radical scavenging activities of both vegetables. The results concluded that *A. sessilis* have relatively more phytochemicals and radical scavenging activities and it also reveals that the leaf fraction has more flavonoid and phenolic content than the stem fractions in both vegetables. It is clear that both plants have definitely more antioxidant properties making it an ideal dietary antioxidant supplement.

Key words: Phytochemicals, antioxidants, total flavonoids, total phenolics, radical scavenging activity.

INTRODUCTION

The knowledge of herbal medicine comes from the traditional system of medicine, where the ethnic knowledge was verbally passed on from one generation to another without any proper documentation (Puspangadan and Atal, 1984). Currently the people are destitute of traditional knowledge, which led to the slowdown of usage of the herbal medicine. India with its rich biodiversity, its major priority is to explore the bioactive compounds present in some of the neglected medicinal plants/vegetables. This will give the opportunity to develop novel drugs, as various recent drugs for the deadly disorders had come from the plants. For example, alkaloids vincristine and vinblastine isolated from *Catharanthus roseus*, Taxol from *Taxus brevifolia* are used as the effective agents for the treatment of cancer (Machana et al., 2011). Conventionally, the food and health are strongly interrelated, as rural people used the plants/plant extracts to cure various human disorders. Many plant species are also been used as food as well as for medicinal purposes (Velu et al., 2012).

Fruits and vegetables have generally high amount of flavonoids, phenolics and other antioxidant components like ascorbic acid, tocopherol, etc (Cartea et al., 2011). Routine or habitual intake of antioxidant rich fruits and vegetables has been well correlated with lower risk of neurodegenerative diseases and certain cancers.

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Antioxidants are the beneficial compounds that will inhibit the oxidative chain reaction initiated by the free radicals thereby reducing the extent of destruction of biomolecules (Kerchev et al., 2008). Plant derived compounds especially phenolics and flavonoids are reported to have antiaging and anticancer properties (Velu et al., 2012). Free radicals are generated in the aerobic organisms as byproducts of body metabolism. These free radicals are active, having unpaired electrons in the outer shell that will react with the biomolecules resulting in cellular damage and lipid peroxidation (Moreira da Silva et al., 2010). Antioxidants counter attack the toxic effects of oxidants in the body; hence, there is an increase in antioxidants research due to its beneficiary activities. Now the research is budged towards the identification of natural antioxidants from the herbal sources as synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are shown to be carcinogenic (Moussa et al., 2011).

Green leafy vegetables are regarded as 'natures anti-aging wonders' due to its medicinal properties beyond essential sustenance (Gupta and Prakash, 2009). Leafy vegetables are the major component in traditional diet due to its health benefits, which is mainly because of the presence of more phytochemicals with potential antioxidant properties (Khanam et al., 2012). Both *Amaranthus tristis* and *Alternanthera sessilis* belongs to the family Amaranthaceae, having the history of medicinal usage. *A. tristis* and *A. sessilis* have been used as food as well as for combating various disorders traditionally. Both the plants have been reported to possess unique bioactive compounds that contribute to the pharmacological activities of that plant. *A. tristis* is a common edible plant that contains amarantin, isoamarantin, betaine, aminoacids and sterols (Velu et al., 2012). It has great medicinal value, as it is used as an astringent in dysentery, diarrhea, hemorrhagic colitis and also used in cough and bronchitis.

A. sessilis commonly known as sessile joy weed, found in humid and warm regions of the world and it is used to relieve headache and dizziness (Arshad et al., 2011). The leaf sap of *A. sessilis* is used for the treatment of neuralgia. It is also considered to be viable for the treatment of snakebites and to stop blood vomiting (Swapna et al., 2011). *A. sessilis* is also used for the treatment of dysentery, gastrointestinal troubles, chronic liver obstruction, leucorrhoea, gonorrhea and to reduce body temperature (Puspangadan, 1984; Panda and Misra, 2011). Leafy vegetables are commonly consumed by lower economic group people than the comfortable counterparts (Rensberg et al., 2007). Nowadays, consumption of leafy vegetables has significantly reduced, which may be due to its taste, cultural background and geographical location (Uusiku et al., 2010). Extensive documentation of neglected/under-utilized green leafy vegetables is required since they are proven sources of essential components like flavonoids, phenolics and vitamins. Hence, the aim of the present study is to scientifically validate the phytochemical content and radical scavenging activities of both *A. tristis* and *A. sessilis* leaf and stem extracts.

