# Total Phenolics and Antioxidant Activity of *Swintonia floribunda* (Griff.)

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**Abstract**

The present study describes the *in vitro* antioxidant activity, total phenolic content and antioxidant capacity of different extractives of *Swintonia floribunda.* The antioxidant activity was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay while the total antioxidant capacity was assessed by Phosphomolybdenum method. On the other hand, the total phenolics were determined by Folin-Ciocalteu reagent and were expressed as gallic acid equivalent. The carbon tetrachloride and aqueous soluble fractions of the methanolic extract showed significant activities in all antioxidant assays when compared to commercial antioxidants BHT and ascorbic acid. In DPPH scavenging assay, the aqueous soluble partitionate demonstrated the highest antioxidant activity with $IC_{50}$ of 9.5µg/ml as compared to 3.25 and 24.5µg/ml for ascorbic acid and BHT, respectively. Total antioxidant capacity was also found to increase in a dose dependent manner and the total phenolic content of aqueous soluble fraction of the crude extract was 42.66g of GAE/100 gm of dried extract. A positive correlation was observed between the phenolic content and total antioxidant activity with correlation coefficient ($R^2$) value of 0.9697. These results suggested that *S. floribunda* could be considered as a chemopreventative agent, providing antioxidant properties and offering effective protection from free radicals.

**Keywords:** *S. floribunda*, antioxidant, total antioxidant capacity, phenolic content

## Introduction

In the past few years, there has been a renewed interest in evaluating the antioxidant activity of various plants and herbs including fruits and vegetables. This change occurred after it was discovered that antioxidant phytonutrients (carotenoids and phenolics) are important for two main reasons. Firstly, they are responsible for the sensory properties of food and secondly, they have protective activity against a variety of degenerative diseases (Flood *et al*., 2002; Ruiz *et al.,* 2006). Currently, there is overwhelming evidence indicating that free radicals cause oxidative damage to lipids, proteins and nucleic acids. Free radicals may lie at the heart of the etiology or of the natural history of a number of diseases, including cancer and atherosclerosis (Ames *et al*., 1983; Ness *et al*., 1997; Poulsen *et al.,* 1998). Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of these diseases (Wang *et al*., 1996). In fact, higher plants exhibit significant potency against human bacterial and fungal pathogens. Apart from being the primary food source of some essential nutrients, fruits and vegetables also contain a variety of bioactive components, which might have other beneficial health effects (Cos *et al.*, 2006).

*Swintonia floribunda* (Family-Anacardiaceae) locally known as civit, is a medium-sized evergreen tree distributed mainly pantropically. In Bangladesh, it is found in hill tracts and commonly with resin that causes irritation to skin. A comprehensive literature search showed no reports on the biological evaluation of this plant except antibacterial activity (Mohammad *et al*., 2008). Thus, *S. floribunda* was investigated for its antioxidant properties and we, here in, report the results of our preliminary screenings.

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## Materials and Methods

***Plant Materials:*** The leaves of *S. floribunda* were collected from National Botanical Garden, Bangladesh, at June, 2009. The taxonomic identification of the plant was conducted by a taxonomist. A voucher specimen for this collection has been deposited in the Bangladesh National Herbarium, Mirpur, Dhaka.

***Chemicals and reagents:*** Deionized water, 2.0M Folin-Ciocalteu phenol reagent, gallic acid, anhydrous sodium carbonate, Butylated hydroxytoluene (BHT), 90% 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Aldrich Co. USA. Ascorbic acid was obtained from SD Fine Chem. Ltd., Boisar, India.

***Preparation of crude extract:*** The sun dried and powdered leaves (600gm) of *S. floribunda* was macerated in 2.5L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40-45$^{o}$C) and reduced pressure. An aliquot (5gm) of the concentrated methanolic extract was partitioned by modified Kupchan method (Van *et al*., 1993) and the resultant partitionates i.e., n-hexane (HSF, 1.405gm), carbon tetrachloride (CTSF, 0.905gm), chloroform (CHSF, 0.505gm) and aqueous (AQSF, 0.355gm) soluble fractions were obtained and used for the experimental purpose.

