IN VITRO ANTIOXIDANT STATUS OF AQUEOUS EXTRACT OF *INDIAN GINSENG* D. AND *PANAX GINSENG* D.

Kanhiya Mahour[1*] and Prabhu N. Saxena[2]

[1]Experimental Laboratory, Department of Zoology, R.P. (P.G.) College, Kamalganj, Farrukabad (U. P.)
[2]Toxicology Laboratory, Department of Zoology, School of Life Sciences, Khandari Campus, Dr.B.R.Ambedkar University, Agra-282002, India.
E-mail: kris_mathura@yahoo.com

Summary

Free radicals have been implicated in causation of many ailments. Free radical scavenging properties of plant extract showed their antioxidant potential in therapeutics. Comparative free radical scavenging property of Indian ginseng (*Withania somnifera* D. (Solanaceae)) and *Panax ginseng* D. (Araliaceae) was studied under three *in vitro* models viz. DPPH method, Nitric oxide method and Lipid peroxidation method. The aqueous extract were prepared, evaporated under vacuum and subjected to qualitative analysis for active ingredients. IC_{50} µg/ml was calculated. It was 523.89, 7.629 and 270.43 µg/ml in Indian ginseng while, it was 199.22, 7.56 and 130.72 µg/ml in *Panax ginseng* D. with DPPH, Nitric oxide and Lipid peroxidation groups respectively. Result showed scavenging property in Indian ginseng as well as in *Panax ginseng* D. in all three methods with variation due to presence of different flavinoids and saponin (glycosides) components. The statistical analysis revealed a difference in IC_{50} at same concentration, indicating that scavenging property is dependent on the reactivity of active constituents present in plant extracts.

Key words: *Panax ginseng* D., *Withania somnifera D.*, antioxidant activity

***Corresponding author**
Dr. Kanhiya Mahour
Department of Zoology, R.P. (P.G.) College,
Kamalganj, Farrukabad (U. P.).
Mb. +91-9412404655
E-mail: kris_mathura@yahoo.com

Introduction

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They have been implicated in causation of ailments such diabetes, liver cirrhosis, nephrotoxicity etc (1). Together with other derivatives of oxygen they are inevitable byproducts of biological redox reaction (2). Reactive oxygen species (ROS) such as super oxide anions (O_2^{--}), hydroxyl radical (.OH) and nitric oxide (NO) inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation (3). The increased production of toxic oxygen derivatives is considered to be a universal feature of stress conditions. Plants and other organisms have evolved a wide range of mechanism to contend with this problem, with a variety of antioxidant molecules and enzymes.

The roots or rhizome of many kinds of plants have been used in traditional oriental medicine. Among them *Panax ginseng* D. is one of the most widely used medicinal plants in far eastern countries including China, Korea and Japan. It has recently been reported that chronic intake of *Panax ginseng* D. has been associated with decreased incidence of cancers such as lung, gastric, liver and colorectal tumors (4). On the other hand Indian ginseng (*Withania somnifera D.*) root extract has a positive effect on mental functions and memory (5). Besides of this Indian ginseng have significant anxiolytic and antidepressant effects (5-7). The likely active principles of Indian ginseng are glycowithanolides, consisting of sitoindosides VII to X, and withaferin (8-9).

The present study was performed to comparison *in vitro* antioxidant status of Indian ginseng and *Panax ginseng* D. along with their phytochemistry.

Materials and methods

All the chemicals and solvents were of analytical grade and procured from SRL chemicals Mumbai, India and Sigma chemicals, USA.

Plant material: The root powder of *Panax ginseng* D. was gifted by Prof. Ashok Kumar, cancer radiation laboratory, Rajsthan University, Jaipur, India. However, *Withania somnifera* D. was collected from Mathura -Agra local region and authenticated by literature.

Plant extract: About 100 gm root powder was exhaustively extracted with distilled water using Soxhlet apparatus. The residue was filtered and concentrated in vacuum with the help of rotatory evaporator.