MATERIALS AND METHODS

Chemicals

Aluminium chloride, potassium acetate, quercetin, sodium carbonate, gallic acid, 2, 4, 6-tripyridyl-2-triazine (TPTZ), sodium acetate, ferric chloride, ammonium molybdate, Folin–Ciocalteau reagent and 2, 2-diphenyl-1-picryl hydrazyl (DPPH) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Solvents and other reagents used in this study were of analytical and HPLC grades.

Sample collection

A. tristis and *A. sessilis* leafy vegetables were collected from the local market in Coimbatore, Tamil Nadu, India.

Preparation of extracts

The vegetables were cleaned and cut into small pieces. Sample (0.5 g) was weighed and homogenized in 5 ml of methanol, until the tissue gets into a fine paste in a mortar and pestle. The samples were then centrifuged at 4000 rpm for 10 min. The aforementioned procedure was repeated twice and the extract was concentrated by evaporating the solvent at 40°C. The aforementioned procedure was repeated twice and the supernatant was collected and stored for the further analysis.

Determination of total flavonoid content

Total flavonoids were quantified using aluminium chloride method (Chang et al., 2002). The reaction mixture containing 0.5 ml of 2% w/v $AlCl_3$ in methanol and 0.5 ml potassium acetate (120 mM) were added to 1 ml of the methanolic extract and incubated at room temperature for 30 min. Absorbance was read at 415 nm and the calibration curve was obtained using quercetin (100 to 1000 µg/ml) as a standard.

Estimation of total phenolics

Methanolic extract (100 µl) was mixed with 1 ml of 10% Folin-Ciocalteau reagent and 2 ml of 5% sodium carbonate. The mixture was incubated in dark for 45 min at room temperature. Then the absorbance of the sample was measured at 765 nm using gallic acid (100 to 1000 µg/ml) as a standard (Singleton et al., 1965). The concentration of total phenolics was expressed in gallic acid equivalents mg/g of fresh weight.

DPPH radical scavenging activity

To the methanolic extract, added 2 ml of DPPH (0.1 mM in methanol) and mixed vigorously. Then the mixture was incubated for 30 min at room temperature and the absorbance was measured at 517 nm (Gyamfi et al., 1999). The percentage of DPPH scavenging activity was calculated using the formula:

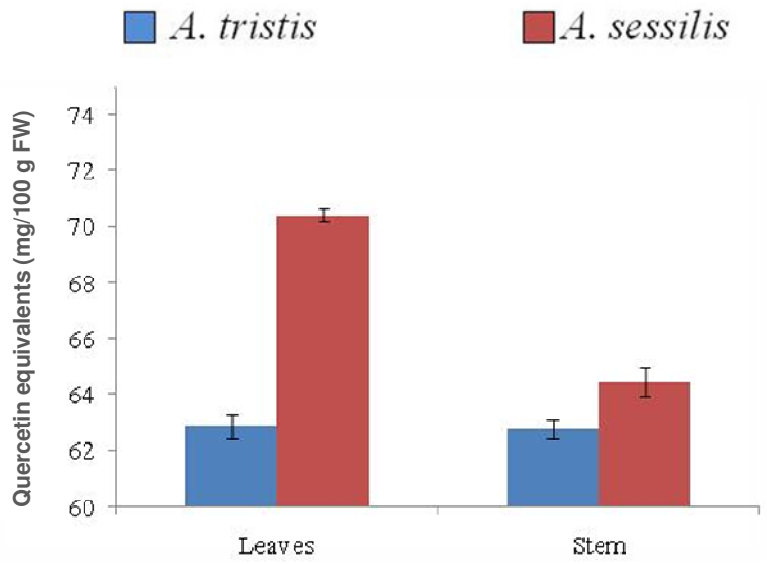

Figure 1. Total flavonoid content in the leaves and stem fractions of *Amaranthus tristis* and *Alternanthera sessilis*.