## Assays for antioxidant activity

### *DPPH radical scavenging activity*

*Qualitative analysis*: The methanol extract was applied on a Silica gel ($F_{254}$) TLC plate as a spot (100µg/ml) and the plate was developed by using the mobile phase methanol-chloroform (5:95, v/v). After development of the chromatogram, the plate was sprayed with DPPH (0.15 % w/v) solution using an atomizer. The change of color on the TLC plate (yellowish on pinkish background) was noted as an indicator of the presence of antioxidant substances.

*Quantitative analysis*: An aliquot of a methanol solution of the sample (extractive and standard) at different concentration (500µg/ml to 0.977µg/ml) were mixed with 3.0ml of a DPPH solution in methanol (20µg/ml). After 30min of reaction at room temperature in dark place the absorbance was measured with a UV-Visible spectrometer at 517nm against methanol as blank (Brand-Williams *et al*., 1995). Then the inhibition of free radical DPPH in percent (I%) was calculated as follows:

$(I\%) = (1 - A_{sample}/A_{blank}) \times 100$, where $A_{sample}$ and $A_{blank}$ are the absorbance of the sample and control (containing all reagents except the test material), respectively. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotted by percentage of inhibition against extractive/standard concentration.

### *Total antioxidant capacity*

The total antioxidant activity of the extract was evaluated by the phosphomolybdenum method (Prieto *et al*., 1995) based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition. A 0.3ml extract (2mg/ml) was combined with 3.0ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate), and the reaction mixture was incubated at 95$^{o}$C for 90min. Then the absorbance of the solution was measured at 695nm using a UV–visible spectrophotometer against blank after cooling to room temperature. The antioxidant capacity was expressed as the number of gram equivalents of ascorbic acid.

### *Total phenolic content*

The total phenolic content of the extractive was measured by using Folin-Ciocalteu reagent (Skerget *et al*., 2005) as an oxidizing agent and gallic acid as a standard. To 0.5ml of extract solution (2mg/ml) in water, 2.5ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0ml of sodium carbonate (7.5 % w/v) solution were added. The mixture was incubated for 20 minutes at room temperature and the absorbance was measured at 760nm using a UV-visible spectrophotometer. The total phenolics was quantified by calibration curve obtained from measuring the absorbance of known concentrations of gallic acid (0-100µg/ml) and was expressed as gm of GAE (gallic acid equivalent)/100gm of the dried extract.

### *Statistical analysis of data*

Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

## Results and Discussion

The methanolic extract of *S. floribunda* and its organic soluble partitionates demonstrated varying degrees of free radical scavenging activity, total antioxidant capacity and total phenolic contents (Table 1).

**Table 1: Free radical scavenging activity, total antioxidant capacity and total phenolics of different partitionates of *S. floribunda***

| Sample | **Free radical scavenging activity** ($IC_{50}$ µg/ml) | **Total antioxidant capacity** (mg of ascorbic acid /100 gm of dried extract) | **Total phenolics** (gm of GAE /100 gm of dried extract) |
|---|---|---|---|
| BHT | 24.5±0.35 | N/A | N/A |
| ASA | 3.25±0.25 | N/A | N/A |
| MESF | 17.40±0.31 | 583.18048±0.47 | 32.796±0.90 |
| HSF | 31.75±0.42 | 398.89524±0.37 | 22.191±0.85 |
| CTSF | 53.30±0.58 | 523.68048±0.52 | 31.45±1.26 |
| CHSF | 56.0±0.48 | 412.55095±1.53 | 24.327±1.12 |
| AQSF | 9.5±0.23 | 675.68952±0.64 | 42.66±1.35 |

The free radical scavenging activity of *S. floribunda* in the DPPH assay is depicted in Figure 1 and most potent activity was found for the aqueous soluble fraction of the methanolic crude extract (AQSF) having $IC_{50}$ value of 9.5µg/ml. On the other hand, methanol extract (MESF) and its hexane soluble fraction (HSF) demonstrated free radical scavenging activity with $IC_{50}$ value of 17.5µg/ml and 31.75µg/ml, respectively (Table 1). The total antioxidant capacity of *S. floribunda* extract expressed as the mg of ascorbic acid/100g of plant extract was determined by Phosphomolybdenum method where the highest value was detected in aqueous soluble fraction followed by crude methanolic extract as evident from 675.68mg and 583.18mg equivalent of ascorbic acid, respectively.

