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Phenolic Contents and Antioxidant Capacities of Different Parts of *Withania somnifera* (L.) Dunal. from Three Different Growth Stages

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ABSTRACT

***Withania somnifera* (L.) Dunal (Family: Solanaceae) is a therapeutically important medicinal herb widely used in Ayurvedic and traditional systems of medicine in Sri Lanka. Present study describes the Total Phenolic Content (TPC) , Total Antioxidant Capacity (TAC) of different parts (leaves, stems, roots, flowers and fruits) of 3 different growth stages (just before flowering, just after flowering and fully matured stage) of *W. somnifera* gown under 3 different spacing levels (60cm × 30cm, 60cm × 45cm and 60cm × 60cm). The TAC of leaf extracts of *W. somnifera* in all 3 different growth stages were significantly higher ($p<0.05$) compared to all other parts of the plant. Out of 3 maturity stages tested the highest TAC of leaf extract for all three spacing levels (19.86±1.13, 27.21±1.55, 29.53±1.27 mg TE/g DW) was observed at just after flowering stage and slight reduction was observed at fully matured stage (19.66±2.04, 22.64±2.25, 21.23±0.99 mg TE /g DW). Leaf extracts of just after flowering stage exhibited the significantly higher TPC ($p<0.05$) followed by fully matured stage and just before flowering stage. Order of total phenol content was leaf>flower>fruit>stem>root. Presence of higher TPC and TAC in just after flowering stage scientifically validate traditional claims of harvesting of *W. somnifera* after flowering stage. The higher TPC and TAC in leaf in all three stages demonstrated the value of leaf for the development of newer effective drugs instead of roots and this creates an avenue for use of leaves in addition to roots.**

KEYWORDS: Antioxidant capacity, Growth stages, Phenolic content, *Withania somnifera.*

INTRODUCTION

Withania somnifera (L.) Dunal (Family: Solanaceae) is known as Amukkara in Sinhala, Ashwagandha in Sanskrit and, Indian Ginseng/Winter Cherry in English is a therapeutically important medicinal herb widely used in Ayurvedic and traditional systems of medicine over 3,000 years (Sangawan *et al.*, 2004). Due to its diverse therapeutic effect, it has been used in many groups of formulations for the treatment of tuberculosis, dyspepsia, leprosy, nervous disorders, intestinal infections, rheumatism, tumors, inflammations, bronchitis, ulcers, scabies, ophthalmus, syphilis and to overcome all kinds of weaknesses and increase the vigour and stamina (Anon, 1979; Jayaweera, 1981; Singh *et al.*, 2010).

Moreover, the most of the therapeutic effects such as promotion of physical and mental health (Battacharya, 1993). hypnotic and an anthelmintic (Nadkarni, 1976), antitumor, anti-bacterial, anti-genotoxic, anthelmintic, anti-inflammatory and anti-ulcer (Anon, 2002), anti-ageing process (Singh *et al.*, 2001), antitumor, antiangiogenesis and radiosensitizing activity (Shohat *et al.*, 1967; Bargagna-Mohan *et al.*, 2007) have been scientifically validated. Withanolides, steroidal lactones, phenols and flavonoides as active chemical groups and trace elements such as Fe, Cu, Ni, Mn, and Zn have been reported (Dhanani, 2013; Mirjalili *et al.*, 2009; Shirin *et al.*, 2009). Further, phytochemical studies revealed that Withaferin A, the active ingredient with the anticancer activity in *W. somnifera*, is well distributed in leaves, bark and stems in addition to the roots (Siriwardane *et al.*, 2013).

Economic value of *W. somnifera* is always on rise due to its broad spectrum of medicinal usage. However, Sri Lanka imports over 45,000 kg of *W. somnifera* roots by spending considerable foreign exchange annually due to lack of systematic cultivation in the country. On the other hand chemical distribution and antioxidant activity of different parts of different growth stages are key parameters determining the proper harvesting stage and the therapeutic activity of different parts of the plants.

