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Der Pharma Chemica, 2011, 3 (5):255-262
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Antioxidant and antibacterial activity of *achyranthes aspera* : An *in vitro* study

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ABSTRACT

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. The aim of the present study was to evaluate the antioxidant and antibacterial activities of the Achyranthes aspera plant extract in different organic solvents. The radical scavenging activity of the different extracts of root, stem, leaf and inflorescences was evaluated by DPPH assay and the antibacterial activity against Staphylococcus aureus a gram positive and Escherichia coli a gram negative bacteria was studied by Agar well cut diffusion method. All of the extracts exhibited different antioxidant and antibacterial activities and the activities varied from solvent to solvent and the activities are concentration and time dependant. The antioxidant and antibacterial activities were compared with the positive control Ascorbic acid and Gentamycin. A qualitative phytochemical analysis was carried out and found to possess bioactive compounds like alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins.

Keywords: antioxidant, antibacterial, DPPH, *Achyranthes aspera*, Gentamycin.

INTRODUCTION

In Biological systems, free radicals are generated as part of the body's normal metabolic process, the free radical chain reactions are usually produced in the mitochondrial respiratory chain, atmospheric pollutants and from drugs. [1, 2] In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting can result in increased radical activity and damage [3, 4]. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes [5]. Infectious diseases are the world's leading cause of premature deaths [6]. Therefore, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [6-8]. The antimicrobial properties of plants

have been investigated by a number of researchers worldwide. In recent years the interest to evaluate plants possessing antibacterial activity for various diseases is growing [9] and it has also been proved that various plants extracts posses bacteriostatic and bactericidal effects [10] and most of the plants contains many bioactive compounds. Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc.,[11]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct .Therefore, it is worthwhile to use modern science and technology tools for verifying therapeutic potential of medicinal plants as antioxidant as per international standards. Such information may be of potential value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals-induced tissue damage [12]. In view of the above, the current study was carried out to evaluate antioxidant and the antibacterial activity of *Achyranthes aspera* Linn. belonging to Amaranthaceae family.

MATERIALS AND METHODS

Chemicals

DPPH was purchased from Sigma Aldrich Ltd, Mumbai and Muller Hinton agar media no 173 was purchased from Hi media Pvt. Ltd., Mumbai, and the solvents used are of AR Grade and they were distilled before use. The microorganisms employed in the current study were procured from the Department of Microbiology, Auxilium College, Vellore which includes clinical isolates of *Staphylococcus aureus* (*S. aureus*) the gram positive *bacteria* and the *Escherichia coli* (*E. coli*) gram negative bacteria.

Collection of plant materials:

Achyranthes aspera Linn. commonly known Rough chaff flower in English, is an annual herb that grows throughout India [13]. The plant material was collected in the month of August from Auxilium College campus, Vellore, Tamil Nadu, India and was taxonomically identified by the Department of Botany, Auxilium College, Vellore, Tamil Nadu, India and a voucher specimen was retained in herbarium for future reference.

Extraction and fractionation:

The freshly cut plants were sorted out as root, stem, leaf, inflorescences and shade dried in the room with active ventilation at ambient temperature and pulverized to a coarse powder in a mechanical grinder and sieved using a mesh of 40 mesh size. The powders (2Kg) were separately percolated using solvents like Hexane, chloroform, Ethyl acetate and Methanol. The miscella was then concentrated using a Vacuum rotary vapor under reduced pressure. The dark brownish semisolid extract was preserved in tightly closed container and used for the analysis.

Antioxidant Activity

DPPH free radical scavenging activities were measured according to method of Shimada [14] with a slight modification of Cakir [15]. Briefly, 2 ml extracts in various concentrations (100-700 µg/ml) of *Achyranthes aspera,* 1ml DPPH solution (methanolic 0.1 mM DPPH) was added. The mixture was shaken vigorously, and the absorbance was measured in 517 nm by UV Spectrophotometer at different concentrations and time intervals. All experiments were performed in triplicate and ascorbic acid was used as standard.

Antibacterial Study

The bacterial strains *Staphylococcus aureus* and *Escherichia coli* species were pre-cultured in Mueller-Hinton Agar overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically. The antibacterial activity of the extracts was determined by using the Agar well cut diffusion technique [16]. Mueller- Hinton agar media plates were each seeded with 0.1 ml of an overnight culture of each bacteria (equivalent to 10^7 –10^8 CFU/mL). After the incubation period they were used as inoculum and the standard procedure was used to measure antibacterial activities [17].

