SMU
Sikkim Manipal University
Manipal
INSPIRED BY LIFE
SMU Medical Journal
I2OR
SIS Scientific Indexing Services
ISSN : 2349 – 1604 (Volume – 3, No. 2, July 2016) Research article
Indexed in SIS (USA), ASI (Germany), I2OR & i-Scholar (India) and SJIF (Morocco) databases. Impact Factor: 3.835 (SJIF)

In vitro anti oxidant activity of chromatographically separated fractions from the leaves of *Aastilbe rivularis* buch. – Ham. Ex D. Don

Prasanta Kumar Mitra[1*], Tanaya Ghosh[1], Prasenjit Mitra[2#],
[1]Department of Medical Biotechnology, [2]Department of Biochemistry,
Sikkim Manipal University, Sikkim Manipal Institute of Medical Sciences,
Gangtok, Sikkim, India.
[#] Present address, Department of Biochemistry, All India Institute of Medical Sciences,
Jodhpur

* Corresponding author
Phone: 9434063026

Manuscript received : 10.05.2016
Manuscript accepted: 21.06.2016

Abstract

In vitro antioxidant activity of chromatographically separated fractions from powdered leaves of A*astilbe rivularis* buch. – Ham. Ex D. Don was studied. Antioxidant activity was measured by superoxide anion generation with help of xanthine-xanthine oxidase assay and with linoleic acid peroxidation assay as well as DPPH photometric assay. Total phenol, flavonoids ,

ascorbic acid and carotenoid contents in the separated fractions were also estimated. Out of five fractions separated, the third fraction had maximuum antioxidant activity which was comparable to that of known synthetic antioxidant. Antioxidant activity was related with high content of total phenol, flavonoid, ascorbic acid and carotenoids in the fraction.

Keywords: . *Astilbe rivularis*, Buch. – Ham. Ex D. Don, chromatographic separation, anti oxidant activity, total phenol, flavonoid, ascorbic acid.

Introduction

Astilbe rivularis, Buch. – Ham. Ex D. Don (*A. rivularis)* is one of the medicinal plants of Sikkim Himalaya. The plant has different names. In Nepali it is called Buriokahti and in Lepcha the plant is known as Pango [1]. In Common Temperate of Himalaya *A. rivularis* is distributed at a range of 5000 – 9000 feet. The plant is also found on sloppy waste place. *A. rivularis* has tall herb stem and leaves are covered with hairs [2,3]. The juice of the plant is applied traditionally to sprains and muscular swelling. Rhizome of this plant is used in curing hemorrhages, diarrhoea, dysentery, headache, prolapse of uterus and to improve fertility [4]. Rajbhandari et al. established anti viral activity of the plant [5,6]. Ethnic use of *A. rivularis*, as reported in literature, is in peptic ulcer [1]. Root juice of the plant, two tea spoonful thrice a day, is given to patients suffering from peptic ulcer [7]. We also found anti peptic ulcer activity of *A. rivularis* leaves in experimental animals [8].

Antioxidant activity of *A. rivularis* is known in literature [9, 10]. Recently we intended to isolate active ingredient(s) from *A.rivularis* leaves responsible for antioxidant effect. In present communication antioxidant activity of chromatographically separated fractions of dried leave powder of *A. rivularis* is being reported.

Materials and Methods

Plant Material

From the medicinal plants garden of the University of North Bengal *A. rivularis* leaves were collected. Leaves were authenticated by the experts of the department of Botany of the said

University. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references. Leaves were shade dried and powdered. The powder was used for extraction and isolation studies.

Fig. 1: *Astilbe rivularis* Buch. – Ham. Ex D. Don

Chemicals

Chemicals required for the study were purchased from Loba Chem. Lab, Himedia Lab and from Merck.

Test material

Leaves of *A. rivularis* were shade dried and powdered. The powder as test material was used for extraction and isolation studies.

Extraction and Isolation

This was done by standard methods of isolation of chemicals from plant sources [11-14].

