International Journal of Drug Development & Research
| Oct-Dec 2010 | Vol. 2 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
©2010 IJDDR

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

# PHARMACOGNOSTIC AND FREE-RADICAL SCAVENGING ACTIVITY IN THE DIFFERENT PARTS OF ASHWAGANDHA [*Withania somnifera* (L. Dunal)]

**Mandal Palash[1,*], Ghosal Mitali[1], Misra Tarun Kumar[2], Das Abhaya Prasad[1]**

[1]Department of Botany, North Bengal University, Darjeeling, West Bengal, 734013, India.
[2]Department of Tea Management, North Bengal University, Darjeeling, West Bengal, 734013, India.

## ABSTRACT

*Different parts of Ashwagandha [Withania somnifera (L. Dunal)] were evaluated for their antioxidant against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and anti-lipid peroxidation activity. Strong antioxidant scavenging activities were observed in mature root and young root bark portion. Anti-oxidative efficiency to inhibit anti-lipid peroxidation of these plant extracts in goat liver was investigated. Mature root bark of Ashwagandha showed higher class of anti-lipid peroxidation against thioburbituric acid but the stelar part of young root have low anti-lipid peroxidation activity. The correlation was also drawn with antioxidants, its attributes and soil nutrients profile. Pharmacognostic evaluation including examination of microscopical characters and determination of leaf constants (stomata frequency, stomatal index, palisade ratio, vein islets and vein termination number) were done for determining the authenticity of drug. The observations from this study suggest the ethno-medicinal use of Ashwagandha which could be commercially exploited by the pharmaceutical industry for natural antioxidant.*

***Keywords:*** *Withania somnifera, Free-radical scavenging, Anti-lipid peroxidation, TLC fingerprint, quantitative microscopy.*

## INTRODUCTION

Ashwagandha [*Withania somnifera* (L. Dunal)], also called winter cherry, is an erect branched shrub, cultivated widely in central and western parts of India and North America. The shrub has traditionally been used for cheering the mind, relieving weakness, nervous exhaustion and arthritis, and for building sexual energy. It is also beneficial to people who do physical labour or exercise a lot to help the body adapt to physical stress. In western world it is frequently referred as 'Indian Ginseng' [1]. Ashwagandha roots are used as tonic for brain and nervous system and in preventive health care in Ayurveda and described as Medhya Rasayana (beneficial to the brain) in Ayurvedic literatures [2].

*W. somnifera* contains many alkaloids like aswagandhine, cuscohygrine, anahygrine etc.; steroidal compounds including ergostane type steroidal lactones, withaferin A, withanolides A-Y etc.; other constituents like saponins with an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 [3,4]. The withanolide class of phenolic compounds acts to prevent or reduce oxidative stress by scavenging free radicals [5,6].

The free radicals and reactive oxygen species are generated in living organisms during metabolism, leading to several human diseases such as cancer, stroke, myocardial infraction, diabetes, and the aging process. In recent years much attention has been devoted to natural antioxidant and their association

**For Correspondence:**
E-mail: nbubotanypalash@rediffmail.com

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

with health benefits [7]. Plants are potential sources of natural antioxidants. It produces various secondary metabolites to counteract reactive oxygen species (ROS) in order to survive [8]. Natural antioxidants tend to be safer and they also possess antiviral, anti-inflammatory, anti-cancer, anti-tumor and hepato-protective properties [7]. Numerous studies over the past two decades indicate that Ashwagandha provides potent antioxidant protection [9,10,11] and stimulates the activation of immune system cells, such as lymphocytes and phagocytes [12,13,14,15].

Although many works on antioxidant activity in root bark of *W. somnifera* were recorded but almost no efforts were taken for assessing this property in different parts of this plant and their variability with agronomic traits. Therefore, a study has been undertaken to evaluate *in vitro* antioxidant activity in different parts of *W. somnifera* and related phyto-pharmaceutical attributes were also correlated with their soil profile. The authenticity of this drug was determined through pharmacognostic characteristics and antioxidants were further evaluated through HPTLC based fingerprint analysis.

## Materials and Methods

### Plant materials

Different parts of *W. somnifera* cv. JN-20 like bark of mature root, senescent leaves, fruits, mature leaves, stellar part of young roots, calyx, young stems, young root bark portion, stellar part of mature roots and immature leaves were collected and separated from different cultivated plots of Medicinal Plant Garden of the North Bengal University. The plant material was authenticated from Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. The material was deposited in the 'NBU Herbarium' and recorded against the accession no 9584 dated 12.03.2010.

### Animal material

Goat liver, which was used for anti-lipid peroxidation assay, was collected from slaughter house immediately after slay. Experiment was conducted within one hour after collection.

### Chemicals

Methanol (M), 2.2-diphenyl-1-picryl hydrazyl (DPPH), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide phosphate sodium salt monohydrate (NADPH), phenazine methosulphate (PMS), trichloroacetic acid (TCA), thiobarbituric acid (TBA), $FeSO_4.7H_2O$, KOH, $KH_2PO_4$, ethylene-diamine tetra acetic acid (EDTA), ascorbic acid, vitamin-E, 2-deoxyribose, ferric chloride ($FeCl_3$), hydrogen peroxide ($H_2O_2$), sodium nitroprusside and sodium carbonate ($Na_2CO_3$) were either purchased from Sigma Chemicals (USA), or of Merck analytical grade.

### Soil sampling and determination of physicochemical properties

Soil samples were collected from different *W. somnifera* cultivated plots of Medicinal Plant Garden of the North Bengal University (top and sub soil with 0-15 cm and 15-30 cm depth respectively) and composite soil was prepared as per the standard method of soil analysis [16]. Physicochemical analysis like pH, electrical conductivity, moisture contents, organic matter, available form of nitrogen, potash as $K_2O$, phosphorus as $P_2O_5$ and sulphur as $SO_4^{-2}$ were made with the samples as per method described by Jackson, 1968 [17].

