Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(4):166-171

In vitro Antioxidant & Phytochemical Investigations of Ethanolic extracts of *Viola serpens & Morus nigra*

Anu Kumar*[1], P. K. Chauhan[2], V.S. Bhardwaj[3] , Ramesh Kumar[1] and Ankur Tyagi[1]

[1]*Department of Biotechnology, HILS, Paonta Sahib Himachal Pradesh*
[2]*Department of Biochemistry, HILS, Paonta Sahib Himachal Pradesh*
[3]*Department of Microbiology, HILS, Paonta sahib Himachal Pradesh*

ABSTRACT

This study investigates the influence of extraction system on the extractability of phytochemical compounds and antioxidant activity of ethanolic extracts of Viola serpens & Morus nigra. It can be concluded that the solvent used affects significantly the phytochemical content and the antioxidant activity of the extract and therefore it is recommended to use more than one extraction system for better assessment of the antioxidant activity of natural products. Several of the investigated herbs contain substantial amounts of free radical scavengers and can serve as a potential source of natural antioxidants for medicinal and commercial uses. The phytochemical screening of ethanolic extract of M.nigra showed the the presence of tannins and terpenoids where as alkaloids, saponins, flavonoids, amino acids, reducing sugars and glycosides were absent. Ethanolic extract of V.serpens showed the absence of alkaloids, saponins, tannins, terpenoids and glycosides, flavonoids amino acids and reducing sugars.The antioxidant screening of ethanolic extract of both plants showed the presence of enzymatic antioxidants such as catalase, peroxidase and ascorbate oxidase and non- enzymatic antioxidant such as ascorbic acid.Present study revealed that out of two working plants V.serpens is more effective in its medicinal value.

Keywords: antioxidants, phytochemicals, medicinal plants.

INTRODUCTION

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing in different parts of the country. In India thousands of species are known to have

medicinal values. The medicinal actions of plants are unique to a particular plant species, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [1]. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases**.** In search of novel sources of antioxidants in the last years, medicinal plants have been extensively studied for their antioxidant activity. From ancient times, herbs have been used in many areas, including nutrition, medicine, flavoring, beverages, cosmetics, etc. The ingestion of fresh fruit, vegetables and tea rich in natural antioxidants has been associated with prevention of cancer and cardiovascular diseases [2]. The higher intake of plant foods correlates with lower risk of mortality from these diseases. Antioxidants are substances that are able to prevent or retard oxidation of lipids, proteins and DNA; and to protect the compounds or tissues from damage caused by oxygen or free radicals. Therefore, their health promoting effects reduce the risk of various diseases [3]. Recent reports indicated that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human diseases [4]. Many studies have demonstrated the antioxidant activities and health benefits of the anthocyanins occurring in various fruits and vegetables [5]. As many antioxidants are supplied from the diet, attention has been paid to intake of the micronutrient antioxidants (vitamins A, C, and E, polyphenols and carotenoids) and to know how it may help to protect individuals from an oxidizing environment and/or inflammatory airway disease. Approximately 60 % of the commercially available anti-tumoral and antiinfective agents are of natural origin [6].The objective of this study was to investigate the scavenging capacities towards superoxide anion radicals and reducing power of the Berry's anthocyanin extract as a potential source of natural functional substances for use as dietary antioxidants. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs[7].

EXPERIMENTAL SECTION

Collection of plant materials

The plant material used was the dried leaves of medicinal plants which were *Viola serpens, Morus nigra* collected from forest region of Paonta sahib and were identified by Botanical Survey of India Dehradun.

Extraction of plant material:

The plant material taken for the study was stored under refrigerated condition till use. The samples were prepared by extraction of plant material with ethanol solvent by Soxhlet apparatus. By evaporating on water bath a crude extract was obtained of medicinal plants.

Storage:

Plant extracts were stored at the temperature of 4^0C till use for investigation.

Phytochemical investigations:

Alkaloid was investigated according to procedure given by [8].
Saponin and Flavonoids: were investigated according to the procedure of [8].
Tannins: Tannins were investigated according to the procedure of [9].
Glycoside: Glycosides were investigated according to the procedure of [10].

Terpenoids: Terpenoids were investigated according to the procedure of [10].
Reducing sugar was investigated according to the procedure of [8].
Amino acids were investigated according to the procedure of [8].