A sample of 100 g of powdered leaves of *A. rivularis* was soaked in 1000 ml of methanol after 1h stirring at room temperature overnight. The solvent was decanted and concentrated to 10 ml under reduced pressure using a rotary evaporator. This was then subjected to acid hydrolysis by refluxing with 10 ml N/10 hydrochloric acid at 100^0C for 10 mins to obtain a brown mass. The mass was extracted with 20 ml ethyl acetate for 10 mins. Material was centrifuged at 3000 rpm for 10 mins and the supernatant was subjected to column chromatography using silica gel G as adsorbent. Five bands were separated. Elution was done by 50% methanol-chloroform mixture. Five bands were separately collected and evaporated to dryness under reduced pressure using a rotary evaporator. Materials obtained were assayed for anti oxidant activity and for total phenol, flavonoid, ascorbic acid and carotenoids content.

Antioxidant assays

Antioxidant activity of chromatographically separated fractions was assayed by superoxide anion generation by xanthine- xanthine oxidase assay [15], linoleic acid peroxidation assay [16] and by DPPH photometric assay [17]. Catechin and quercetin were used as anti oxidant reference compound [16].

Flavonoids content

Flavonoids content of chromatographically separated fractions was determined using Aluminum chloride colorimetric method [18].

Total phenols content

Total phenols content of chromatographically separated fractions was determined by Folin Ciocalteu reagent [19].

Ascorbic acid content

Ascorbic acid content of chromatographically separated fractions was determined by the

method of Cakmak and Marschner [20].

Carotenoids content

Total carotenoids of chromatographically separated fractions were determined by the method of Jensen[21]

Statistical Analysis

The statistical significance between antioxidant activity values of the extracts was evaluated with a Duncan's multiple range test (DMRT) at 5 % were considered to be statistically significant [22].

Results

Results on antioxidant activity of chromatographically separated fractions of powdered leaves of *A. rivularis* by superoxide anion generation by xanthine- xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay were given in Table. 1

Table 1: Inhibitory activity of xanthine oxidation and linoleic acid peroxidation and scavenging capacity of DPPH by chromatographically separated fractions of powdered leaves of *A. rivularis*

Chromatographically separated fractions of powdered leaves of *A. rivularis*/ Flavonoids	**Xanthine oxidase (% inhibition)**	**Linoleic acid peroxidation (% inhibition)**	**DPPH (% inhibition)**
Fraction : 1	42 ± 1.3	27 ±0.5	35 ± 0.9
Fraction : 2	38 ±1.1	30 ±0.9	29 ± 0.6
Fraction : 3	98 ±2.7*	96 ± 2.1*	97 ±2.2*
Fraction : 4	25 ±0.8	38 ±1.1	32 ±0.7
Fraction : 5	37 ±1.0	40 ±1.6	32 ±0.6
Catechin	100 ±0.01	70 ±1.1	100 ±0.01
Quercetin	100 ±0.01	85 ±1.2	100 ±0.02

Concentration used : 100 µg / ml . Results were a mean of triplicate experiments ± SD
*significant

It appears from the table that chromatographically separated fractions of powdered leaves of *A. rivularis* had more or less in vitro antioxidant activity but maximum activity was with fraction -3 where 98%, 96% and 97% inhibition in xanthine oxidase, linoleic acid peroxidation and DPPH inhibitions respectively were noticed. Results were compared with catechin and quercetin where inhibition in both xanthine oxidase and scavenging capacity of DPPH came 100%.