Preparation of rat liver homogenate: Adult albino rat of both sex and approximately the same weighting about 120-130 g was used. The rats were fed on pellet (Pranav Agro Industries Ltd., Maharastra) and water was provided *ad libitum*. They were housed in polypropylene cages at the 20±5º C temperature, 50±5% relative humidity and 12 hrs/day photoperiod. Randomly selected rats were fasted overnight. They were scarified by cervical dislocation, dissected and the whole liver was removed quickly. It was further processed to get 10% homogenate in 0.15 M KCl (10) using a Teflon homogenizer. The homogenate was filtered to get a clear solution and used as a source of polyunsaturated fatty acids for determining the extent of lipid peroxidation.

***In vitro* antilipid peroxidation assay:** The extent of lipid peroxidation in rat liver homogenate was measured *in vitro* in terms of formation of thiobarbituric acid reactive substances (TBRAS). Different concentrations of the extract were made with ethanol. These samples were individually added to the liver homogenate (0.5 ml). This mixture was incubated with 0.15 M KCl (100µl). Lipid peroxidation was initiated by adding 100 µl of 15 m M $FeSO_4$ solution. The reaction mixture was incubated at 37º C for 30 min. an equal volume of TBA: TCA (1:1, 1 ml) was added to the above solution followed by the addition of 1 ml BHT. This final mixture was heated on a water bath for 20 min at 80º C and cooled centrifuged and absorbance read at 532 nm (11) using a spectrophotometer (Cintra-5). The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls not treated with the extract as per the formula. The experiment was repeated in triplicate.

$$\text{Inhibition (\%)} = \frac{\text{(Control-Test)}}{\text{Control}} \times 100$$

DPPH radical scavenging activity: DPPH scavenging activity was measured by the spectrophotometer (12). To an ethanolic solution of DPPH (200 µM), 0.05 ml of the test compounds dissolved in ethanol were added at different concentrations. An equal amount of ethanol was added to the control. After 20 min the decrease in absorbance of the test mixtures (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated[11]. Each trial has been done in triplicate.

Scavenging of nitric oxide radical (13-14)**:** Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction as described previously (15-16). Sodium nitroprusside (5 m M) in standard phosphate buffer solution was incubated with different concentrations of the aqueous extract dissolved in phosphate buffer (0.025 M, pH: 7.4) and the tubes were incubated at 25º C for 5 hrs. Control experiments with out the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 hrs, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess' reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrile with sulphailamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiment was repeated in triplicate.

All experimental results were statistically treated for mean±S.Em, paired t-test and p values.

Results and Discussion

Ten concentrations of the aqueous extract of Indian ginseng and *Panax ginseng* D. were tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models (Table-1). The maximum percentage inhibition in all models viz. DPPH, nitric oxide and lipid peroxidation were found to be 52.67, 75.31 and 52.81 respectively at 1000 µg/ml concentration in Indian ginseng, while in *Panax ginseng* D. it was 60.56, 77.89 and 69.66 at the same concentration. On a comparative basis the *Panax ginseng* D. extract showed better activity than Indian ginseng. IC_{50} value also suggests better activity in *Panax ginseng* D. than Indian ginseng. The IC_{50} µg/ml was 523.89, 7.629 and 270.43 µg/ml in Indian ginseng extracts with DPPH, Nitric oxide and Lipid peroxidation sets while it was 199.22, 7.56, and 130.72 µg/ml in *Panax ginseng* D. *vide -supra* (Table-1).

The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl-perferyl complex (17) or through OH radical by Fenton reaction (18) there by initiating a cascade of oxidative reactions. The results obtained in the present studies may be attributed to several reasons viz. reducing the rate of conversions of ferrous to ferric or by chelation of the iron itself (19). The moderate activity of the extract may probably due to the rapid and extensive degradation of the antioxidant principles in an *ex vivo* state. It is also known that the ˙ OH radical which initiates lipid peroxidation has a short life time (10^{-9} s at 37º C) and hence very difficult to investigate by conventional methods (20).

Nitric oxide is free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases (21-22). In the present study the nitrile produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25º C was reduced by the aqueous extract of *Panax ginseng* D. This may be due to the antioxidant principles in the extract which complete with oxygen to react with nitric oxide (23) thereby inhibiting the generation of nitrile.

