*Indian J. Plant Physiol.*, Vol. 13, No. 4, (N.S.) pp. 394-399 (Oct.-Dec., 2008)

**SHORT COMMUNICATION**

# ANTIOXIDANT ACTIVITIES, PHENOLS AND FLAVONOID CONTENTS OF *WITHANIA SOMNIFERA* AND *RAUWOLFIA SERPENTINA*

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Received on 25 Dec., 2008

**The oxidative damage caused by free radicals to lipids, proteins and nucleic acids can trigger various chronic diseases such as coronary heart diseases, atherosclerosis, cancer, AIDS and ageing. In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. In the present study, we carried out a systematic record of the relative antioxidant activity in two selected medicinal plant species extracts. Our findings clearly indicated higher levels of both flavonoid (160.88mg $g^{-1}$ dry weight of leaf) and phenolic (mono di and total, 196.32, 65.25 and 268.61mg $g^{-1}$ dry weight of leaves, respectively) contents in the extracts of *Withania somnifera* compared to *Rauwolfia serpentina* (flavonoid 127.7mg, monophenol 170.58mg, diphenol 43.13mg and total phenol 223.32mg $g^{-1}$ dry weight of leaf, respectively). Similar results (*W. somnifera*: 47.34 and *R serpentina*: 35.54 % inhibition $g^{-1}$ dry weight) were also obtained for *in-vitro* superoxide anion scavenging capacity.**

***Key words:*** **Antioxidant capacity, flavonoid, phenol, *Rauwolfia serpentina*, *Withania somnifera*.**

Majority of the diseases / disorders are mainly linked to oxidative stress due to free radicals (Gutteridgde 1995). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari 2001). The most common Reactive Oxygen Species (ROS) include superoxide anion ($O^{\cdot-}_2$), hydrogen peroxide ($H_2O_2$), peroxyl ($ROO^{\cdot}$) and hydroxyl ($OH^{\cdot}$) radicals. The oxidative damage caused by these ROS to lipids, proteins and nucleic acids can trigger various chronic diseases such as coronary heart diseases, atherosclerosis, cancer, AIDS and ageing (Finkel and Holbrook 2002). In treatment of these diseases, antioxidant therapy has gained an immense importance. Antioxidants have been reported to prevent oxidative damage caused by free radicals / ROS, and may prevent the occurrence of diseases (Velavan *et al.* 2007). It can interfere with the production of free radicals and also with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Buyukokuroglu *et al.* 2001). Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the current scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (Auudy *et al.* 2003).

Flavonoids and phenolic compounds, widely distributed in plants, have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic *etc*. (Miller 1996). Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties (Koleva *et al.* 2002, Oke and Hamburger 2002).

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In Indian system of medicine, both *Withania somnifera* (Family Solanaceae) and *Rauwolfia serpentina* (Family Apocynaceae) are two important plants and their roots and leaves have been used in various ailments and as health tonic. The roots of *W. somnifera* are categorized as rasayanas, and are reputed to promote health and longevity (Weiner and Weiner 1994). Also, used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory, astringent and more recently to treat ulcers, bacterial infection, venom toxins, neurological disorders and Parkinsons diseases (Machiah *et al.* 2006, Rasool and Varalakshmi 2006). Its chemo preventive properties make it a potentially useful adjunct for patients undergoing radiation and chemotherapy (Ichikawa *et al.* 2006). Like *W. somnifera*, *R. serpentina* is also used in the treatment of hypertension, anxiety, worry, and irritability, and have come to be popularly known as "tranquillizing" drug (Malamud *et al.* 1957). It is also known as an antidote to snake bite, to remove white spots in the eyes, against stomach pains, fever, vomiting, blood pressure, control nerve impulse and headache and to treat insanity (Gupta and Rana 2007). Therefore, the objectives of the present study were to investigate the antioxidant potentials of both *W. somnifera* and *R. serpentina* through their superoxide anion scavenging activities and also to correlate this with their respective phenolics and flavonoid contents.