### Extraction and determination of methanol extractive value

Fresh parts of *W. somnifera* were extracted by standard solvent extraction method [18,19]. Every plant parts were dried in the sun for seven days and finally in an oven below 60°C. The dried plant materials were ground into fine powder and separately extracted with methanol : water : : 4 : 1, under soxhlet extractor for

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

eight hours. The solvents were completely removed by vacuum evaporator at 50°C. These crude extracts were used for further investigation. The extractive value of the pant materials were calculated on dry weight basis from the formula given below:

$$\text{Percent extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

**Antioxidant activity assay**

*DPPH scavenging activity assay*

The free radical scavenging capacity of different parts of *W. somnifera* was determined by using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) [20]. 0.1 mM solution of DPPH in methanol was prepared. 1.8 ml of this solution was added to 0.2ml of test solutions at different concentrations (0.1 mg/ml-1mg/ml) in different test tubes. Thirty minutes later, the absorbances were measured at 517 nm. Methanol was used as a blank. The percentage of free radical scavenging activity was calculated as follows:

$$\text{Percent inhibition of DPPH radical} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

*Anti-lipid peroxidation (ALP) assay*

Lipid peroxidation was measured *in vitro* with the extracts of liver homogenate of male goat in terms of formation of thioburbituric acid reactive substances by the method of Dhawal *et al.* 2005 [21]. 0.l ml of the plant samples were individually added to 2.8 ml of 10% liver homogenate and 0.1 ml of 50 mM $FeSO_4$. The reaction mixture was incubated at 37°C for 30 min. This reaction mixture (1 ml) was mixed with 10% TCA-0.67% TBA (2 ml) in acetic acid. Then the combination was boiled for one hour at 100°C and centrifuged for 5 minutes at 10000 rpm. Supernatant was used for measuring OD value at 535 nm. Blank contained all reagents except liver homogenate and extract. Control was prepared by mixing all reagents without extract and $FeSO_4$ and iron induced sample was organized by mixing all reagents devoid of extract. Vitamin E was used as standard. ALP percentage was calculated by using the following formula:

$$\text{ALP \%} = \frac{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{Abs. of sample}}{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{Abs. of control}} \times 100$$

**Thin Layer Chromatography**

The powdered drug of mature root bark of *Withania somnifera* (2 g.) was extracted with chloroform and ethyl acetate successively. For thin layer chromatography, ten micro-liters (conc. 100 mg/ml extractive) of extracts was spotted on silica gel precoated TLC plates (Merck, Germany) and placed in solvent mixture (chloroform : ethylacetate : methanol : benzene :: 74:4:8:24), which acted as mobile phase. Chromatograms were evaluated first under UV light at 254 nm for observing the pattern of phytochemical profile and then visible light to detect the presence of antioxidant compounds by spraying with 0.2% DPPH (in methanol) and vanillin-$H_2SO_4$ reagent for detection of polyphenols.

**Determination of pharmacognostic characteristics-Anatomical and microscopic study of fresh and powdered drug**

For the anatomical study, leaf, petiole, stem, root of fresh plant were collected, sectioned and stained with safranin and light green. For quantitative microscopy, stomatal number, stomatal index, vein islet and veinlet termination number were determined by using fresh leaves of plant after removal of pigments [22]. Powder of the dried root was used for the observation of powder microscopic characters.

**Statistical analysis**

The data were pooled in triplicate and subjected to analysis of correlation co-efficient matrix using SPSS (Version 12.00) for drawing the relation between soil physicochemical properties and antioxidant attributes and MS Excel of Microsoft Office, 2007 was used for comparing the antioxidant attributes of different plant parts collected from different cultivated plots. Smith's

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

Statistical Package (Version 2.5) was used for determining the $IC_{50}$ values of antioxidants and their standard error of estimates (SEE). In order to examine and visualize relationships between soil attributes and antioxidant traits, a principal component analysis (PCA) based on the correlation matrix was calculated using Multivariate Statistical Package (MVSP 3.1).

**Results and Discussion**

In this present study the antioxidant activity of the methanolic extracts of the different parts of ashwagandha were investigated by using DPPH scavenging assay and anti-lipid peroxidation of the extracts. Methanolic extracts of every parts of *W. somnifera* have got profound antioxidant activity. Although the stellar part of mature root, young root bark portion and bark of mature root have very less $IC_{50}$ values (0.4764, 0.529 and 0.5166 µg/ml respectively) and higher extractive values (11.38 mg/ml, 15.78mg/ml and 13.98 mg/ml respectively) but other parts also contain high free radical scavenging activity. The level of the antioxidant activity of different plant parts were as follows: stellar part of mature root > young root bark portion > bark of mature root > senescent leaf > fruits > mature leaf > young stem > immature leaf > stellar part of young root > calyx. The antioxidants react with the stable free radical DPPH (deep purple colour) and convert it to 2, 2-diphenyl-1-picryl hydrazine with decoloration [23]. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance [24]. Peroxidized free radicals of lipid molecules were highly scavenged by the methanolic extract of the bark of mature root, senescent leaf, fruit and mature leaf of *W. somnifera*. Other parts of this plant also have minute amount of scavenging activity. The extracts that showed encouraging response in $IC_{50}$ values were given (Fig. 1).

With liver homogenate the extracts undergo rapid peroxidation when incubated with $FeSO_4$ and produce peroxide [25] and they attack the biological material. This leads to the formation of malonaldehyde and other aldehydes, which form a pink colour with TBA, absorbing at 535nm [26]. It was observed (Fig. 2) that methanol extract of *W. somnifera* have high anti-lipid peroxidation effect against goat liver. The highest anti-lipid peroxidation activity was found ($IC_{50}$ value 0.44 ± 0.077 µg/ml) in bark of mature root. The level of the ALP of different plant parts were as follows: bark of mature root > senescent leaf > fruits> mature leaf > young root bark portion > stellar part of mature root > calyx > young stem > immature leaf > stellar part of young root.

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help for estimation of specific constituents soluble in a particular solvent [27]. In the present investigation alcohol soluble extractive values increased with maturity of roots.

For anatomical identification, root was sectioned transversely and the section showed that epidermis is uniseriate and consists of thin walled, i.e., non-cuticularised tubular cells, outer walls of some epidermal cells are prolonged outward to form unicellular root hairs, cortex is multiseriate, Parenchyma consists of larger and polyhedral cells with conspicuous intercellular spaces, vascular bundles are radial. In microscopic vision, powdered root showed pitted and lignified parenchyma, two types of parenchyma cells were observed viz. larger polyhedral cells with intercellular space and smaller cells with no intercellular spaces. Vessels were present with scalariform thickenings, fibers with enlargement at the ends (Fig. 3). The microscopical characters of the root can serve as diagnostic parameters. The microscopic studies of the transverse section showed presence of

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

scalariform thickening in vessels and pitted, lignified parenchyma cells, which are the characteristics of the family Solanaceae [28].