Table 2: Total phenol, flavonoids, ascorbic acid and carotenoids content of chromatographically separated fractions of powdered leaves of *A. rivularis*
0.7

Chromatographically separated fractions of powdered leaves of *A. rivularis*	**Total phenol content (mg/mg dry wt)**	**Total flavonoids content (mg/mg dry wt)**	**Ascorbic acid content (mg/g dry wt)**	**Carotenoids content (mg/g dry wt)**
Fraction : 1	20.2 ±1.2	17.2±0.9	7.2±0.6	8.1±0.7
Fraction : 2	7.5 ±0.6	10.5±0.5	4.3±0.4	5.2±0.4
Fraction : 3	47 ± 1.4*	88 ± 2.9*	20.1 ±0.9*	24.2 ± 0.9*
Fraction : 4	5.5 ±0.5	12.8±0.6	8.5±0.7	6.7±0.4
Fraction : 5	6.1±0.7	2.5±0.7	3.3±0.2	1.9±0.2

Results were a mean of triplicate experiments ± SD.
*significant

It appears from Table – 2 that total phenol content was maximum and statistically significant in fraction -3 (47 mg/mg) in comparison to that of other chromatographically separated fractions of powdered leaves of *A. rivularis.* For fractions 1, 2 , 4 and 5 total phenol content came 20.2 mg/mg, 7.5 mg/mg, 5.5 mg/mg and 6.1 mg/mg respectively. Total flavonoids, ascorbic acid and carotenoids contents were also maximum and significantly higher in fraction -3 in comparison to that of other chromatographically separated fractions of powdered leaves of *A. rivularis.*

Discussion

Endogenous cause like metabolism and exogenous causes like chemicals and ionizing

radiation lead to the formation of reactive oxygen species (ROS) in living organisms. Generation of ROS in body is now considered responsible for formation of several diseases like arthritis, liver disorders, diabetes, connective tissue disorders, cancer, neurodegenerative disorders, chronic inflammation as well as the ageing process [23]. Generation of ROS can be protected by antioxidants defence mechanisms operating in body. In case there is an imbalance between generation of ROS and antioxidants defence mechanisms, oxidative stress occurs. This causes irreversible oxidation of biomolecules like proteins, DNA and lipids, leading to inactivation of many enzymes and cell death [24]. Antioxidants provide protection to the living organisms from the damage caused by uncontrolled production of ROS.

Since time immemorial Indian medicinal plants have been used in the Ayurveda to maintain health and to cure diseases in humans. This is probably due to production of lot of antioxidants by the plants. These antioxidants protected formation of ROS thereby keeping body healthy by reducing oxidative stress [25].

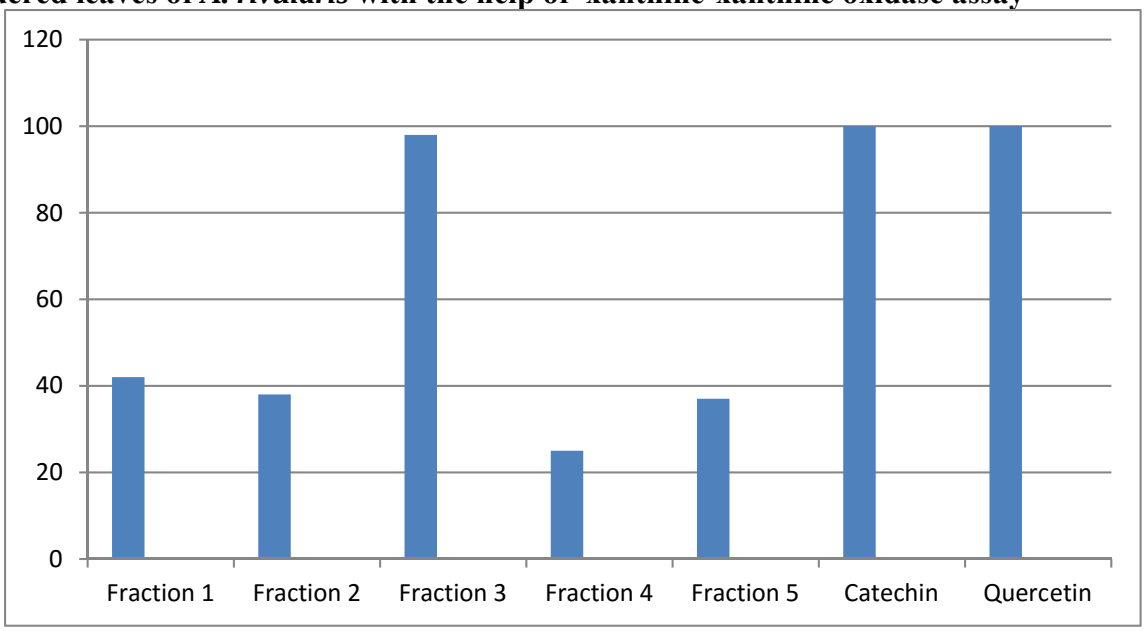
Phenolic compounds, flavonoids, ascorbic acid and carotenoids are widely distributed in plants and these chemicals are considered good antioxidants. Besides, they are responsible for multiple biological effects like free radical scavenging abilities, anti inflammatory and anticarcinogenic activities [26].