Data analysis

Results were expressed as mean ± SD. In all cases antioxidant activity was based on at least three independent experiments performed in duplicate. Regression analysis was followed to check the linearity for the mean absorbance for all concentration. ANOVA was used to compare significance between the concentrations. A difference was considered to be significant when P value <0.05.

RESULTS AND DISCUSSION

The plant kingdom represents a rich store house of organic compounds, many of which have been used for medicinal and other purpose. There exists a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. Oxidation reactions are necessary part of life, unfortunately they can also be damaging because of the production of reactive oxygen species (ROS). In the past few years, there has been growing interest in the involvement of ROS in several pathological situations [18]. ROS are by-products of basic metabolic processes, immune reaction against pathogens, air pollution, tobacco smoke, herbicides, and pesticides. In biological systems, phenolic compounds and flavonoids are associated with scavenging ROS [19, 20]. In invitro condition DPPH is considered as a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule [21]. The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm and also by the colour change from purple to yellow, which is induced by anti-oxidants [19]. The degree of discoloration indicates the scavenging potential of the antioxidant compound in the extracts [22].

In this work various fractions obtained from the different parts of the plant in different extracts were tested for their anti oxidant capacity and the results are reported in Table 1- 4. DPPH was reduced with the addition of all fractions in a concentration and time dependent manner. The work is designed to analyze which part of the plant exhibits enhanced antioxidant activity which was lacking in the previous work. The different plant extracts at various concentrations induced a rapid decrease in the optical density. The decrease in absorbance of DPPH radical caused by antioxidants is visually noticeable as a change in colour from purple to yellow when measured at 517 nm.

Antioxidant activities of Hexane extract of *Achyranthes aspera* for different parts of the plant was given in Table. 1. The antioxidant activity varied from parts to parts and it increased as the time and the concentration increased and the order was: root > stem > inflorescences > leaf.

Antioxidant activity of Chloroform extract of *Achyranthes aspera* for different parts of the plant was shown in Table. 2. In Chloroform extract, the stem showed high radical scavenging potential and it almost closer to standard Ascorbic acid [17]. The Inflorescence exhibited a higher activity

(82 %) and the order of activity was: Stem> Inflorescences > leaf. Root did not show any antioxidant activity and this may be due to the interference of individual chemical components present in the chloroform extract.

Table.1 Antioxidant activities of different parts of *Achyranthes aspera* in Hexane extract

S.No	Concentration (µg / mL)	Time (min)	Antioxidant Activity (%)			
			Root	Stem	Leaf	Inflorescences
1	100	0	14.35±0.43	02.82±0.33	00.12±0.35	5.05±0.09
		30	22.69±0.62	10.94±0.62	02.22±0.66	14.03±0.99
		60	28.42±0.43	15.86±0.43	04.85±0.00	17.25±0.78
2	200	0	22.26±0.00	05.77±0.69	01.27±0.99	8.51±0.02
		30	28.42±0.01	10.94±0.11	02.81±0.59	13.52±0.00
		60	31.08±0.33	15.86±0.99	05.98±0.33	18.3±0.44
3	300	0	23.30±0.56	20.18±0.48	03.14±0.40	11.77±1.00
		30	30.26±0.77	23.74±0.62	05.18±0.25	18.68±0.40
		60	36.51±0.99	26.49±0.77	07.54±0.30	25.9±0.88
4	400	0	24.22±0.84	26.60±0.43	03.43±1.90	20.49±0.76
		30	33.66±0.64	32.13±0.62	07.51±0.99	25.96±0.39
		60	37.33±0.44	34.21±0.08	12.81±0.35	28.63±0.25
5	500	0	26.43±0.31	26.43±0.45	4.94±0.21	23.77±0.18
		30	34.99±0.28	34.58±0.23	12.74±0.38	30.67±0.59
		60	41.24±0.51	37.67±0.62	19.72±0.49	34.61±0.44
6	600	0	30.94±0.71	32.51±0.44	05.05±0.82	24.52±0.29
		30	35.99±0.59	34.95±0.23	14.03±0.10	31.48±0.18
		60	43.87±0.27	37.33±0.39	20.25±0.50	35.81±0.33
7	700	0	33.12±0.11	33.12±0.28	05.77±0.29	34.47±0.27
		30	42.87±0.36	40.01±0.78	16.61±0.33	36.51±0.37
		60	51.08±0.33	46.32±0.23	24.33±0.23	38.09±0.24