Therefore, present study was undertaken to determine the antioxidant capacity and total phenolic content of different parts (leaves, stems, roots, flowers and pods) of *W. somnifera* from the three different growth stages (*viz.* just before flowering, just after flowering, and fully maturity).

MATERIALS AND METHODS

Location

Experiment was carried out in the experimental plots and the laboratory of the

Department of Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) from January to April 2013.

Cultivation and Collection of Samples

Withania somnifera seedlings were planted in 1 m x 3 m beds in 3 different spacing (60 cm× 30 cm, 60 cm × 45 cm and 60 cm × 60 cm) and maintained in similar soil and climatic conditions in university research plots. Samples of leaf, stem, root, flowers and pods were harvested in 3 different growth stages i.e. just before flowering, just after flowering, and fully maturity. Each treatment was replicated 3 times. Voucher specimen (HTS A- 20) was prepared and deposited in herbarium at Industrial Technology Institute (ITI), Sri Lanka.

Sample Preparation

All samples were washed and dried first at room temperature (28 ^{0}C ±2) for 3-5 days and then using an oven for 2 hr at 40 ^{0}C. Then dried samples were ground into powder using a grinder. Ground samples were packed in polythene bags and stored in a refrigerator until use.

Preparation of Extracts

Pre-prepared samples (0.1g) were accurately weighed into a 15 ml centrifuge tube and add 5 ml of 80% methanol. The sample was vortexed for 15 minutes and placed in a water bath at 60 ^{0}C for 40 minutes and vortex procedure was repeated in 10 min interval. Then samples were centrifuged at 4,000 rpm for 5 min and supernatant was decanted into 15 ml centrifuge tube and the remaining was re-extracted with 5 ml of 80% methanol. Both supernatants were collected and stored at -20 ^{0}C.

Determination of Total Antioxidant Capacity

Total antioxidant capacity was determined using Ferric Reducing Antioxidant Power (FRAP) assay as described by (Benzie and Stain 1996). Methanolic extract (100 µl) of extract was mixed with 900 µl of freshly prepared FRAP reagent of pH 3.6 containing 2.5 ml of 10 mmol/l, 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution in 40 mmol/l, HCl plus 2.5 ml of 20 mmol/l $FeCl_3$ and 25 ml of 300 mol/l acetate buffer. Absorbance of the reaction was measured at 593 nm using the spectrophotometer (Shimadzu, UV Mini 1240, Japan) after incubating for 4 minutes. The Trolox was used as the standard solution.

Determination of Total Phenolic Content

The Total Phenolic content was determined by modified Folin-Ciocalteu colorimetric method (Abeysinghe *et al.*, 2007). Four milliliter of distilled water and 0.5 ml of extract were added into a test tube. Then the same amount of (0.5 ml) of 0.5 N Folin-Ciocalteu reagent was added and allowed to react for 3 min. Then 1 ml of saturated sodium carbonate solution was mixed and samples were incubated in a water bath for 2 hr at 30 ^{0}C. The absorbance was measured at 760 nm using UV visible spectrophotometer (Shimadzu UV-160). Gallic acid was used as the standard. The total phenol content in one gram of plant extract was calculated and expressed as Gallic Acid Equivalents (GAE).

Statistical Analysis

All data were expressed as mean value means ± S.D. Data were analyzed using the general linear model (GLM) procedure of SAS statistical package followed by Duncan's Multiple Range Test (DMRT) for mean separation.

RESULTS AND DISCUSSION

Present study describes the total phenolic content (TPC), total antioxidant capacity (TAC) of different parts (leaf, stem, root, flowers and fruits) of 3 different growth stages (Just before flowering, just after flowering and fully matured stage) of *W. somnifera* gown under 3 different spacing levels (60 cm × 30 cm, 60 cm × 45 cm and 60 cm × 60 cm). As shown in Figure 1, the TAC of leaf extracts of *W. somnifera* in all 3 different growth stages were significantly higher ($p<0.05$) compared to all other parts of the plant. This may due to presence of high content of phenolics, flavonoids and variety of other pigments in the leaf. Our results are in agreement with (Sharma *et al.*, 2011) who found significantly higher TAC in leaf extracts of *W. somnifera*. Further, (Siriwardana *et al.*, 2013) was reported that the highest antioxidant capacity is well distributed in leaves then bark and stem and the roots.

