

Comparative Study of Antioxidant Activities of the Leaves and Stem of *Ipomoea aquatica* Forsk (Water Spinach)

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ABSTRACT: Leaves and stem extracts of *Ipomoea aquatica* Forsk were analysed for their antioxidant activities. Folin-Ciocalteu and AlCl₃ methods were used to quantify total phenolic and flavonoid contents while 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical assay was used to examine the antioxidant activity of the extracts. The respective total phenolic and flavonoid contents of the stem (18.00 ± 1.20 and 20.00 ± 1.40 µg Catechin equivalent, CEQ g⁻¹ DW) show no significant (p > 0.05) difference when compared to leaves (16.00 ± 1.10 and 30.00 ± 2.1 µg CEQ g⁻¹ DW). In DPPH scavenging assay, the reference standard i.e. vitamin C (IC₅₀ = 0.0660 µg/ml) had significantly (p < 0.05) higher scavenging activity than the stem (IC₅₀ = 35.96 µg/ml) which in turn is significantly higher (p < 0.05) than the leaves (IC₅₀ = 176.92 µg/ml). From the results it can be concluded that stem and leaves of *Ipomoea aquatica* had similar antioxidants activity.

Keywords: *Ipomoea aquatica*, antioxidant, DPPH Assay, Scavenging activity, phenolic, flavonoid.

INTRODUCTION

Antioxidants are substances that are capable of preventing cells from damages caused by unstable molecules known as free radicals (Hamid *et al.*, 2010). Antioxidants thus interact with and stabilize free radicals and may prevent some of the damages free radicals may otherwise cause. The application of synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), tertiary butylated hydroquinone and gallic acid esters have been restricted as they are suspected to cause or prompt adverse health effect (Maurya and Singh, 2010). The importance of substituting these antioxidants with those of natural origin has therefore become a priority in recent years. Thus, phytochemicals have been isolated from plant materials such as bark, leaves, stem, roots, fruits, seeds, fruit rid, flower and whole plants (Gobalakrishnan *et al.* 2013).

Some medicinally important plants have been effectively used against nose bleed, reduction of blood sugar levels and as antibiotic against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Hamid *et al.*, 2011). Bioactive compounds are usually accumulated as secondary metabolites in all plant cells; however, their concentrations could vary from one plant part to another (Gobalakrishnan *et al.*, 2013).

Water spinach (*Ipomoea aquatica* Forsk) belongs to the family Convolvulaceae. It is a tender trailing or floating

perennial aquatic plant found on moist soil along the margins of fresh water and is being cultivated as an edible green leafy vegetable in Hong Kong, Taiwan, China and Nigeria (Prasad *et al.*, 2005; Umar *et al.*, 2007; James *et al.*, 2009).

It was therefore the aim of the present study to compare the antioxidant activities of the leaves and stems of *I. aquatica* with a view to understanding which of the two has better medicinal antioxidant activities.

Materials and Methods

Plant Materials

Fresh *Ipomoea aquatica* Forsk was obtained from a local market in Sokoto metropolis. It was authenticated at the herbarium, Department of Biological Sciences of the Usmanu Danfodiyo University Sokoto. The leaves and stems were cleaned with water and dried in a hot air oven for 36h at 48°C. The leaves and stems were subsequently powdered using a mortar and pestle.

Sample Preparation

Thirty grammes each of the dry powdered and stems of *I. aquatica* were extracted with 200 cm³ of 95% methanol for 6h at 60°C using a soxhlet apparatus. The extracts were then filtered through Whatman No. 41 filter paper to remove suspended particles. The filtrates were concentrated under vacuum in a rotary evaporator at 40°C.

Determination of Total Phenolic Flavonoid and Free Radical Scavenging Activity

The total phenolic compounds were determined by Folin-Ciocalteu method according to Ragazzi and Veronese (1973). For flavonoid, aluminium (III) chloride method as described by Lamaison and Carnet (1990) was used. The Free radical scavenging activity was determined using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical assay as described by Adesegun *et al.* (2008).

Statistical Analysis

Analyses were carried out in triplicates of each set up and results expressed as mean \pm standard error of mean. Statistically significant difference ($p < 0.05$) was determined using Student's t-test. All data were analyzed using Statistical Package for the Social Sciences (SPSS version 15.0).

RESULTS

The total phenolic and flavonoid contents of *I. aquatica* are presented in Table 1. From the results, both the phytochemicals in the stem show no significance ($p > 0.05$) difference from the respective leaves values.

The free radical scavenging activities of the extracts of the plant are presented in Table 2. The stem had

significantly ($p < 0.05$) higher scavenging activity ($IC_{50} = 35.96 \mu\text{g/ml}$) than the leaves ($IC_{50} = 176.92 \mu\text{g/ml}$) at all concentrations. All the samples have significantly ($p < 0.05$) lower level of activities when compared to reference standard (ascorbic acid) at concentrations of 25-100 $\mu\text{g/ml}$.