DPPH Radical Scavenging Activity (%) = $[(A_{control}-A_{sample})/A_{control}]$

Reducing power: Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant activity was determined by following the method of Benzie and Strain (1996). The FRAP solution was prepared by mixing 25 ml acetate buffer (300 mM, pH=3.6), 2.5 ml TPTZ (10 mM in 40 mM HCl), 2.5 ml $FeCl_3.6H_2O$ (20 mM) and it was pre-warmed to 37°C before use. Methanolic extracts (100 µl) was added to 2 ml of the FRAP solution and incubated at 37°C for 30 min. $FeSO_4$ (200 to 1000 µM) was used as the reference standard and were expressed as ferric reducing ability of the antioxidants equivalent of 1 mM $FeSO_4$.

RESULTS

Phytochemical content

The total flavonoids of both vegetables were assessed and the results were expressed in quercetin equivalents. Our results indicated that the flavonoid content is present at substantial levels in both the leafy vegetables (Figure 1). The flavonoids levels in *A. sessilis* were, 70.42 mg/100 g in leaves and 64.45 mg/100 g in stem. On the other hand, *A. tristis* had relatively lower levels of flavonoids in leaf (62.87 mg/100 g) and stem (62.78 mg/100 g) fractions. Results clearly showed that the leaf fraction have more flavonoid content in both vegetables. The total phenolic content in the methanolic extracts of *A. tristis* and *A. sessilis* were evaluated and the results were expressed in gallic acid equivalents (Figure 2). Results revealed that *A. sessilis* (103.75 and 43.2 mg/100 g) had the highest level of phenolics in leaf and stem fractions while *A. tristis* (96.89 and 37.61 mg/100 g) had relatively lower level of phenolics in leaves and stem, respectively.

Determination of radical scavenging activity

The results of radical scavenging activity of stem and leaf fraction of both vegetables are as shown in Figure 3. The highest DPPH radical scavenging activity was found in the leaves of *A. sessilis* (79.42%), whereas it was lower in *A. tristis* (69.45%). With regard to FRAP values, the leaves and stem fractions of *A. sessilis* (32.56 and 17.45 mM/100 g) showed remarkable reducing power as compared to *A. tristis* (Figure 4).

DISCUSSION

Antioxidants play a major role in the protection of cells from lethal effects of free radicals and their derivatives (Majewska et al., 2011). A diet rich in antioxidant helps to strengthen the antioxidant based defense system in the human body (Stangeland et al., 2009). Oxidative damage occurs, if there is an imbalance between the antioxidants and oxidants in the system. In the present study, phytochemical content and radical scavenging activities were performed in stem and leaf portions of both vegetables, as stem portion are also edible in these plants.

In the present study, flavonoid content of the vegetable extracts were determined by aluminium chloride method, which is based on the reaction of $AlCl_3$ with the C-3 Keto and C-3 or C-5 hydroxyl group of the flavones and formed the acid stable complex that showed the