The *W. somnifera* and *R. serpentina* plants were collected from the Botanical Garden of School of Life Sciences, Pt. Ravishankar Shukla University, Raipur 492 010 (CG). Plants were washed carefully under tap water to make them free from the contaminants. These were then sun dried completely. Dried leaf (both young and old) and root tissues were ground separately in to fine powder. Five grams of each plant powder was extracted in 50 ml of methanol by intermittent maceration up to 48 hrs. The solvent was evaporated and reduced up to 5 ml at room temperature. After evaporation, samples were centrifuged at 10,000 rpm for 15 min at room temperature; supernatants were collected and used for various estimations.

Aluminium chloride spectrophotometric method was used for flavonoids determination (Chang *et al.* 2002). The reaction mixture comprised of 0.1 ml extract, 0.1 ml aluminium chloride (10%), 0.1 ml potassium acetate (1M) and 2.7 ml distilled water. It was kept at room temperature ($37^0$C) for 30 min. The absorbance was measured at 415 nm by using a spectrophotometer. The calibration curve was prepared using different concentrations of quercetin. Flavonoid content was expressed as mg total flavonoid $g^{-1}$ dry weight of the leaf or root tissues.

Monophenols were estimated by the method of Keshavkant (2000). The reaction mixture consisted of 0.1 ml extract, 0.9 ml distilled water, 1.0 ml sodium hydroxide (0.5N), 1.0 ml 4-amino-antipyrene (0.6%), 1.0 ml sodium bicarbonate (saturated solution) and 1.0 ml potassium ferrocyanide (2.4%). Tubes were incubated for 45 min at room temperature ($37^0$C). The absorbance was recorded at 520 nm using a spectrophotometer. The Hydroxy benzene was used to prepare a standard curve. O-Dihydroxyphenol content was assayed by the method of Mahadevan (1975). The reaction mixture consisted of 0.1 ml extract, 0.9 ml distilled water, 1.5 ml hydrochloric acid (0.5N), 1.5 ml Arnous reagent and 1.0 ml sodium hydroxide (1N). After incubation of 45 min at room temperature; absorbance was measured at 525 nm using spectrophotometer. Catechol was used to prepare standard curve. Total phenols were determined by Folin ciocalteu reagent (McDonald et al. 2001). The extract (0.1 ml) was mixed with 0.9 ml distilled water, 2.0 ml Folin ciocalteu reagent (1N) and 2.0 ml of saturated sodium bicarbonate. The absorbance of the mixture was measured at 765 nm by using a spectrophotometer after incubating it for 45 min at room temperature. Standard curve was prepared using chlorogenic acid and all the phenolic contents were expressed as mg mono/di/total phenol $g^{-1}$ dry weight of the plant samples.

The scavenging activity was measured by the method of Liu *et al.* (1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments, the superoxide anion was generated in 2 ml of Tris-HCl buffer (100mM, pH 7.4) containing 0.25

ml of NBT (300 µM) solution, 0.25 ml of NADH (936 ìM) solution and 0.15 ml of plant extract. The reaction was initiated by adding 0.25 ml of PMS (120 ìM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging capacity was calculated according to the following equation:

$$\% \text{ Inhibition} = (A_o - A_1) / A_o \times 100$$

Where, $A_o$ = absorbance of the control and $A_1$ = absorbance in the presence of the extract.

All the values obtained were tested (t-test) for their significant differences (P = 0.05) (Steel and Torrie 1980) and expressed as mean±SD of five independent tests.