Determination of antioxidant activity

Assay of Catalase activity: Catalase activity was assayed by the method of[11].
Assay of Peroxidase activity: The assay was carried out by the method of [12].
Assay of ascorbate oxidase activity: Assay of ascorbate oxidase activity was carried out according to the procedure of [13].
Quantification of vitamins: The determination of ascorbic acid was carried out by the procedure given by [14].

RESULTS AND DISCUSSION

Phytochemical screening of plant materials

The phytochemical screening of ethanolic extract of *M.nigra* showed the the presence of tannins and terpenoids where as alkaloids, saponins, flavonoids, amino acids, reducing sugars and glycosides were absent. Ethanolic extract of *V.serpens* showed the absence of alkaloids, saponins, tannins, terpenoids and glycosides, flavonoids amino acids and reducing sugars **Table 1**. Polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits, which include lowering of blood lipids. Thus these plants have been used to lower the blood lipid content.

Table 1: Phytochemical constituents of *viola serpens* & *morus nigra*

Phytochemicals	*M. nigra*	*V.serpens*
Alkaloids	_	_
Saponins	_	_
Tannins	+	_
Amino acids	_	+
Terpenoids	+	_
Reducing sugars	_	+
Glycosides	_	_
Flavonoids	_	+

Antioxidant Activity of Ethanolic extracts of *Morus nigra & Viola serpens*:

The levels of antioxidant enzymes assessed in both plants ethanolic extracts of were collectively represented in **Table 2.** Among the ethanolic extracts of both plants the highest activity of Catalase was observed in ethanol extract of *Viola serpens* (0.40 units/mg protein) and lowest in ethanolic extract of *Morus nigra* (0.14 units/mg proteins).

Table 2: Enzymatic Antioxidant Analysis in ethanolic extracts of *Morus nigra & Viola serpens*

Samples	**Catalase** µ/moles of H_2O_2 decomposed /min/g extract	**Peroxidase** 1U/L	**Ascorbate oxidase** µ mole /ml
Ethanolic extracts of *Morus nigra*	0.14	4.46×10^3	1.855
Ethanolic extracts of *Viola serpens*	0.40	11.9×10^3	0.153
	1 unit = µ/moles of H_20_2 decomposed /min/g extract	1 unit = µmoles pyrogallol oxidized/ min	1 unit = 0.01 O.D change /min

In plants, antioxidant enzymes namely catalase [15] and peroxidase have been shown to increase when subjected to stress conditions. The Peroxidase activity was observed to be low in ethanolic extract of *Morus nigra* (4.46×10^3 units/mg protein), while the activity increased in ethanolic extract of *Viola serpens* (11.9×10^3). The ascorbate oxidase activity was highest in ethanolic extract of *Morus nigra* (1.855 units/mg protein), and lowest in ethanolic extract of *Viola serpens* (0.153 units/mg protein). The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity [16]. The reducing ability of a compound generally depends on the presence of reductants which possess antioxidative potential by breaking the free radical chain, by donating a hydrogen atom. Ethanolic extract *A. lamarckii* and its sub-fractions exhibited a good reducing power. Leaves of this plant are useful for curing diabetes . Decoction of bark has been used as an emetic in India . Methanol extract of *Alangium salviifolium* flowers have shown to antibacterial activity against both gram-positive and gram-negative bacteria.

Methanolic extract of root of *A. salviifolium* have shown analgesis and anti-inflammatory activities in albino mice [17].

Non –Enzymatic Antioxidant Activity (Ascorbic acid)

The concentration of non-enzymatic antioxidant (Ascorbic acid**)** in ethanolic extracts of both plants was also assessed and the results are represented in **Table 3**.

Table 3. Non –Enzymatic Antioxidant Activity (Ascorbic acid)

Samples	**Vitamin C (mg/g)**
Ethanolic extract of *Morus nigra*	0.173
Ethanolic extract of *Viola serpens*	0.051

Vitamin C content was high in ethanolic extract of *Morus nigra* (0.173 mg/ g tissue), whereas in ethanolic extract of *Viola serpens*, it was (0.051 mg/ g tissue). Ascorbate has been found in the chloroplast, cytosol, vacuole and extracellular compartments of the plant cells and shown to function as a reductant for many free radicals [18]**.** Oxidative damage to cellular components such as lipids and cell membranes by free radicals and other reactive oxygen species is believed to be associated with the development of a range of degenerative diseases including heart diseases, cancer, inflammation, arthritis, immune system decline, brain dysfunction. Blackberries are a good source of anthocyanins in which the anthocyanin contents were reported to be 67.4–230 mg/100 g fresh weight [19]. Furthermore, the anthocyanin pigment in blackberry has also

exhibited a strong scavenging activity towards nitrite and thereby prevents the formation of nitrosamine and reduces the carcinogenesis induced by nitrosamines. Therefore, the anthocyanin pigment in blackberry is a natural, edible colorant with excellent antioxidant properties and health benefits and seems applicable in both healthy food and medicine. Both the Plants may play an important role in the prevention of human diseases related to oxidative damage.