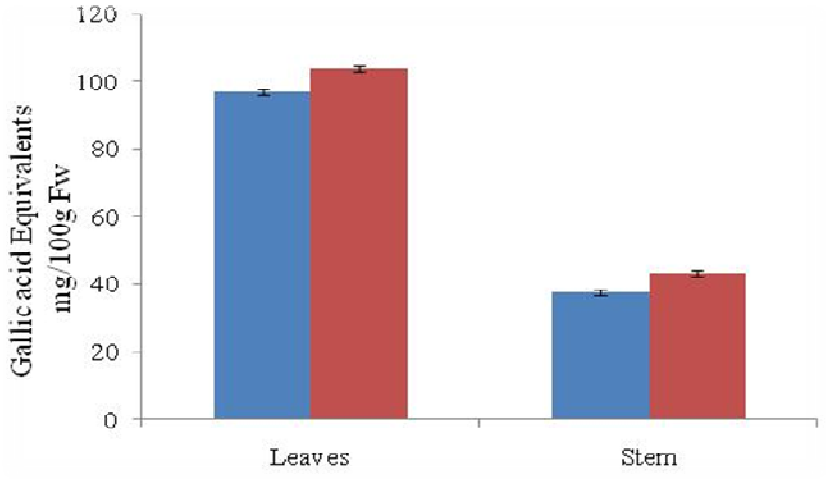

Figure 2. Total phenolic content in the leaves and stem fractions of Amaranthus tristis and Alternanthera sessilis

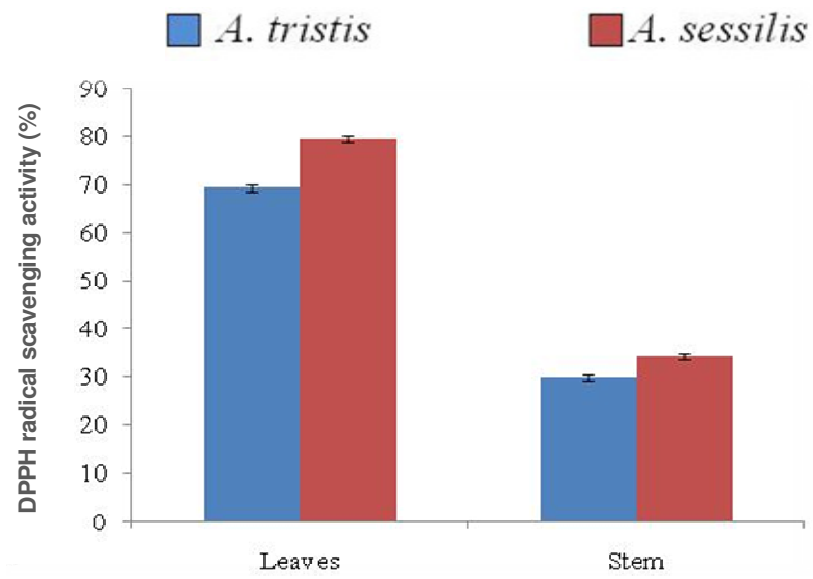

Figure 3. DPPH radical scavenging activity in the leaves and stem fractions of *Amaranthus tristis* and *Alternanthera sessilis*.

absorption maximum at 415 nm (Chang et al., 2002). The results showed that *A. sessilis* had more flavonoid content than *A. tristis*. Previous reported, Borah et al. (2011) showed that the flavonoid content of *A. sessilis* was 37 mg/100 g, which is slightly lower than the present study, that may be due to the difference in the harvesting season and geographical location in which the plant grown as flavonoid content is influenced by multifactorial influence of the environment (Siatka and Kasparova, 2010). The accumulation of flavonoid content is mainly attributed to the activity of phenylalanine ammonia lyase, which is the rate limiting enzyme in the synthesis of flavonoids and phenolic compounds shown to be enhanced by sunlight/UV radiation or other environmental stresses (Alothman et al., 2009). The results of this study revealed that the leaf fractions have higher flavonoid content than the stem portion of both vegetables, which may be due to accruing of flavonoids in leaves in order to protect the leaves from various biotic and abiotic stresses (Jaakola et al., 2004).