DISCUSSION

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and also involved in the retardation of oxidative degradation of lipids (Pourmorad *et al.*, 2006). Our findings of higher levels of phenolic compounds in the stem methanolic extracts agrees with the findings of James *et al.* (2009) who reported values of $0.35 \pm 0.07\%$ for the stem and $0.31 \pm 0.01\%$ for the leaves. In a similar study by Huang *et al.* (2005), the stem phenolic compounds ($37.40 \pm 1.70 \mu\text{mol/g D.W.}$) were reported to be higher than that of the leaves ($27.60 \pm 1.57 \mu\text{mol/g D.W.}$). According to Maurya and Singh (2010), the antioxidant activity of plants might be due to the presence of phenolic compounds in them. Thus, the presence of phenolic compounds in the leaves and stem of *I. aquatica* may be an added value to its nutritional and medicinal potential.

Table 1: Total Phenolic and Flavonoid Contents of *I. aquatica* Leaves and Stem ($\mu\text{g CEQ g}^{-1}\text{DW}$)

Parameter	Leaves	Stem
Total phenolic	16.00 ± 1.10	18.00 ± 1.20
Total flavonoid	30.00 ± 2.10	20.00 ± 1.40

The data are mean \pm standard error of mean of triplicate determinations.

Table 2: Free radical scavenging activity of *I. aquatica* leaves, stem and standard ascorbic acid.

Concentration ($\mu\text{g/ml}$)	Scavenging Activity (I%)**		
	Stem	Leaves	Ascorbic acid
25	$46.1 \pm 0.28^*$	$26.6 \pm 0.33^*$	$89.5 \pm 0.43^*$
50	$54.9 \pm 0.01^*$	$34.1 \pm 0.16^*$	$97.4 \pm 0.16^*$
75	$64.7 \pm 0.12^*$	$36.1 \pm 0.16^*$	$98.0 \pm 0.03^*$
100	$74.0 \pm 0.17^*$	$37.9 \pm 0.16^*$	$99.0 \pm 0.01^*$
IC_{50}	35.96	176.92	0.0660

The data are Mean \pm Standard error of mean of triplicate determinations; *significantly different at $P < 0.05$. **I = inhibition of DPPH

The trend of flavonoid accumulation in stem and leaves is consistent with the findings of Huang *et al.* (2005) where the leaves contained higher flavonoid than the stem. Previous findings have suggested that flavonoids carry out antioxidant action through scavenging or

chelating process, hence, play a preventive role in cancer and heart disease (Ogbunugafor *et al.*, 2011). Thus, the occurrence of flavonoids in *I. aquatica* extracts may similarly improve the economic and health potentials of the vegetable as reports have shown that

the vegetable is cultivated commercially for nutritional and medicinal applications (Prasad *et al.*, 2005). The observed differences in the concentrations of phenolic and flavonoid contents of the leaves and stem is in agreement with a previous study (Gobalakrishnan *et al.*, 2013) which suggested that bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration could vary in different parts of the plant. According to Hemm *et al.* (2004), phenolics and flavonoids synthesis in plants is influenced by environmental factors, leaf maturity, plant age etc. Light intensity regulates not only plant growth and development, but also the biosynthesis of both primary and secondary metabolites Ghasemzadeh *et al.* (2010). Phenolic biosynthesis requires light or is enhanced by light, and flavonoid formation is absolutely light dependent and its biosynthetic rate is related to light intensity (Xie and Wang, 2006).

The antioxidant potential of phytochemicals is studied using the quenching of free radicals. The *I. aquatica* leaves and stem was further highlighted by the quenching of DPPH – free radicals, which is a proton free radical commonly used to determine the free radical scavenging power of antioxidants (Adesegun *et al.*, 2008). The decrease in absorbance of DPPH – *I. aquatica* extracts mixture in this study which measured the extent of radical scavenging activity of the extracts supported the findings of AsokKumar *et al.* (2009). In the present study, it was observed that the stem extract exhibited better scavenging activity than the leaves. This observation is consistent with the findings of Huang *et al.* (2005). Similar results have also been reported by James *et al.* (2009). The stem extract had better scavenging activity ($IC_{50} = 35.96 \mu\text{g/ml}$) than the leaves ($IC_{50} = 176.92 \mu\text{g/ml}$). The higher scavenging activity of the stem may be as a result of the increased phenolic contents of the stem compared to the leaves. Olukemi *et al.* (2005) has reported a strong relationship between phenolic content and antioxidant activity in selected fruits and vegetables. Phenolic compounds are the major contributors to antioxidant activity (Hamid *et al.*, 2011; Zainol *et al.*, 2003). This was confirmed in this study whereby the stem extract with more phenolic compounds exhibited greater antioxidant activity. The extracts had significant effects on the DPPH radical.

CONCLUSION

In the current study, *I. aquatica* leaves and stem were compared in terms of their antioxidant properties. The leaves and stem had antioxidant properties which were

higher in the stem extract although not significant ($p > 0.05$); and this suggest *I. aquatica* stem and leaves had similar antioxidant activities and hence may play a role in the treatment of free radical related disorders.

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