Table.2 Antioxidant activities of different parts of Achyranthes aspera in Chloroform extract

S.No	Concentration (µg/mL)	Time (min)	Antioxidant Activity (%)		
			Stem	Leaf	Inflorescences
1	100	0	09.33±0.66	1.05±0.66	10.10±0.65
		30	33.33±0.77	20.3±0.56	29.62±0.43
		60	45.83±0.55	42.05±0.90	47.03±0.7
2	200	0	11.60±0.67	4.62±0.12	14.08±0.76
		30	40.05±0.22	21.07±0.89	33.33±0.76
		60	58.65±0.32	43.22±0.77	49.04±0.32
3	300	0	12.50±0.89	4.94±0.45	16.67±0.43
		30	54.66±0.98	23.05±0.08	38.53±0.5
		60	72.83±1.45	44.95±0.90	60.65±0.67
4	400	0	14.14±3.60	5.45±0.54	18.67±0.60
		30	56.56±0.65	28.01±0.90	46.61±0.90
		60	85.13±0.56	46.82±0.76	65.02±0.32
5	500	0	15.33±0.87	5.85±0.45	30.02±0.90
		30	58.09±0.88	32.07±0.54	48.61±0.09
		60	86.13±0.33	52.04±0.54	72.76±0.12
6	600	0	20.09±0.87	6.03±0.98	34.14±2.87
		30	60.02±0.54	33.4±1.76	49.78±0.69
		60	88.89±0.65	53.45±2.47	75.08±0.32
7	700	0	22.22±0.67	7.05±0.65	35.54±0.54
		30	63.15±0.32	34.09±0.78	52.91±0.21
		60	89.03±0.22	55.67±0.18	82.48±0.17

Antioxidant activity of Ethyl acetate extract of *Achyranthes aspera* for different parts of the plant was shown in Table. 3. The antioxidant activities of root was 65%, stem 51%, leaf 71% and inflorescences was 78%. and the order followed was inflorescences > leaf > root > stem and they were concentration and time dependant.

Table. 3 Antioxidant activities of different parts of Achyranthes aspera in Ethyl acetate extract

S.No	Concentration (µg/mL)	Time (min)	Antioxidant Activity (%)			
			Root	Stem	Leaf	Inflorescences
1	100	0	01.84±0.99	08.06±0.6	17.18±0.78	20.64±0.87
		30	09.64±0.67	15.35±0.65	23.05±0.56	28.39±0.78
		60	24.95±0.78	22.29±0.32	34.68±0.56	46.01±0.67
2	200	0	03.73±0.67	09.01±0.43	20.14±0.45	24.59±0.67
		30	16.60±0.99	16.26±0.90	38.41±0.36	36.88±0.60
		60	28.58±0.12	26.49±0.32	52.55±0.90	49.77±0.87
3	300	0	14.02±0.34	20.53±0.32	26.54±0.32	32.48±0.54
		30	22.42±0.36	29.95±0.98	44.59±0.32	44.63±0.43
		60	41.50±0.98	36.69±0.90	57.45±0.98	61.70±0.68
4	400	0	14.47±0.23	26.80±0.65	26.95±0.90	35.60±0.34
		30	26.09±0.98	33.94±0.21	39.73±0.21	52.98±0.53
		60	42.50±0.43	40.18±0.90	59.64±0.23	67.89±0.48
5	500	0	18.29±0.21	30.45±0.38	33.09±0.09	36.01±0.59
		30	31.61±0.90	36.14±0.47	45.14±0.43	49.17±0.43
		60	45.58±0.89	43.77±0.32	62.95±0.39	67.89±0.32
6	600	0	26.84±0.89	33.66±0.32	36.59±0.34	41.83±0.59
		30	40.01±0.23	42.43±0.98	49.64±0.56	59.13±0.47
		60	58.50±0.28	50.58±0.21	68.18±0.87	72.98±0.68
7	700	0	30.22±0.90	34.16±0.90	40.14±0.86	43.58±0.67
		30	49.06±0.90	43.33±0.32	55.86±0.78	63.53±0.78
		60	65.21±0.22	51.56±0.10	71.27±0.11	77.80±0.55