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present results it may be postulated that *Panax ginseng* D. reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles (24). DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour spectrophotometrically depending on the number of electron taken up (25).

The *in vitro* antioxidant activity was also tested with the help of thin layer chromatography. The result revealed that both the plant root extract have same active constituents (Fig.-1).

From the above it can be concluded that root of Indian ginseng and *Panax ginseng* D. is rich in natural active constituents like saponin and flavinoids, which have been shown to possess various biological properties. Hence, the comparative *in vitro* antioxidant potential of Indian ginseng and *Panax ginseng* D. observed in the present study may be attributed to the presence of these natural active constituents.

Table1 Effect of aqueous extract of Indian ginseng and *Panax ginseng* on different antioxidant models

Concentration	**Indian ginseng (% inhibition)**			***Panax ginseng* (% inhibition)**		
	DPPH	**Nitric oxide**	**Lipid peroxidation**	**DPPH**	**Nitric oxide**	**Lipid peroxidation**
1000 µg/ml	52.67	75.31	52.81	60.56	77.89	69.66
500 µg/ml	49.72	75.31	50.12	54.18	63.45	66.69
250 µg/ml	47.56	74.81	48.98	51.68	61.79	60.50
125 µg/ml	43.08	73.06	40.38	49.78	60.56	48.21
63 µg/ml	42.52	72.56	28.69	44.32	60.33	36.40
32 µg/ml	34.05	70.32	26.45	39.12	57.30	35.00
16 µg/ml	28.69	65.33	22.19	34.10	54.94	26.00
8 µg/ml	26.45	58.10	19.25	30.16	51.68	22.00
4 µg/ml	18.30	46.13	12.18	24.45	49.73	20.00
2 µg/ml	13.11	15.20	10.22	20.08	30.16	19.26
IC_{50}	**523.89**	**7.629**	**270.43**	**199.22**	**7.56**	**130.72**

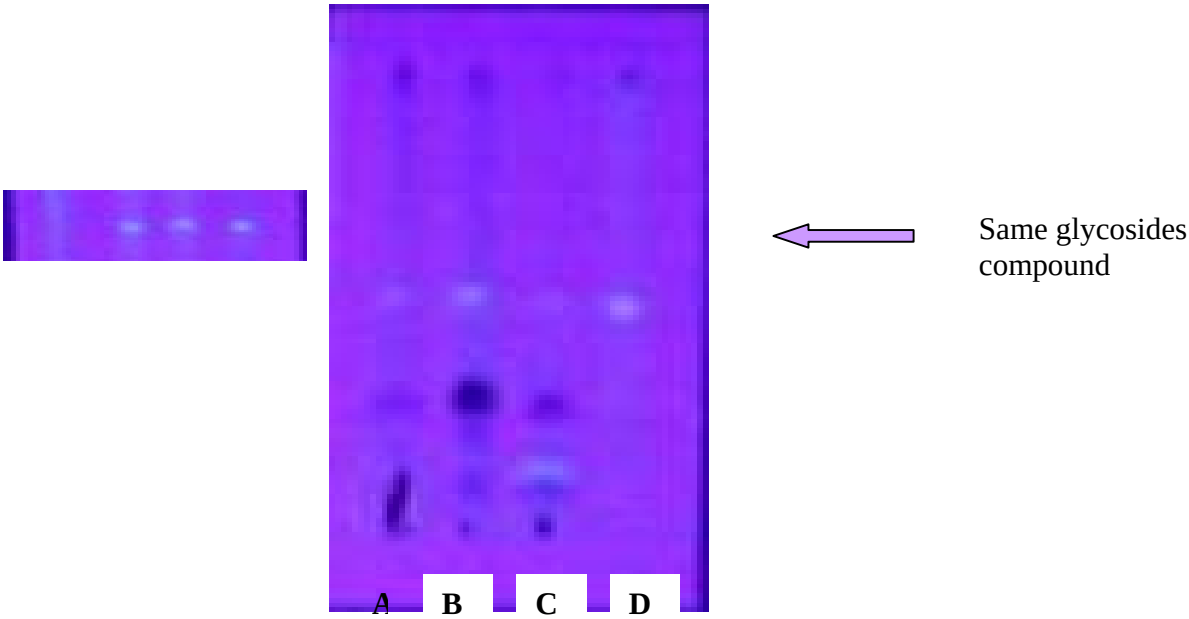

Fig.-1

Mobile phase: Chloroform: Methanol: Water
13:7:2

Figure legends

A= *Withania somnifera* D.
B= *Withania somnifera* D.
C= *Panax ginseng* D.
D= *Panax ginseng* D.