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are the synthetic antioxidants. They are commercially available and commonly used in processed foods, but their safety and toxicity are matters of concern. Researcher claimed that these synthetic antioxidants have many side effects including carcinogenic activity [27]. Hence there is a trend to substitute these synthetic antioxidants with naturally occurring antioxidants.

Astilbe rivularis Buch. – Ham. Ex D. Don (*A. rivularis),* family Saxifragaceae, is known for its antioxidant activity [9,10]. To isolate the antioxidant compound(s) from *A. rivularis,* powdered leaves of *A. rivularis* were processed for solvent extraction, acid hydrolysis and chromatographic separation. Five fractions were separated by Silica gel G column chromatography. Band separation was as under.

Fractions were separately evaluated for antioxidant activity by superoxide anion generation with help of xanthine-xanthine oxidase assay and with linoleic acid peroxidation assay as well as DPPH photometric assay. In all the three methods of assay, fraction -3 showed maximum antioxidant activity (Figures: 3,4,5). Results were comparable to that of standard antioxidants used.

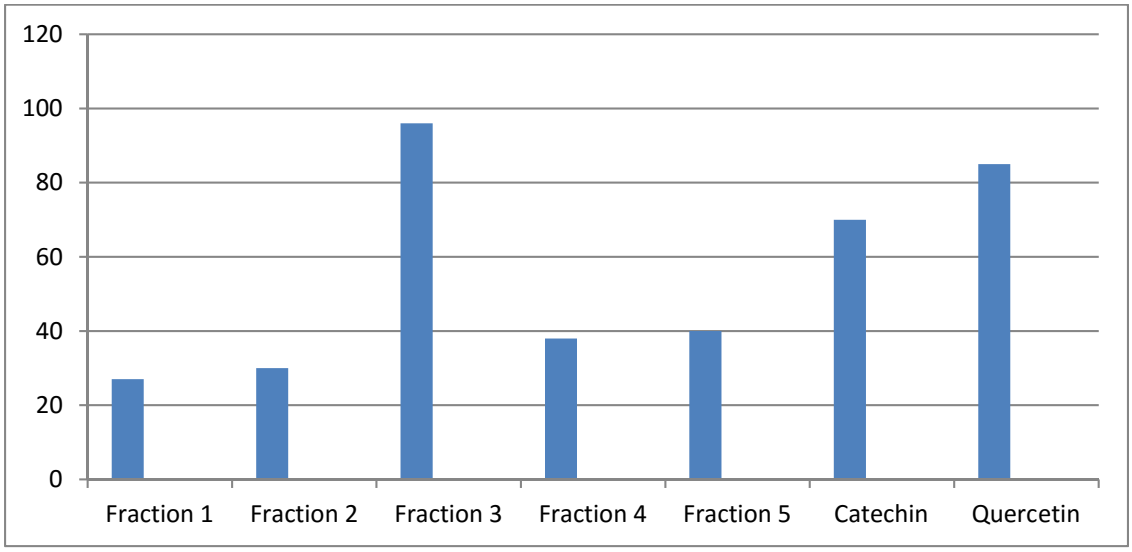
Fig. 3 : Antioxidant activity of chromatographically separated five fractions from powdered leaves of *A. rivularis* with the help of xanthine-xanthine oxidase assay



Xanthine oxidase (% inhibition), Concentration used : 100 µg / ml . Results were a mean of triplicate experiments.