Stomatal frequency, stomatal index, palisade ratio, vein islet and vein termination are the means of qualitative microscopic evaluation of drug plants. These are constant for a particular plant species. Stomatal frequency, stomatal index, palisade ratio, vein islet and vein termination of Aswagandha leaves are 205.63, 26.55, 23.08, 62.20 and 77.46 per $mm^2$ respectively. These character as seen in the transverse section of leaves are the distinguishing features and can be used as anatomical markers.

TLC profiles of an herbal drug remain bears significant chemical signature even when samples are taken from wild, cultivated or from individuals growing under different agro-climatic conditions [29]. For thin layer chromatographic identification, the chloroform and ethylacetate extracts were loaded on Lane A and B respectively of Plate I and also on Lane C and D respectively of Plate II. The ethyl acetate extracted powdered drug of *Withania somnifera* showed the presence of bio-active components appearing as a bright yellow spot at hRf value of 25.11 (Table 3) after spraying with DPPH solution on the TLC plate (Fig. 4 II-D). The corresponding hRf values of polyphenols identified after application of vanillin-$H_2SO_4$ reagent on TLC plate were from 26.45 to 29.89 respectively (Table 2) with colour pattern from blue to olive-green (Fig. 4 I-B). Chloroform extracted samples did not show any remarkable DPPH scavenging spots on TLC plate as expressed from Fig. 4 II-C. Maximum number of spots appeared as blue colour after vanillin-$H_2SO_4$ treatment on plate I, which indicates that monoterpene alcohol and their esters were concentrated as diversified chemical form in *Withania* bark after maturation.

Mineral nutritional status and physical properties, generally pH, EC and macro-nutritional properties of soil greatly influence the phytochemical constituents present in the different parts of the plant. It is also reported that phytochemical constituents directly influence the antioxidant properties of the plant extract [30]. In order to identify the potential relationship between free radical scavenging activity and soil physico-chemical properties, Pearson correlation analysis were performed on average values of antioxidant and soil parametric traits. In case of mature root bark, which is potentially used as drug, only significant positive correlation ($P<0.05$) was obtained between $IC_{50}$ of DPPH radical scavenging and soil pH. Moisture content of soil is another significant determining factor for elicitation of antioxidants. It was observed that moisture content of soil is positively correlated with anti-lipid peroxidation of mature root bark and flower; as well as $IC_{50}$ value of radical scavenging and anti-lipid peroxidation activity of stellar portion of young root and calyx. It means that high moisture content of soil practically reduces the antioxidant property of concerned tissue of Ashwagandha. Macro-nutritional components of soil were also differentially correlated with different parts of the plant. As recorded, DPPH radical scavenging activity was positively influenced by organic matter and phosphates in case of immature root bark and negatively correlated with organic matter and nitrogen for young leaves. Potash significantly determines the performance of anti-lipid peroxidation in case of young leaves. High application of potash may be helpful for enhancing DPPH radical scavenging activity during senescence stage of leaf but the same application may be strongly inhibitory for radical scavenging in flowers. Sulphur applications possibly have little contribution for enhancing these two antioxidant activities as expressed in Table 1.

In order to classify the antioxidant response of different parts of *Withania somnifera* and their relationship with soil parameters, a principal component analysis (PCA) was carried out on whole set of average values. As shown in Fig. 5 the first axis was essentially explained

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

by high concentration phosphates and potash in soil. The location of calyx and mature root bark in Fig. 6 indicated that DPPH scavenging is influenced by phosphates and potash availability in soil. On the other hand young stem which is present in central position in Fig. 6, 7 and 8 revealed intermediate values for antioxidant, anti-lipid peroxidation and extractive values with soil parameters like organic matter, nitrogen, pH and electrical conductivity. Anti-lipid peroxidation activities of mature root bark was associated with phosphates and potash of soil because both have higher values on *x*-coordinate (PC1). Central location of extractive values of young stem, stellar part of young root and stellar part of mature root indicate that they are negatively or positively biased on soil organic matter, nitrogen, pH and moisture content.

## CONCLUSION

This study suggests that the *W. somnifera* (L.) Dunal plant parts like bark of mature root, senescent leaves, fruits, mature leaves, stellar part of young roots, calyx, young stems, young root bark portion, stellar part of mature roots and immature leaves extracts possess antioxidant activity in DPPH assay and lipid peroxidation in goat liver, which might be helpful in preventing or slowing the progress of various oxidative stress-induced diseases. The fresh and powdered drug of this plant can be identified easily by TLC fingerprinting and by microscopic anatomy.

| Soil Properties | MRB | | MRS | | YRB | | YRS | | YS | | YL | | ML | | SL | | C | | F | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DPPH | ALP | DPPH | ALP | DPPH | ALP | DPPH | ALP | DPPH | ALP | DPPH | ALP | DPPH | ALP | DPPH | ALP | DPPH | ALP | DPPH | ALP |
| pH | 0.948* | 0.750 | 0.909* | -0.719 | 0.499 | -0.791 | 0.532 | 0.854 | 0.771 | 0.401 | -0.155 | 0.963 | 0.688 | -0.616 | -0.574 | 0.084 | 0.776 | 0.556 | 0.771 | 0.853 |
| Electrical Conductivity | -0.596 | 0.332 | -0.507 | 0.705 | 0.487 | 0.828 | 0.622 | 0.224 | -0.829 | 0.068 | -0.469 | -0.166 | 0.442 | 0.866 | -0.185 | -0.857 | 0.350 | 0.583 | 0.010 | 0.227 |
| Moisture | 0.362 | 0.950* | 0.383 | -0.046 | 0.864 | -0.033 | 0.986** | 0.994* | 0.007 | 0.373 | -0.556 | 0.733 | 0.961* | 0.140 | -0.693 | -0.642 | 0.972* | 0.957* | 0.720 | 0.942* |
| Organic Matter | -0.125 | 0.722 | -0.296 | 0.611 | 0.914* | 0.34 | 0.811 | 0.527 | -0.499 | -0.337 | -0.945* | 0.352 | 0.488 | 0.287 | -0.802 | -0.970* | 0.527 | 0.545 | 0.641 | 0.476 |
| Nitrogen | 0.000 | 0.0781 | -0.197 | 0.524 | 0.952* | 0.260 | 0.828 | 0.592 | -0.385 | -0.344 | -0.966* | 0.459 | 0.513 | 0.163 | -0.872 | -0.930 | 0.572 | 0.549 | 0.735 | 0.537 |
| $P_2O_5$ | 0.050 | 0.731 | -0.211 | 0.515 | 0.925* | 0.167 | 0.727 | 0.520 | -0.315 | -0.495 | -0.997** | 0.467 | 0.381 | 0.012 | -0.914* | -0.866 | 0.467 | 0.398 | 0.774 | 0.453 |
| $K_2O$ | 0.738 | 0.836 | 0.488 | -0.225 | 0.799 | -0.569 | 0.620 | 0.767 | 0.446 | -0.190 | -0.681 | 0.916* | 0.500 | -0.608 | -0.933* | -0.324 | 0.643 | 0.396 | 0.989** | 0.714 |
| Sulphate Sulphur | -0.478 | 0.202 | -0.735 | 0.890 | 0.543 | 0.560 | 0.295 | -0.066 | -0.699 | -0.786 | -0.846 | -0.129 | -0.135 | 0.279 | -0.531 | -0.807 | -0.089 | -0.052 | 0.285 | -0.134 |