Total antioxidant capacity of leaf extracts at just before flowering stage showed significant different among 3 spacing levels and TAC was also increased when increasing the spacing. The same increase was observed in just after flowering stage. The higher TAC in wider spacing levels might be due to plant exposure to the better light conditions.

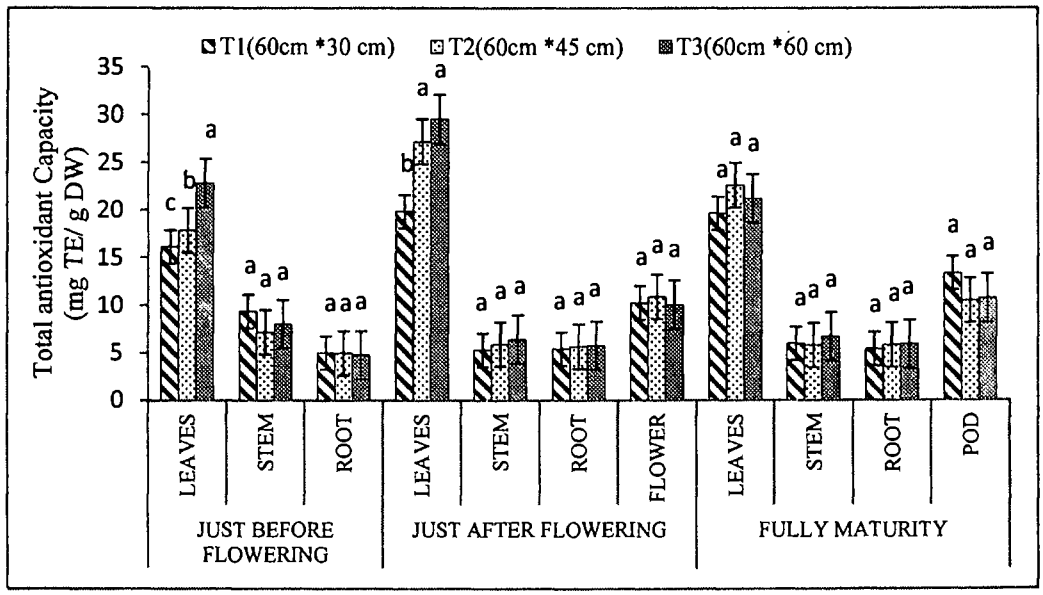

Figure 1. Total Antioxidants Capacity of different parts of *Withania somnifera* grown under 3 different growth stages

Means in a cluster with the same letters are not significantly different at the 0.05 level

These findings are in agreement with (Chutichudet *et al.*, 2011), who reported the higher content of secondary metabolites and antioxidant capacity in plants grown in fully sunlight conditions compared to plants grown under shady conditions. Out of 3 maturity stages tested the highest TAC of leaf extracts for all three spacing levels (19.86±1.13, 27.21±1.55, 29.53±1.27 mg TE/ g DW) were observed at just after flowering stage and slight reduction was observed at fully matured stage (19.66 ±2.04,22.64±2.25,21.23 ±0.99 mg TE/ g DW). This may be due to the production of higher secondary metabolites with maturity. Our findings are in agreement with Vallejo *et al.* (2003) and Navarro *et al.* (2006) who observed the increase of secondary metabolites and antioxidant capacity of Broccoli and *Agastacha rugosa* with the maturity.