CONCLUSION

The results of the present study revealed that the ethanolic extracts of both plants i.e. *Morus nigra* and *Viola serpens* have antioxidant properties since these contains enzymatic and non - enzymatic antioxidants, these can be very effective against microbes causing various diseases. *In vitro* assessment of the antioxidant activity of ethanolic fractions of *M.nigra* and *V.serpens* to scavenge 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and highly reactive hydroxyl radicals showed that the semi - pure compounds present in the fractions are useful potential source of antioxidants and can be used in the therapy of diseases like cancer, coronary heart disease, ageing and any other disease related to oxidative stress. These fractions being non-toxic showed significant antioxidant activity at scavenging free radicals. They also significantly scavenge hydroxyl radical which is known to cause cellular damage.

Acknowledgement
With great pleasure and deep sense of gratitude I express my heartiest thanks to my guide Mr.PANKAJ KUMAR CHAUHAN (Sr.Lecturer, Department of Biochemistry) Himachal Institute of Life Sciences Paonta Sahib (H.P.) for his excellent guidance and support throughout my project work. I am greatful for his untiring and selfless devotion towards the progress of my project work and his immense patience to listen my problems and provide a solution to them.

REFERENCES

.

[1]Wink M. Introduction biochemistry, role & biotechnology of secondary products. In: M. Wink, Ed, Biochemistry of secondary products metabolism. CRC Press. Bocaroton, FL. **1999**, Pp.1-16.
[2] Willcox JK, Ash SL, Catignani GL. *Crit Rev Food Sci Nutr*. **2004**, 44, 275–295.
[3] Manach C, Scalbert A, Morand C, et al. *Am J Clin Nutr*. **2004**, 79, 27–47.
[4] Odukoya, A., Ilori, O., Sofidiya, M., Aniunoh, O., Lawal, B., Tade, I. *EJEAF Che.* **2005**, 4: 6, 1086-1093
[5] Jiao, Z., Liu, j., Wang, S. *Food Technol*. **2005**,43, 1, 97-102.
[6] Cragg GM, Newman DJ, Weiss RB. *Semin Oncol*.**1997**, 24, 156–163.
[7]Shweta Saboo, Ritesh Tapadiya, 1S. S. Khadabadi and 2U. A. Deokate. *J. Chem. Pharm. Res.*, **2010**, 2(3):417-423.
[8] Harborne, J.B. . Phytochemical methods, London. Chapman and Hall, Ltd.**1973**, Pp. 49-188.
[9] Trease, G.E., Evans, W.C. Pharma cognsy.11th Edn. Brailliar Tiridel Can. Macmillan Publishers. **1989**.
[10] Siddiqui, A.A., Ali.Practical Pharmaceutical chemistry.1st ed. CBS Publishers and Distributors, New Delhi.**1997**, pp . 126-131.
[11] Sinha, A.K. *Anal. Biochem.***1972**, 47, 389-394.
[12] Addy, S.K. and Goodman, R.N. *Ind. Phytopath*.**1972**, 25, 575-579.

[13] Vines, H.M. and Oberbacher, M.F. *Nature*. **1965**, 206, 319-320.
[14] Sadasivam, S. and Manickam, A. Vitamins. In: Biochemical methods, Eds. Sadasivam, S. and Manickam, A.2nd Edition, New Age International (P) Limited, New Delhi.**1997**, 185-186.
[15] Hertwig, B. Steb, P.and Feierabend, *Plant physiol*.**1999**, 100, 1547-1553
[16] Foyer, C. Ascorbic acid. In: Antioxidants in higher plants, EDS. Alscher, R.G. and Hess, J.L. CRC press, Boca Raton.**1993**, p. 111-134.
[17] Aruoma OI. *J Am Oil Chem Soc*.**1998**, 75, 199–212.
[18]Rajesh Kumar and S. Hemalatha. *J. Chem. Pharm. Res*., **2011**, 3(1):259-267.
[19] A Kumaran; J Karunakaran. *LWT*., **2006**, 40, 344-352.