**Table 1.** Correlation matrix (Pearson co-efficient) of soil properties and antioxidant traits of different parts of Ashwagandha

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

| PLATE 1: LANE A | | | | | PLATE 1: LANE B | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sl. No. | hRf | Spot Colour | Spot Intensity | Probable phyto-chemical group | Sl. No. | hRf | Spot Colour | Spot Inten-sity | Probable phytochemi-cal group |
| 1. | 0.858 | Black Brown | ++++ | *Complex Phenylpropene and Flavonoid derivatives* | 1. | 0.215 | Black Brown | ++++ | *Complex Phenylpro-pene and Flavonoid derivatives* |
| 2. | 2.146 | Black Brown | ++++ | | 2. | 1.290 | Black Brown | ++++ | |
| 3. | 3.863 | Black Brown | ++++ | | 3. | 2.151 | Black Brown | ++++ | |
| 4. | 7.082 | Black Brown | +++ | | 4. | 4.516 | Yellow Brown | ++ | *Flavonoid glycosides* |
| 5. | 9.657 | Blue | ++ | *Monoterpene alcohol and their esters* | 5. | 5.376 | Yellow Brown | ++ | |
| 6. | 11.588 | Brown | + | *Phenylpropane derivatives* | 6. | 6.667 | Blue | ++++ | *Monoterpene alcohol and their esters* |
| 7. | 15.665 | Brown | + | | 7. | 8.387 | Yellow Brown | ++ | *Flavonoid glycosides* |
| 8. | 16.953 | Brown | + | | 8. | 10.538 | Yellow Brown | ++ | |
| 9. | 18.240 | Brown | + | | 9. | 11.613 | Blue | +++ | *Monoterpene alcohol and their esters* |
| 10. | 19.742 | Brown | + | | 10. | 13.333 | Blue | ++++ | |
| 11. | 21.674 | Brown | + | | 11. | 15.484 | Yellow Brown | ++ | *Flavonoid glycosides* |
| 12. | 27.253 | Blue | +++ | *Monoterpene alcohol and their esters* | 12. | 17.419 | Yellow Brown | + | |
| 13. | 28.541 | Blue | ++ | | 13. | 19.355 | Yellow Brown | + | |
| 14. | 30.472 | Grey Blue | ++ | *Saponin deriva-tives* | 14. | 26.452 | Blue | ++ | *Monoterpene alcohol and their esters* |
| 15. | 36.481 | Orange | ++ | *Flavonoid gly-cosides* | 15. | 27.957 | Olive green | ++++ | *Conjugated Withanolides* |
| 16. | 39.914 | Orange | + | | 16. | 29.892 | Olive green | +++ | |
| 17. | 45.494 | Yellow Brown | ++ | | 17. | 34.409 | Blue | + | *Monoterpene alcohol and their esters* |
| 18. | 47.639 | Yellow Brown | ++ | | 18. | 36.129 | Blue | ++ | |
| 19. | 50.000 | Grey Blue | ++ | *Saponin deriva-tives* | 19. | 37.634 | Blue | + | |
| 20. | 51.717 | Blue | ++ | *Monoterpene alcohol and their esters* | 20. | 41.075 | Blue | ++ | |
| 21. | 53.219 | Blue | + | | 21. | 43.011 | Blue | ++ | |
| 22. | 55.579 | Blue | + | | 22. | 44.516 | Blue | ++ | |
| 23. | 57.082 | Blue | + | | 23. | 46.022 | Orange | + | *Flavonoid glycosides* |
| 24. | 62.446 | Blue | + | | 24. | 48.387 | Bluish orange | ++ | |
| 25. | 67.167 | Blue | + | | 25. | 50.968 | Orange | ++ | |
| 26. | 69.742 | Blue | + | | 26. | 54.194 | Blue | +++ | *Monoterpene alcohol and their esters* |
| 27. | 72.747 | Blue | ++ | | 27. | 58.280 | Blue | + | |
| 28. | 76.824 | Blue | ++ | | 28. | 64.731 | Blue | + | |
| 29. | 99.356 | Blue | ++++ | | 29. | 69.247 | Blue | ++ | |
| | | | | | 30. | 72.688 | Blue | + | |
| | | | | | 31. | 75.484 | Blue | ++ | |
| | | | | | 32. | 81.075 | Blue | +++ | |
| | | | | | 33. | 82.581 | Blue | +++ | |
| | | | | | 34. | 86.022 | Blue | ++ | |
| | | | | | 35. | 99.355 | Blue | ++++ | |

**Table 2.** List of hRf values and other qualitative characters of spots visualized on TLC plates after chromatographic development with Vanillin-$H_2SO_4$ reagent.

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

| Sl. No. | hRf | Spot Colour | Spot Intensity | Probable phytochemical group | Sl. No. | hRf | Spot Colour | Spot Inten-sity | Probable phytochemical group |
|---|---|---|---|---|---|---|---|---|---|
| 1. | 0.66 | Yellow | +++ | Antioxidants | 1. | 0.44 | Yellow | +++ | Antioxidants |
| 2. | 1.97 | Yellow | ++ | Do | 2. | 2.40 | Yellow | ++ | Do |
| 3. | 3.93 | Yellow | ++ | Do | 3. | 7.42 | Yellow | ++ | Do |
| 4. | 6.77 | Yellow | + | Do | 4. | 10.26 | Yellow | ++ | Do |
| 5. | 22.93 | Yellow | + | Do | 5. | 11.35 | Yellow | ++ | Do |
| | | | | | 6. | 13.32 | Yellow | ++ | Do |
| | | | | | 7. | 25.11 | Yellow | +++++++++ | Do |
| | | | | | 8. | 43.89 | Yellow | + | Do |
| | | | | | 9. | 51.31 | Yellow | + | Do |
| | | | | | 10. | 67.69 | Yellow | + | Do |
| | | | | | 11. | 98.69 | Yellow | ++++ | Do |

**Table 3:** List of hRf values and antioxidant characters of spots visualized on TLC plates after chromatographic development with DPPH reagent.