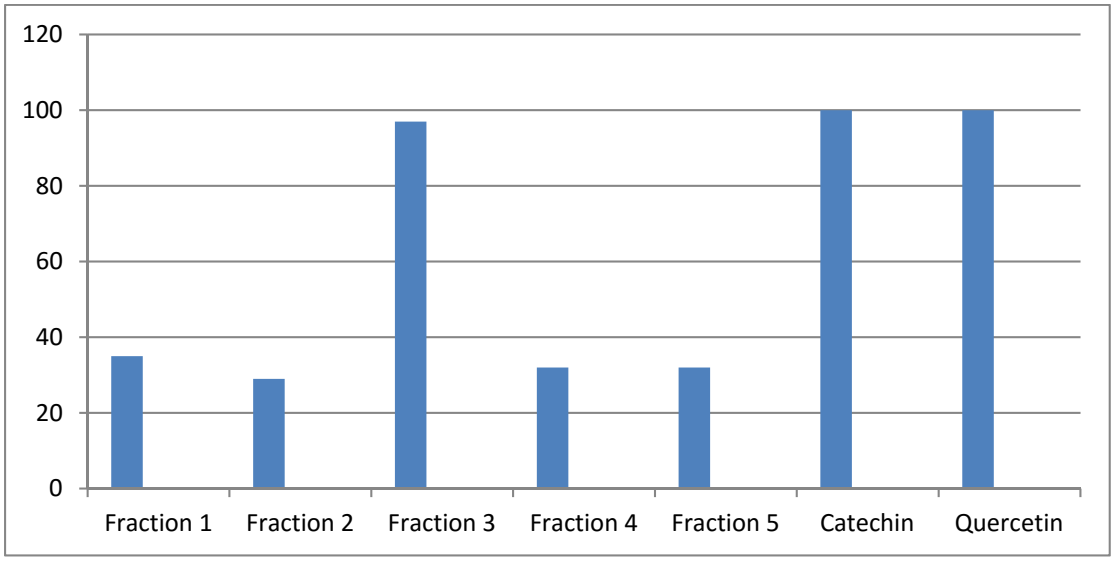
Total phenol, total flavonoids, ascorbic acid and carotenoids content were estimated in all the separated five fractions. Results showed that maximum amount of these compounds were present in third fraction (Figures: 6,7). Antioxidant activity of leaves of *A. rivularis* was, therefore, due to the presence of these chemicals.

Fig. 4 : Antioxidant activity of chromatographically separated five fractions from powdered leaves of *A. rivularis* with the help of linoleic acid peroxidation



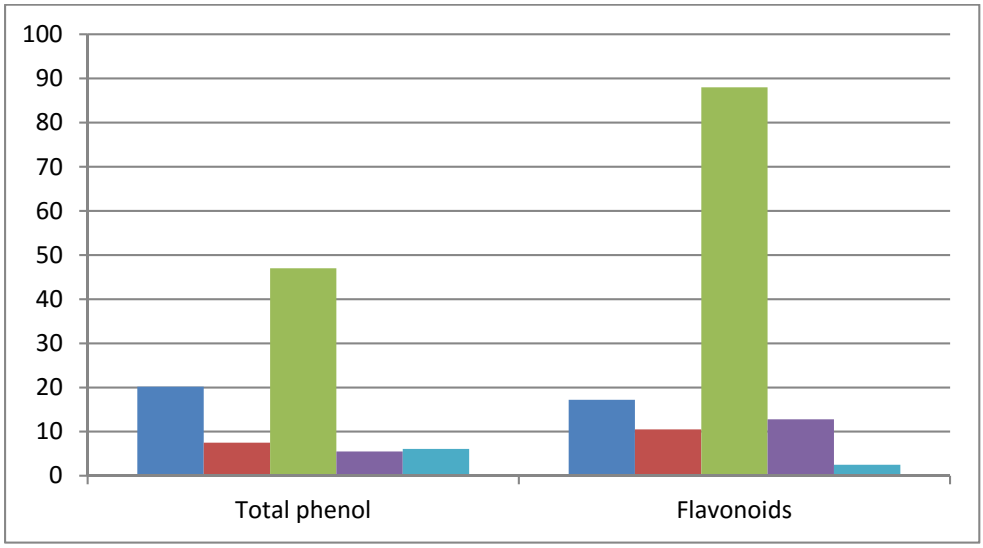
Linoleic acid peroxidation (% inhibition), Concentration used : 100 µg / ml . Results were a mean of triplicate experiments.