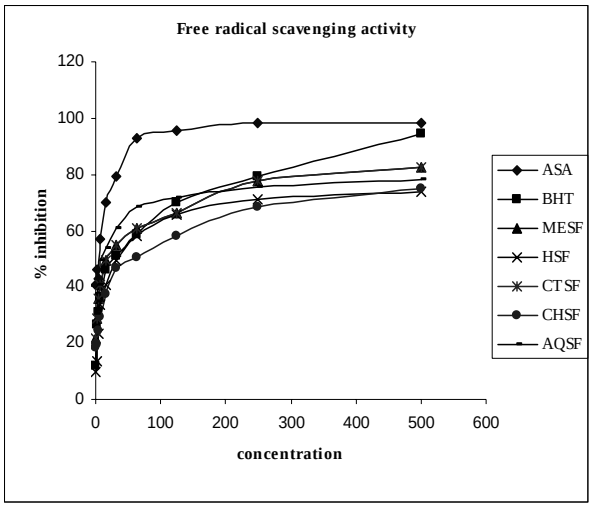


**Figure 1: DPPH free radical scavenging activity of different extractives of *S. floribunda***

The highest total phenolics was found in AQSF (42.66gm of GAE/100gm of dried extract) and the lowest in HSF (22.191gm of GAE/100gm of dried extract). (Table 1) Phytochemicals, especially plant phenolics constitute a major group of compounds that act as primary antioxidants (Hanato *et al*., 1989). They can react with active oxygen radicals, such as hydroxyl radicals (Hussain *et al*., 1987), superoxide anion radicals (Afnaslev *et al*., 1986) and lipid peroxy radicals (Torel *et al*., 1986) and inhibit lipid oxidation at an early stage. They also inhibit cyclooxygenase and lipoxygenase of platelets and microphases, thus reducing throbotic tendencies in vivo (Moroney *et al*., 1988).

The correlation analysis revealed that a correlation exists between total phenolic content and total antioxidant activity. The correlation coefficient ($R^2$) value for the phenolic content and total antioxidant activity (Figure 2) was 0.9697 indicated that 96.97% of the antioxidant activity resulted from the contribution of the phenolic compounds (Hajimahmudi *et al*., 2008). Different secondary metabolites, such as volatile oils, carotenoids and vitamins may also contribute to the antioxidant capacity, which in this case contributed to approximately 3.03% of the antioxidant activity. (Odabasoglu *et al*., 2005)

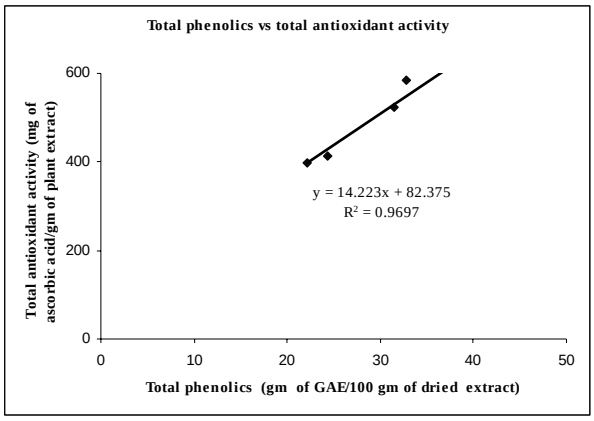


**Figure 2: Correlation between the total phenolics and total antioxidant activity**

## Conclusion

From the above results, it can be concluded that the crude extract of *S. floribunda* and its carbon tetrachloride and aqueous soluble fraction possess potent antioxidant potential which also suggest the presence of secondary metabolites having antioxidant activities. The plant could be subjected for extensive chromatographic separation and purification processes to isolate bioactive lead compounds for the discovery of novel therapeutic agents.