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

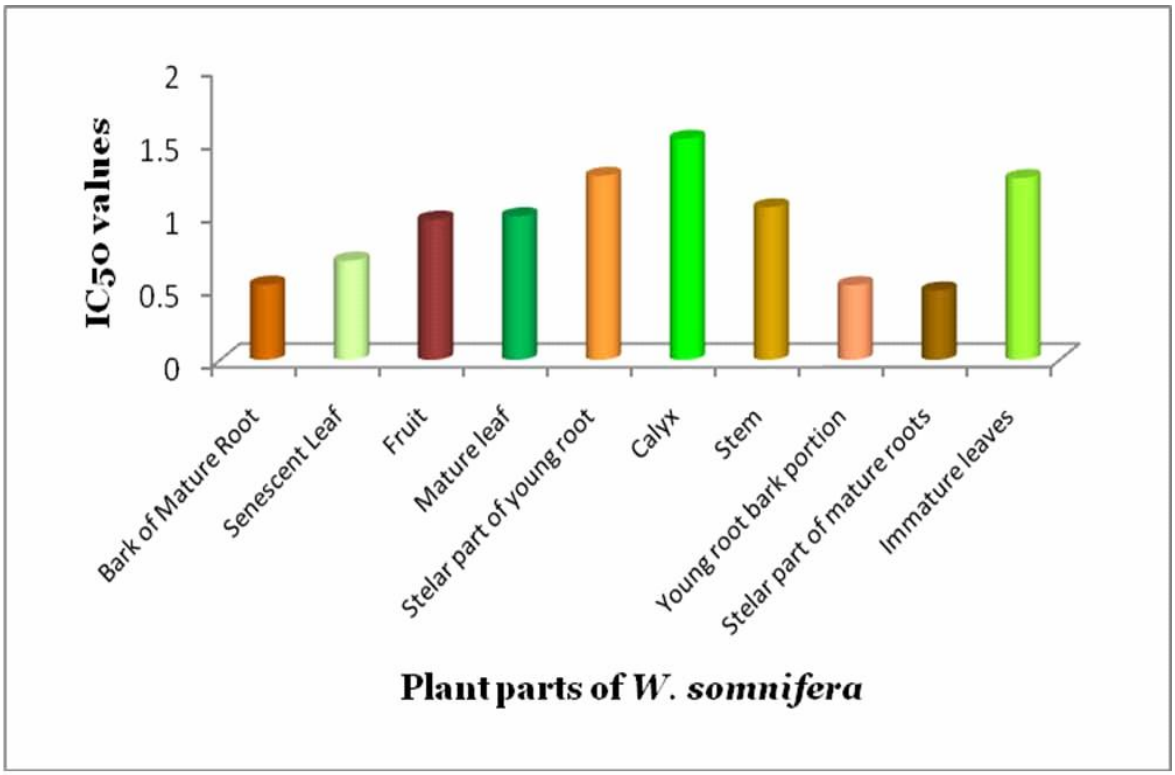



**Fig.1: DPPH free radical scavenging activity (IC 50 values µg/ml)**

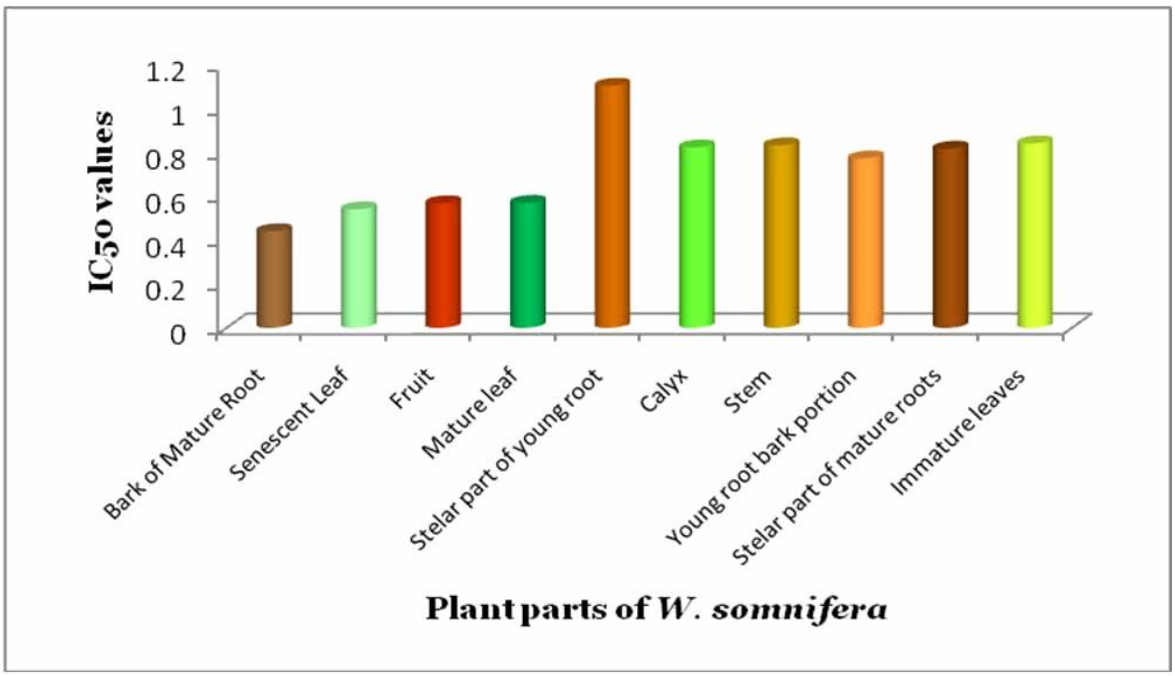