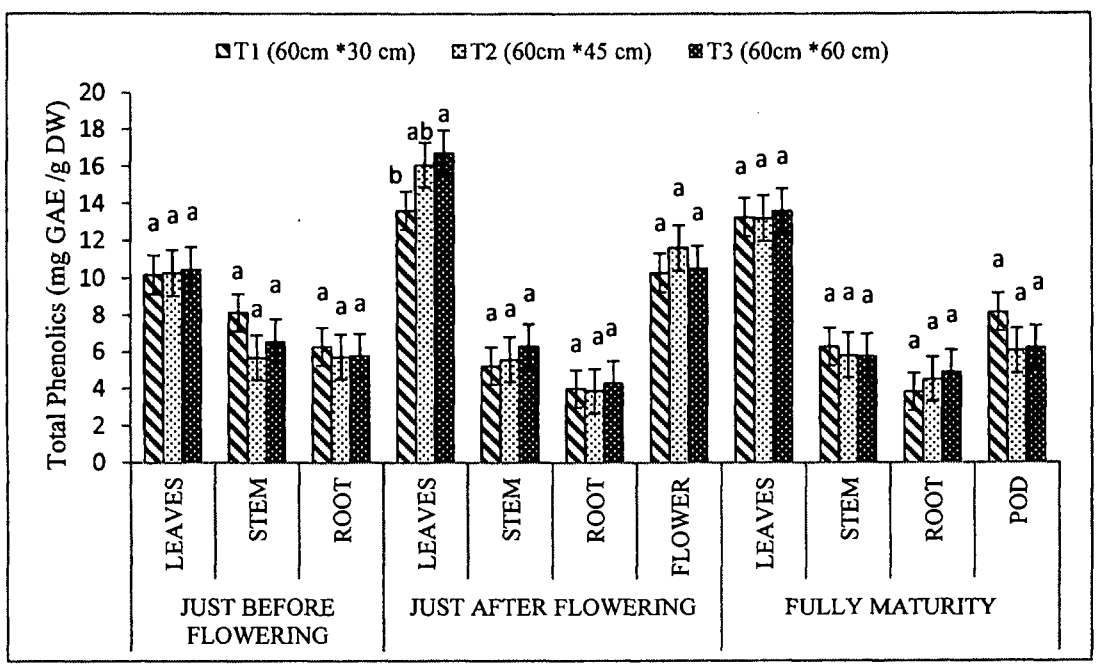

Figure 2. Total polyphenolics content of different parts of *Withania somnifera* grown under 3 different growth stages

Means in a cluster with the same letters are not significantly different at the 0.05 level

However, slight reduction of TAC in fully matured stage may be due to degradation of pigments and subsequently re-absorption into the plants. Moreover, order of increase of TAC in different plant parts of *W. somnifera* were leaf>pod>flower>stem>root.

Polyphenols are group of secondary metabolites which is responsible for an array of physiological properties (Benavente-Garcia *et al.*, 1997). Results of total phenolic content (TPC), of different parts of 3 different growth stages of *W. somnifera* grown under 3 different spacing levels are presented in Figure 2. It was clearly demonstrated that all the parts of *W. somnifera* possess TPC. Leaf extracts of just after flowering stage exhibited the significantly higher TPC ($p<0.05$) followed by fully matured stage and just before flowering stage.

In addition to leaf extracts, flower and fruit extracts also exhibited much higher TPC compared to the stem and root extracts. However, no statistically significant variations were observed for any spacing levels except in leaf extracts in just after flowering stage. Order of total phenol content was leaf>flower>fruit> stem>root.

In general, the antioxidant capacity of plant extracts is associated with group of compounds, such as phenol, flavones, flavonols, carotenoids and pigments like proanthocyanidins etc. (Skerget *et al.*, 2005). Out of above group of compounds, phenolics act as stabilizing agent of scavenging radicals (Rice-Evans *et al.*, 1997). In the present study we have also observed a positive relationship between TPC and TAC of different extracts of *W. somnifera.*

CONCLUSIONS

Present study demonstrated the total phenolics and antioxidant content of locally grown *W. somnifera* for the first time in Sri Lanka. Presence of higher contents of total phenolics and total antioxidant capacity in just after flowering stage scientifically validate traditional claims of harvesting of *W. somnifera* after flowering stage. The higher content of total phenolics and antioxidant capacity in leaf in all three stages demonstrated the value of leaf for the development of newer effective drugs instead of roots and this creates an avenue for use of leaves in addition to roots.

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