## References


Afanaslev, I.B., Dorozhko, A.I., and Bordskii, A.V., (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol*. **38**: 1763–9.

Ames, B.N., (1983). Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. Science, **221**: 1256-1264.

Brand-Williams, W., Cuvelier, M. E. and Berset, C., (1995). Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* **28**: 25-30.

Cos, P., Vlietinck A.J., Berghe, D.V., and Maes, L., (2006). Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. *J Ethnopharmacol*. **106**: 290-302.

Flood, A, Velie E.M., Chaterjee, N., Subar, A.F., Thompson, F.E., and Lacey, J.V., (2002). Fruit and vegetable intakes and the risk of colorectal cancer in the Breast Cancer Detection Demonstration Project follow-up cohort. *Am J Clin Nutr*. **75**: 936-943.

Hajimahmudi, M., Sadeghi, N.N., Jannat, B., Oveisi, M.R., Madani, S., Kiayi, M., Akrami, M.R and Ranibar, A.M., (2008). Antioxidant activity, Reducing power and total phenolic content of Iranian Olive Cultivar. *J Biologic, Sci*. **8 (4)**: 779-783.

Hatano, T., Edamatsu, R., Mori, A., Fujita, Y. and Yasuhara, E., (1989). Effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2-picrylhydrazyl. *Chem Pharm Bull*. **37**: 2016–23.

Hussain, S.R., Cillard, J. and Cillard, P., (1987). Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry*. **26**: 2489–91.

Mohammad, S.R., Mohammad, Z.R., Md, A.W., Raseduzzaman, C. and Mohammad, A.R., (2008). Antimicrobial activity of some indigenous plants of Bangladesh. *Dhaka Univ. J. Pharm. Sci*. **7(1)**: 23-26.

Moroney, M.A, Alcaraz, M.J. and Forder, R.A., (1988). Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J Pharm Pharmacol*. **40**:787–92.

Ness, A.R and Powles, J.W., (1997). Fruit and vegetables, and cardiovascular disease: a review. *Int J Epidemiol*. **26**: 1-13.

Odabasoglu, F., Aslam, A., Cakir, A., Suleyman, H., Karagoz, Y., Bayir, Y., and Halici, M., (2005). Antioxidant activity, reducing power and total phenolic content of somelichen species. *Fitoterapia*. **76**: 216-219.

Poulsen, H.E., Prieme, H. and Loft, S., (1998) Role of oxidative DNA damage in cancer initiation and promotion. *Eur J Cancer Prev*. **7**: 9-16.

Prieto, P., Pineda, M., and Aguilar, M., (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, **269**: 337-341.

Ruiz, D., Egea, J., Gil, M.I. and Tomas-Barberan, F.A. (2006). Phytonutrient content in new apricot (*Prunus armeniaca* L.) varieties. *Acta Horticulturae*.**717**: 363-365.

Skerget, M., Kotnik, P., Hadolin, M., Hras, A., Simonic, M. and Knez, Z., (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food. chem*. **89:** 191-198.

Torel, J., Cillard, J. and Cillard, P., (1986). Antioxidant activity of flavonoids and reactivity with peroxy radicals. *Phytochemistry*. **25**: 383–5.

Van Wagenen, B.C., Larsen, R., Cardellina, J.H. II, Ran dazzo, D., Lidert, Z.C. and Swithenbank, C., (1993). Ulosantoin, a potent insecticide from the sponge Ulosa ruetzleri. *J. Org. Chem*. **58:** 335-337.

Wang, H., Cao, G. and Prior, R.L., (1996). Total antioxidant capacity of fruits. *J Agric Food Chem*. **44**: 701-705.