Phenolics are present ubiquitously in all the plants that are partly responsible for health benefits including as antioxidants, anticancer and against other cardiovascular complications. In this study, phenolic content was

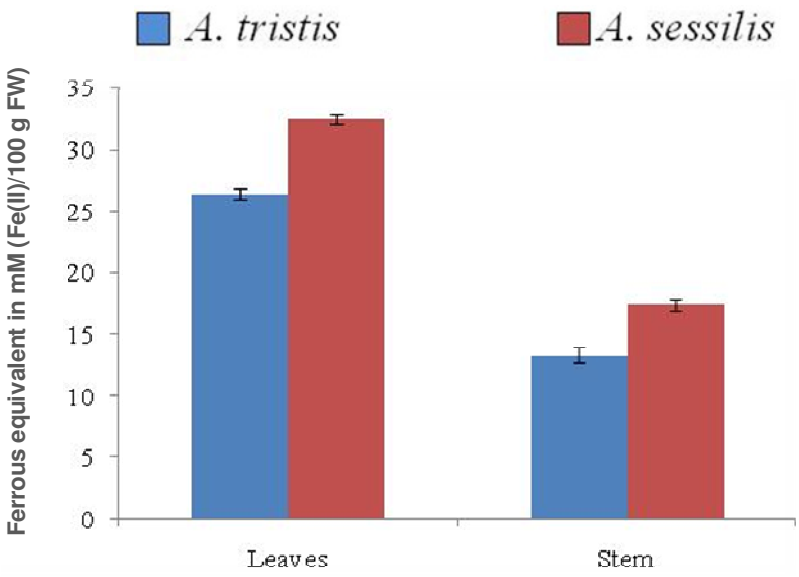

Figure 4. FRAP reducing power in the leaves and stem fractions of *Amaranthus tristis* and *Alternanthera sessilis*.

estimated by Folin-Ciocalteau method, which is rapid and considered standard method for the estimation of phenolic compounds (Khanam et al., 2012). Similar to flavonoid content, *A. sessilis* had more phenolic content, which is mainly attributed to the antioxidant potential of the plant. The phenolic content of both the fractions of *A. sessilis* is about 146.9 mg/100 g, which is consistent with the results reported by Borah et al. (2011). Like flavonoids, phenolic levels could also be affected by temperature and other stresses apart from post harvest handling and storage conditions. Also, the accumulation of phenolics depends on the genetic and environmental factors; hence it may vary between the species and even between the cultivars (Gawlik-Dziki, 2008).

Due to its simplicity, scalability and reproducibility, DPPH radical was used to assess the radical scavenging activity of the vegetable extracts. The hydrogen atom donating ability of the vegetable extracts was assessed based on the bleaching of purple coloured DPPH to yellow color (Subhasree et al., 2009). The radical scavenging activity of *A. tristis* in the leaf fraction was 69.45%, which is in agreement with previous report (Velu et al., 2012). The antioxidant capacity of vegetable extracts could be due to the higher levels of phenolic compounds. The free radical scavenging activity of the extracts depends on the availability and structure of hydroxyl group in the phenolic compounds (Hung et al., 2012).

The antioxidant potential of both vegetable extracts were further assessed by their ability to reduce Fe (III) to Fe (II). Leaves of *A. sessilis* had more FRAP values than the *A. tristis*. *A. sessilis* showed more radical scavenging activity and FRAP values, which may be due to the accumulation of flavonoids and other phenolics that are directly proportional to the free radical scavenging activity of the extracts. FRAP assay was used to evaluate the reducing power of several other fruits and vegetables like tomato cultivars and in African, leafy vegetables (Ji et al., 2011; Chandra and Ramalingam, 2011; Katerere et al., 2012).

The phytochemical and radical scavenging activity of *A. tristis* and *A. sessilis* leaf and stem fractions were carried out in this study. From the results, it was concluded that leaf fraction had more phytochemical content than the stem fractions that eventually contribute to the radical scavenging activity of the leaf extract. Results showed that, both plants possess high antioxidant properties in general and hence it can be recommended to be included in our daily diet, as it will protect us from commonly occurring chronic diseases. Still, extensive *in vitro* and *in vivo* studies are essential in order to use these plants as a source of production of natural antioxidants. This work will also be a prelude to replenish our ancestral knowledge about these underutilized vegetables that will be helpful for the betterment of upcoming generations.

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