In general, higher levels of flavonoid contents were observed in young leaves (127.70±1.49 and 160.88±1.70 mg $g^{-1}$ dry weight) as compared to both old leaves (123.96±1.07 and 156.31±1.15 mg $g^{-1}$ dry weight) and roots (41.56±1.04 and 82.07±1.29 mg $g^{-1}$ dry weight) of *R. serpentina* and *W. somnifera*, respectively (Table 1). If, we compare the flavonoid contents between both the species, then it can be inferred that higher values were obtained in the extracts of *W. somnifera* (160.88±1.70 mg $g^{-1}$ dry weight) compared to the *R. serpentina* (127.70±1.49 mg $g^{-1}$ dry weight) (Table 1).

Like flavonoids, all the three phenolics (mono, di and total) estimated were exhibited higher values in the *W. somnifera* as compare to *R. serpentina* (Table 1). Highest phenolic (mono, di and total) contents were determined in the young leaves (Mono: 196.32±3.53 & 170.58±1.9, Di: 65.25±1.40 & 43.13±0.21, Total: 268.61±1.76 & 223.32±3.21 mg $g^{-1}$ dry weight) and lowest values in the roots (Mono: 56.93±1.41 & 33.45±2.12, Di: 77.92±1.39 & 24.17±0.70, Total: 111.73±0.98 & 84.13±0.70 mg $g^{-1}$ dry weight) of both *W. somnifera* and *R. serpentina*, respectively (Table 1). In the old leaves of both the species, the phenolic contents estimated were in between the young leaves and roots (Table 1).

Table 1 indicates the superoxide anion scavenging activities of both leaves and root extracts of *W. somnifera* and *R. serpentina* on the PMS-NADH-NBT system. The decrease of absorbance at 560nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. In general, it was noticed that the extracts of *W. somnifera* were having

**Table 1.** Changes in the flavonoids, phenolics and superoxide anion scavenging activities in leaves (young & old) and roots of both *Withania somnifera* and *Rauwolfia serpentina*. Values are mean±SD of 5 separate determinants. Values presented for both the species were significantly different from each other at P = 0.05 level.

| **Parameters** | ***Withania somnifera*** | | | ***Rauwolfia serpentina*** | | |
|---|---|---|---|---|---|---|
| | **Young leaves** | **Old leaves** | **Roots** | **Young leaves** | **Old leaves** | **Roots** |
| Flavonoids (mg $g^{-1}$ dry weight) | 160.88±0.7 | 156.31±0.15 | 82.07±0.29 | 127.7±1.49 | 123.96±0.07 | 41.56±0.04 |
| Monophenol (mg $g^{-1}$ dry weight) | 196.32±3.53 | 159.40±3.74 | 56.93±1.41 | 170.58±1.9 | 111.39±0.70 | 33.45±2.12 |
| Diphenol (mg $g^{-1}$ dry weight) | 65.25±1.40 | 56.22±1.39 | 77.92±1.39 | 43.13±0.21 | 42.83±0.84 | 24.17±0.70 |
| Total phenol (mg $g^{-1}$ dry weight) | 268.61±1.76 | 237.29±1.12 | 111.73±0.98 | 223.32±3.21 | 189.97±1.19 | 84.13±0.7 |
| Superoxide anion scavenging activity (% inhibition $g^{-1}$ dry weight) | 47.34±1.74 | 30.17±0.49 | 26.81±0.96 | 35.54±2.41 | 21.78±2.04 | 11.35±0.67 |

significantly more scavenging capacity compared to the *R. serpentina* (Table 1). If we compare scavenging capacity between different plant parts of a particular plant; it was observed that young leaves were having highest scavenging capacity (47.34±1.74 and 35.54±2.41 % inhibition $g^{-1}$ dry weight) whereas roots are showing least capacity (26.81±0.96 and 11.35±0.67 % inhibition $g^{-1}$ dry weight) in both *W. somnifera* and *R. serpentina*, respectively (Table 1).