Fig. 5 : Antioxidant activity of chromatographically separated five fractions from powdered leaves of *A. rivularis* with the help of scavenging capacity of DPPH



Scavenging capacity of DPPH (% inhibition), Concentration used : 100 µg / ml . Results were a mean of triplicate experiments.

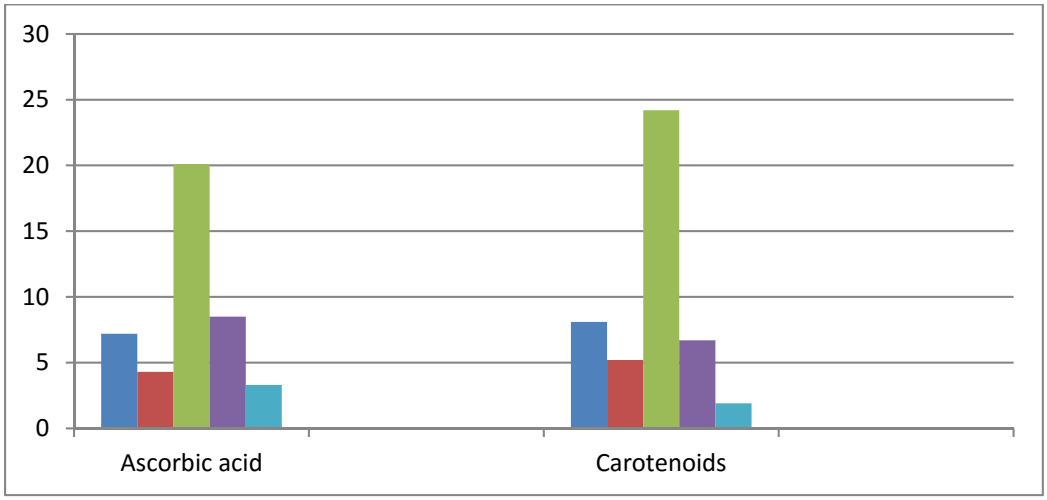
Fig. 6 : Total phenol and flavonoids content of chromatographically separated fractions of powdered leaves of *A. rivularis*



In mg/mg dry weight. Results were a mean of triplicate experiments.

Fraction 1 **Fraction 2** **Fraction 3** **Fraction 4** **Fraction 5**

Fig. 7 : Ascorbic acid and carotenoids content of chromatographically separated fractions of powdered leaves of *A. rivularis*



In mg/g dry weight. Results were a mean of triplicate experiments.

Fraction 1 **Fraction 2** **Fraction 3** **Fraction 4** **Fraction 5**

Numerous medicinal plants are known for their antioxidant activity. In almost all these plants antioxidant potential was found related with their total phenol, total flavonoids, ascorbic acid and carotenoids content [28-30]. In present study antioxidant activity of *Astilbe rivularis* Buch. – Ham. Ex D. Don (*A. rivularis*) was found confined in third fraction of chromatographically separated fractions of powdered leaves of *A. rivularis.* Results also showed that antioxidant activity was due to presence of maximum amount of total phenol, total flavonoids, ascorbic acid and carotenoids in the fraction.