**Fig. 2: Anti-lipid peroxidation scavenging assay (IC 50 values µg/ml)**

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

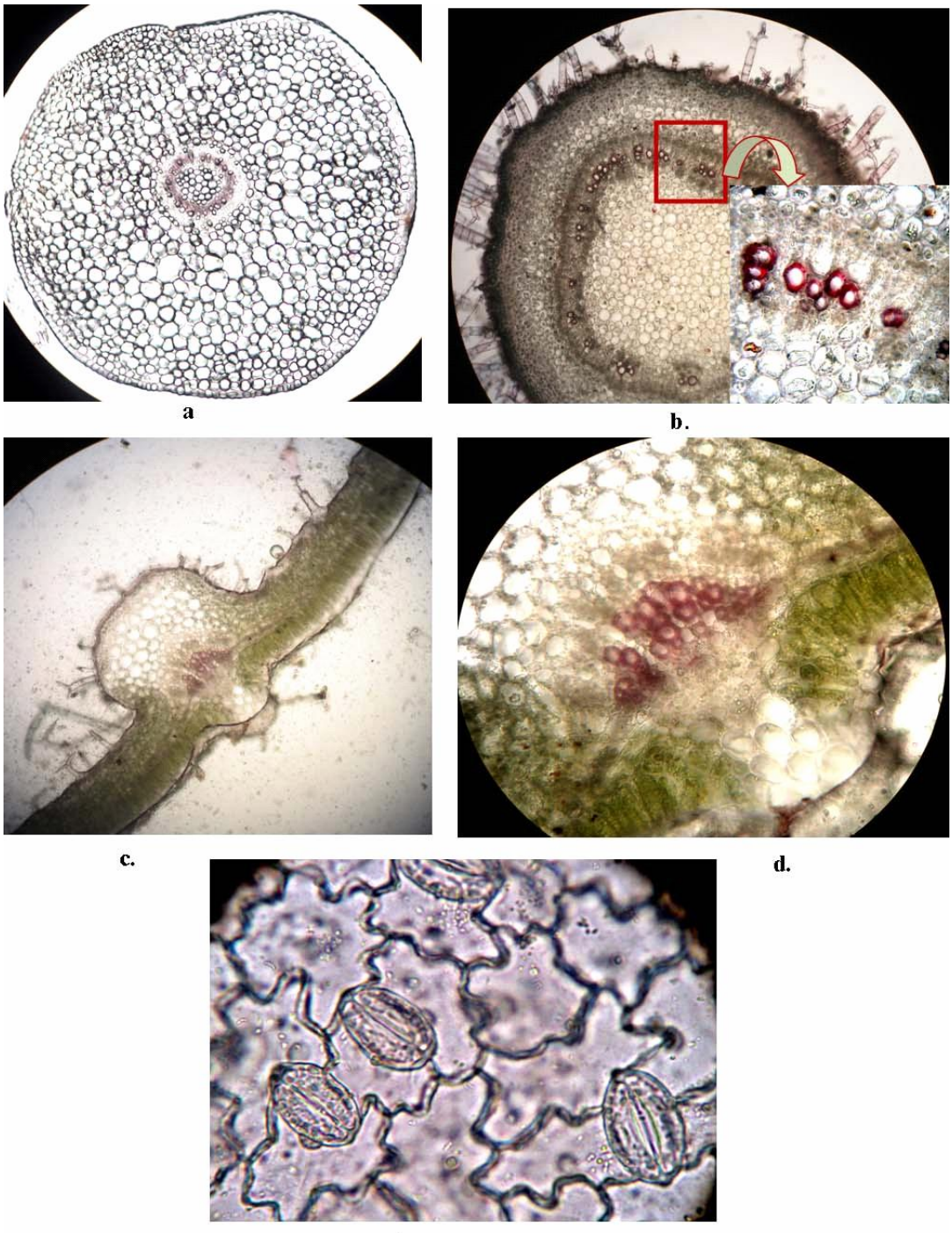
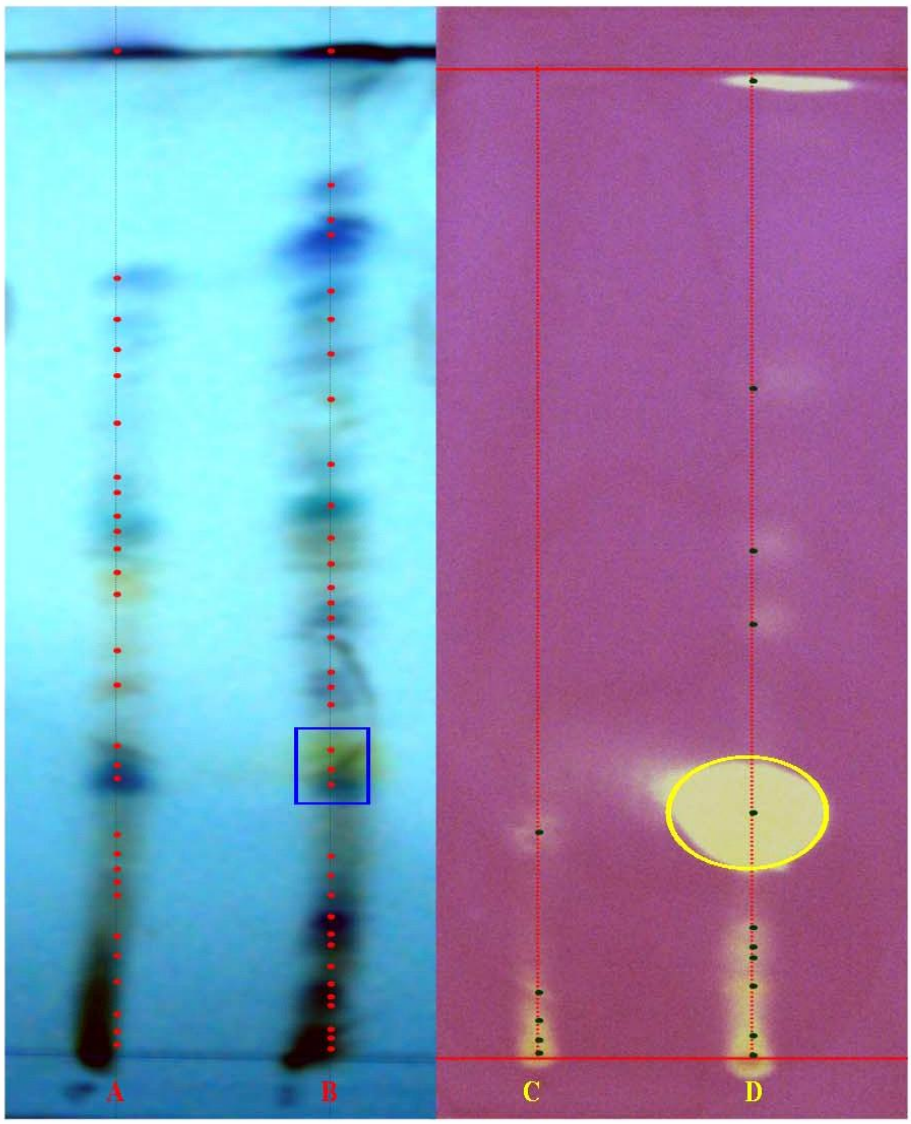