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Among those substances, the flavonoids widely distributed in plants, have the ability to scavenge free radicals, superoxide and hydroxyl radicals (Velavan *et al.* 2007). The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al.* 2003). The highest levels of flavonoid contents, in general, were observed in the extracts of *W. somnifera* compared to *R. serpentina* (Table 1). The flavonoid contents were more in the young leaves of both *W. somnifera* and *R. serpentina* (160.88±1.70 and 127.70±1.49 mg $g^{-1}$ dry weight) and lowest in their respective root tissues (82.07±1.29 and 41.56±1.04 mg $g^{-1}$ dry weight) (Table 1). Plethora of literature shows that, these plants contain several types of biologically active compounds like, alkaloids and steroidal compounds (Elsaka *et al.* 1990, Ganzera *et al.* 2003). It was also established that the flavonoid type of compounds, which contain hydroxyls, are responsible for the superoxide anion scavenging activities (Das and Pereira 1990). According to our findings, the high content of this phytochemicals in young leaves of *W. somnifera* can probably explain its high superoxide anion scavenging activity, compared to *R. serpentina*.

Phenolic compounds can act as antioxidants by radical scavenging, in which they break the free radical chain reaction through hydrogen atom donation (Kosem *et al.* 2007). The resulting phenoxy radical can be reduced to its parent compound by enzymatic or nonenzymatic reactions (Sakihama *et al.* 2002). Another possible antioxidant mechanism is *via* metal chelation and restriction of the accessibility of the metal ion for participation in Fenton-type reactions (Cheng and Chang 2003, Kosem *et al.* 2007). Like flavonoids, the levels of all the three phenols were exhibited more in the *W. somnifera*, compared to the *R. serpentina*, and also maximum in the young leaves and minimum in the roots of the same species (Table 1). Similar findings were also reported for *Acorus calamus*, *Terminalia arjuna*, *Terminalia bellerica*, *Mellilotus officinalis*, *etc*. (Pourmorad *et al.* 2006, Teow *et al.* 2007, Velavan *et al.* 2007). So, it can be assumed that, the higher phenolic contents of young leaves of both the species may play an important role in the superoxide scavenging activities through free radical quenching, electron transfer, radical addition or radical recombination.

In general, lower levels of superoxide scavenging activities were measured in the *R. serpentina* compared to *W. somnifera* (Table 1). Maximum superoxide anion scavenging activity (47.34±1.74 % inhibition $g^{-1}$ dry weight) was measured in young leaves of *W. somnifera* and minimum (11.35±0.67 % inhibition $g^{-1}$ dry weight) in the roots of *R. serpentina* (Table 1). The old leaves of both the plants were having intermediate scavenging capacity (Table 1). The results indicated that, the superoxide anion scavenging activity varied widely among plants and at the same time within the plant parts of the same plant also (Table 1). The data obtained in the above study showed that, the young leaves of *W. somnifera*, which contain maximum flavonoid as well as phenolic compounds, exhibited the greatest superoxide anion scavenging activity (47.34±1.74 % inhibition $g^{-1}$ dry weight). It can be possible that, this high superoxide anion scavenging capacity of *W. somnifera* may be due to hydroxyl groups existing in the phenolic compound's chemical structure that can provide the necessary component as a radical scavenger (Pourmorad *et al.* 2006). Similar findings were also recorded by Pourmorad *et al.* (2006), Teow *et al.* (2007) and Velavan *et al.* (2007) for various species. In conclusion, the results of the present study reveal that, both *W. somnifera* and *R. serpentina* contain higher amounts of flavonoids as well as phenolics and so exhibited the greatest superoxide anion scavenging activities through the scavenging of free radicals, which participate in various pathophysiology of diseases including ageing. Here, we have also proven the relation between flavonoid and phenol contents with superoxide anion scavenging activity, as reported previously (Pourmorad *et al.* 2006,

Velavan *et al.* 2007, Teow *et al.* 2007). Overall, both the plant extracts were potent source of natural antioxidants that can be important in disease prevention, health preservation and longevity promotion.

## ACKNOWLEDGEMENTS

We thank Head, School of Life Sciences, Pt. Ravishankar Shukla University, Raipur, for providing the necessary laboratory facilities.

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