Presently this active fraction is being investigated for isolation of antioxidant chemical(s).

Conclusion

Astilbe rivularis Buch. – Ham. Ex D. Don (*A. rivularis)* has in vitro antioxidant activity. To isolate active ingredient responsible for this activity, powdered leaves of *A. rivularis* were processed for solvent extraction, acid hydrolysis and chromatographic separation. Five fractions were separated by column chromatography. Results showed that the 3rd fraction had maximum in vitro antioxidant activity and contained maximum amount of total phenol, total flavonoids, ascorbic acid and carotenoids. In vitro antioxidant activity of the 3rd fraction seems to be due to the presence of the antioxidant chemicals.

References

[1] Gurung Bejoy (2002) The medicinal plants of Sikkim Himalaya. Pub. Bejoy Gurung, East Sikkim, P. 55.

[2] Das AP and Ghosh Chandra (2009) Germplasm collection in garden of medicinal plants. University of North Bengal, Siliguri, West Bengal, P. 8.

[3] Chopra Col Sir RN & Chopra IC (1958) Indigenous drugs of India, U.N.Dhar and Sons Private Limted, Kolkata, P. 605.

[4] Manandhar NP (2002) Plants and people of Nepal. Timber Press, Inc. Oregon, USA, P.29

[5] Rajbhandari M Mentel MR, Jha PK, Chaudhary RP, Bhattarai S, Gewali MB, Karmacharya N, Hipper Mand U. Lindequist R (2009). Antiviral activity of some plants used in Nepalese traditional medicine,eCAM. 6, 517-522.

[6] Meena Rajbhandari, Michael Lalk, Renate Mentel and Ulrike Lindequist (2011) Antiviral Activity and Constituents of the Nepalese Medicinal Plant Astilbe rivularis Rec. Nat. Prod. 5:2, 138 -142.

[7] Kirtikar KR, Basu BD (2001) Indian Medicinal Plants, vol. 9, 2nd ed.Oriental Enterprises, Rajpur, Dehradun, Uttaranchal, India, P. 2832-2836.

[8] Mitra P and Mitra PK (2008) Use of Astilbe rivularis Buch. – Ham. Ex D. Don as anti peptic ulcer agent**,** Pleione, 2(1), 74 – 76.

[9] Palash Mandal; Misra, T. K. (2009) Remove from marked Records *In vitro* antioxidant potential of *Astilbe rivularis* rhizome. Canadian Journal of Pure & Applied Sciences. 3(1), 649-654.

[10] Subedi L, Timalsena S, Duwadi P, Thapa R, Paudel A, Parajuli K (2014) Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal. J. Tradit. Chin. Med. 34(5), 584-90.

[11] Zarnowski R and Suzuki Y (2004) Expedient soxlet extraction of resorcinolic lipids from wheat grains. Journal of Food composition and Analysis. 17, 649 – 664.

[12] Wang L and Weller C L (2006) Recent advances in extraction of nutraceuticals from plants, Trends in Food Science and Technology. 17, 300-312.

[13] Cannell, RJP (1998) Natural Products Isolation. New Jersey: Human Press Inc; 1998. p 165–208.

[14] Huie, CW (2002) A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. Anal. Bioanal. Chem. 373, 2002, 23-30.

[15] Chang WS, Y.H. Chang, F.J. Lu, H.C. Chiang (1994) Inhibitory effects of phenolics on xanthine oxidase, Anticancer Res. 14, 501- 506.

[16] Chang W. Choi a,b,, Sei C. Kim a,b, Soon S. Hwang a, Bong K. Choi a, Hye J. Ahn a, Min Y. Lee a, Sang H. Park b, Soo K. Kim c (2002) Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Science. 163, 1161-1168

[17] Mensor LL, F.S. Menezes, G.G. Leita˜o, A.S. Reis, T.C. dos Santos, C.S. Coube, S.G. Leita˜o (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, Phytother. Res. 15, 127-130.