**Fig. 3**: Microscopic view of different parts of *Withania somnifera*

a. T.S. of mature root
b. T.S. of mature stem ( vascular bundle inside )
c & d. T.S. of mature leaf
e. Stomata of leaf

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands




I. II.

**Fig. 4**:

I. TLC plate shows polyphenol profile of *W. somnifera*

II. TLC plate shows antioxidant activity of *W. somnifera*

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

**Fig. 7**

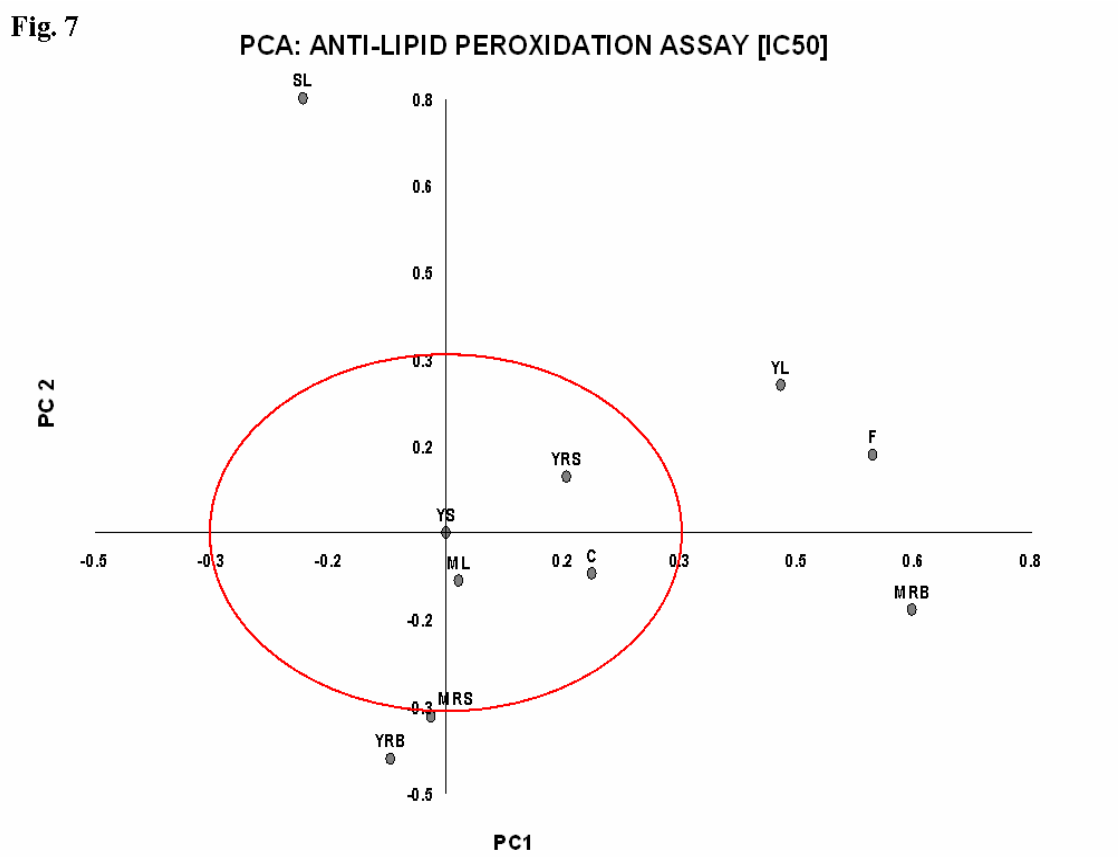
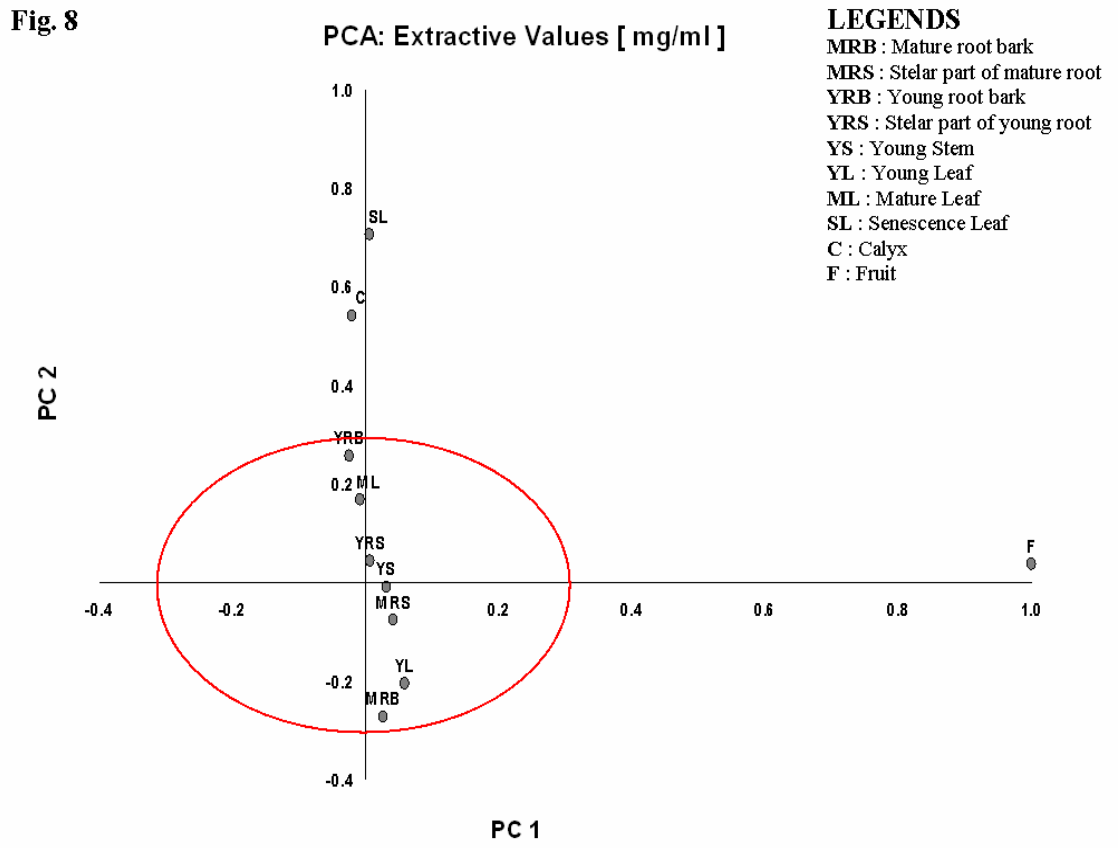