References

1. Marx JL. Oxygen free radicals linked to many diseases. Sci 1987; 235:529-532.
2. Arora A, Sairam RK and Srivastava GC. Oxidative stress and antioxidative system in plants. Curr Sci 2002; 82:1227-1230.
3. Geesin JG, Gorden JS and berg RA. Retinoids affect collagen synthesis through inhibition of ascorbic acid induced lipid peroxidation in cultured human dermal fibroblasts. Arch Biochem Biophys 1990; 278: 352-357.

4. Yun TK. Experimental and epidemiologic evidence of cancer preventive effects of Panax ginseng. Nutr Rev 1996; 54:571-581.
5. Singh RH and Malviya PC. J Res Ind Med Yoga Homeo 1978; 13:15-17.
6. Singh RH and Mehta AK. J Res Ind Med Yoga Homeo 1977; 12: 3.
7. Singh RH, Nath SK and Behere PB. J Res Ayu Siddha 1989; 11:1-5.
8. Bhattacharya SK, Goel RK, Kaur RR and Ghosal S. Phytother Res 1987; 1:32-34.
9. Ghosal S, Srivastava RS, Bhattacharya SK, Upadhyay SN, Jaiswal AK and Chattopadhyay U. Phytother Res 1989; 3:201-205.
10. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thio barbituric acid reaction. Anal Biochem 1979; 95:351-355.
11. Kumar PV, Shashidhara S, Kumar MM and Sridhara BY. Effect of *Luffa echinata* on lipid peroxidation and free radical scavenging activity. J Pharm Pharmacol 2000; 52:891-895.
12. Sreejayan N and Rao MNA. Free radical scavenging activity of curcuminoids. Drug Res 1996; 46:169-172.
13. Sreejayan N and Rao MNA. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 1997; 49:105-108.
14. Shirwaikar A and Somashekar AP. Anti-inflammatory activity and free radical scavenging studies of *Aristolochia bracteolate* Lam. Ind J Pharm Sci 2003; 65:68-71.
15. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS and Tanncubaum SR. Analysis of nitrate and N^{15} in biological fluids. Anal Biochem 1982; 126:131-134.
16. Marcocci L, Maguire JJ, Droy-Lefaix MT and Packer L. The nitric oxide scavenging property of *Ginko biloba* extract EBG 761. Biochem Biophys Res Commun 1994; 201:748-752.
17. Gutteridge JMC. Age pigments and free radicals: fluorescent lipid complexes formed by iron and copper containing proteins. Biochem Biophys Acta 1985; 834:144-147.
18. Halliwell B. Speroxide-dependent formation of hydroxyl free radicals in the presence of iron chelates. FEBS Lett 1978, 92:321-325.
19. Brraugghler JM, Duncan CA and Chase LR. The involment of iron in lipid peroxidaiton. Importance of ferrous and ferric ratio in initiation. J Biol Chem 1986; 261:10282-87.
20. Pryor WA. Oxy-radicals and related species, their formation, lifetimes and reactions. Annu Rev Physiol 1986; 48: 657-662.
21. Ialenti A, Moncada S and Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacol 1993; 110:701-705.
22. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Natur 1993; 362:801-811.
23. Marcocci L, Packer L, Droy-Lefaiz MT, Sekaki A and Gardes-Albert M. Antioxidant action of Ginko biloba extract Egb761. Meth Enzymol 1994; 234:462-470.
24. Sanchez- Moreno, C. Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci Tech Int 2002; 8:122-127.
25. Blos MS. Antioxidant determinations by the use of stable free radical. Nature 1958; 26:1199-1207.