[18] Chang, C., M. Yang, H. Wen and J. Chern (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Analaysis. 10, 178-182.

[19] McDonald, S., P.D. Prenzler, M. Autolovich and K. Robards (2001) Phenolic content and antioxidant activity of olive extracts. Food Chemistry. 73, 73-84.

[20] Cakmak I, Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiol. 98, 1222-1227.

[21] Jensen A (1978) Chlorophyll and carotenoids. In: Hallebust JA, Craigie JS. (eds). Handbook of Physiochemical and Biochemical Methods. Cambridge University Press, Cambridge, UK, pp. 5-70.

[22] Bliss, CI (1967) Statistics in biology, Statistical methods for research in the natural sciences, Vol. 1, McGraw Hill Book Company, NY, pp: 558.

[23] Vinay R. Patel, Prakash R. Patel and Sushil S. Kajal (2010) Antioxidant Activity of Some Selected Medicinal Plants in Western Region of India. Advances in Biological Research. 4 (1), 23-26.

[24] Farber, JL (1994) Mechanisms of cell injury by activated oxygen. Environmental Health Perspectives, 102, 17-24.

[25] Katiyar Shikhar, Patidar Devendra, Gupta Shailja, Singh RK, Singh Poonam (2013) Some Indian Traditional Medicinal Plants With Antioxidant Activity: A Review. International Journal of Innovative Research in Science, Engineering and Technology. 2(112), 7303 – 7314.

[26] Miller, AL (1996) Antioxidant flavonoids: structure, function and clinical usage. Alt. Med. Rev., 1, 103.

[27] Branen A L (1975) Synthetic anti oxidants. Journal of American Oil Chemists Society. 52, 59–63.

[28] Sharma SK, Singh Lalit , SinghSuruchi (2013) A review on medicinal plants having antioxidant potential. Indian Journal of Research in Pharmacy and Biotechnology. 1(3), 404 – 409.

[29] Paolo Scartezzini, Ester Speroni (2000) Review on some plants of indian traditional medicine with antioxidant activity, Journal of Ethnopharmacology. 71, 23-43.

[30] Gopal TK, Harish G, Chamundeeswari D, Reddy C Umamaheswara (2010) *In-vitro* Anti-Oxidant Activity of Roots of Boerhaavia diffusa Linn, Research journal of Pharmaceutical, Biological and Chemical Sciences 4, 782-788.

Authors Column

Prof. (Dr.) Prasanta Kumar Mitra is a very senior medical teacher and researcher. He has completed thirty eight years in medical teaching and more than forty years in research. His research area is 'Medicinal plants of India'. He has four Ph.D.s to his credit and published one hundred fifty one research papers in national & international peer reviewed journals. Fifteen students did Ph.D. work under his guidance. He was co-supervisor of the research projects of five MD students.

Prof. Mitra was Editor-in-Chief of the European Journal of Biotechnology and Biosciences. He is now Editor, SMU Medical Journal and giving service as Editor, Associate Editor, Member of Editorial Board and Reviewer of many national and international research journals. He is the member of many scientific & research organizations of India and abroad.

On behalf on Govt. of West Bengal Prof. Mitra worked as Coordinator of World Bank and GTZ projects for Health Sector Development in North Bengal.

Presently, Prof. Mitra is heading the Dept. of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences (SMIMS) of the Sikkim Manipal University, India. He is research coordinator of SMIMS and Member Secretary of Institutional Ethics Committee (SMIMS). He is also a member of the International Forum of Teachers of the UNESCO Chair in Bioethics and Chairman of the committee for preparation of Bioethics Curriculum in Biotechnology under UNESCO Chair in Bioethics.

Prof. Mitra is a well known writer and science popularizer. He has written sixteen hundred ninety nine popular science articles in different newspapers / magazines. He is the recipient of Rajiv Gandhi Excellence award for his academic excellence