**Fig. 8**



FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

**References:**


1) *Gandhi A. Comparative pharmacological investigation of ashwagandha and ginseng. Journal of Ethnopharmacology 1994; 3: 131-135.*
2) *Singh RH, Narsimhamurthy K, Singh G. Neuronutrient impact of Ayurvedic Rasayana therapy in brain aging. Biogerontology 2008; 9(6): 369-374.*
3) *Elsakka M, Grigoresu E, Slanesu U, Dorneanu V. New data referring to chemistry of Withania somnifera species. Rev. Med, Chir, Soc. Nat. Med. Iasi. 1990; 92(2): 385-387.*
4) *Ganzera M, Choudhury MI, Khan IA. Quantitative HPLC analysis of withanolides in Withania somnifera . Fitoterapia 2003; 74(1-2): 68-76.*
5) *Velioglu YS, Mazza G, Gao YL, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem 1998; 46: 4113-4117.*
6) *Kahkonen MP, Hopia AI, Vuorela HI. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Chem 1999; 47: 3954-3962.*
7) *Arnous A, Makris DP, Kefalas P. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J Agric Food Chem. 2001; 49: 5736-5742.*
8) *Lu F, Foo LY. Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK (eds). Food antioxidants, New York, Marcel Dekker, 1995.*
9) *Panda S, Kar A. Evidence for free radical scavenging activity of Ashwagandha root powder in mice. Indian J Physiol Pharmacol 1997; 41(4): 424-426.*
10) *Gupta SK, Dua A, Vohra BP. Withania somnifera (Ashwagandha) attenuates antioxidant defense in aged spinal cord and inhibits copper induced lipid peroxidation and protein oxidative modifications. Drug Metabol Drug Interact 2003; 19(3): 211-222.*
11) *Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. Systemic administration of defined extracts from Withania somnifera (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. Neurochem Int 1997; 30(2): 181-190.*
12) *Wagner H, Norr H, Winterhoff H. Plant adaptogens. Phytomed 1994; 1: 63-76.*
13) *Anabalgan K, Sadique J. Antiinflammatory activity of Withania somnifera. Indian J Exp Biol 1981; 19: 245-249.*
14) *Davis L, Kuttan G. Immunomodulatory activity of Withania somnifera. J Ethnopharmacol 2000; 71(1-2): 193-200.*
15) *Davis L, Kuttan G. Effect of Withania somnifera on CTL activity. J Exp Clin Cancer Res 2002; 21(1): 115-118.*
16) *Misra TK, Saha A, Nanda AK, Mandal P. Soil sampling in tea plantation for fertility evaluation: A guideline. Assam review and tea news 2009; 97: 12-15.*
17) *Jackson ML. Weathering of primary and secondary minerals in soils. Trans Int Soc Soil Sci 9th Congr (Adelaide) 1968; 4: 281-292.*
18) *Saha MN, Alam MA, Aktar R, Jahangir R. In vitro free radical scavenging activity of Ixora coccinea L. Bangladesh Journal Pharmacology 2008; 3: 90-96.*
19) *Hennebelle T, Sahpaz S, Joseph H, Bailleul F. 2006. Phenolics and iridoids of Lippia alba. Nat Prod Commun 1: 727-730.*
20) *Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181: 199-1200.*
21) *Dhalwal K, Deshpandey YS, Purohit AP, Kadam SS. Evaluation of antioxidant activity of Sida cordifolia. J Pharmaceutical Biology 2005; 43: 754-761.*
22) *Anonymous. Pharmacopoeias of India. New Delhi, India, Controller of Publication of Govt. of India, 1985, pp 88 –90.*
23) *Deore SL, Khadabadi SS, Baviskar BA, Khadabadi SS. In vitro Antioxidant activity and Phenolic Content of Croton caudatum. International Journal of ChemTech Research 2009; 1(2): 174-176.*
24) *Ara N, Nur H. In vitro antioxidant activity of methanolic leaves and flowers extracts of Lippia alba. Research Journal of Medicine and Medical Sciences 2009; 4(1): 107-110.*
25) *Aruma OI. Characterization of drugs as antioxidant prophylactics. Free Rad Biol Med 1996; 20: 675-705.*
26) *Kosugi H, Kato T, Kikugawa K. Formation of yellow, orange and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. Anal Biochem 1987; 165: 456-464.*

FULL Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

27) *Ozarkar KR. Studies on anti-inflammatory effects of two herbs Cissus quadrangularis Linn. and Valeriana wallichi DC using mouse model [Ph.D. Thesis]. Mumbai, University of Mumbai (2005).*

28) *Metcalfe CR, Chalk L. Anatomy of Dicotyledons. USA, Oxford University Press Inc., 1979, pp 191.*

29) *Vogel H, Gonzalez M, Faini F, Razmilic I, Rodrıguez J, Martın JS, Urbina F. Antioxidant properties and TLC characterization of four Chilean Haplopappus-species known as bailahuen. Journal of Ethnopharmacology 2005; 97: 97–100.*

30) *Misra TK, Saha A, Nanda AK, Mandal P. Variation of antioxidant properties and phytochemical constituents of Tea cultivated under various Agronomic conditions of North Bengal. NBU Journal of Plant Sciences 2008; 2: 58-66.*

**Article History:----------**

**Date of Submission: 23-09-10**

**Date of Acceptance: 26-11-10**

**Conflict of Interest: NIL**

**Source of Support: NONE**