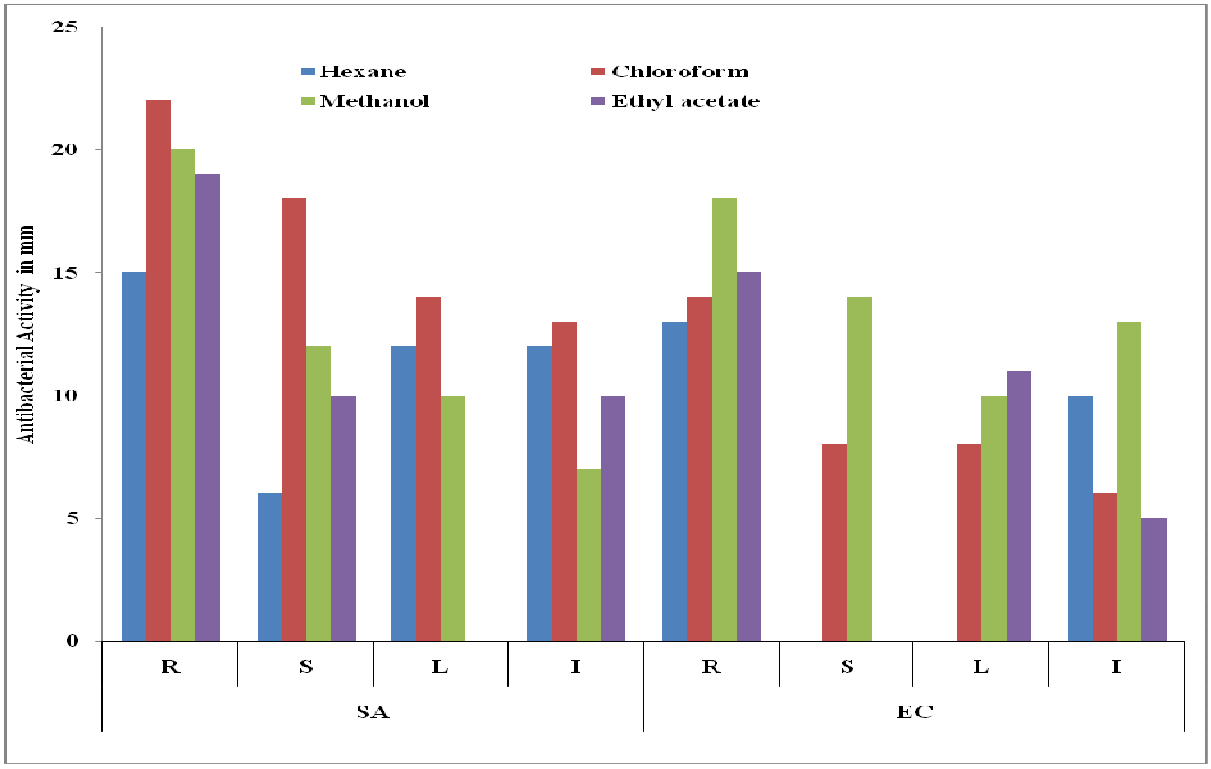
Table. 4 **Antioxidant activities of different parts of Achyranthes aspera in Methanol extract**

S.No	Concentration (µg/mL)	Time (min)	Antioxidant Activity (%)			
			Root	Stem	Leaf	Inflorenscence
1	100	0	03.58±0.99	5.68±0.23	6.61±0.23	4.12±0.56
		30	27.09±0.46	25.93±0.23	19.46±0.65	37.36±0.65
		60	43.88±0.43	48.42±0.56	45.34±0.67	71.84±0.56
2	200	0	6.97±1.78	7.68±0.98	15.18±0.32	17.05±0.56
		30	33.86±0.78	31.94±0.98	33.99±0.32	44.96±0.65
		60	46.21±0.43	61.13±0.65	58.15±0.09	80.27±0.09
3	300	0	9.33±0.34	9.78±0.45	18.39±0.32	22.75±0.32
		30	43.82±0.14	44.04±0.45	40.46±0.57	54.36±0.65
		60	54.58±0.46	73.04±0.34	64.15±0.23	82.18±0.65
4	400	0	11.11±0.33	11.87±0.43	22.86±0.87	24.71±0.54
		30	51.85±0.47	45.76±0.32	46.50±0.68	55.56±0.86
		60	73.83±0.87	78.40±0.23	67.68±0.56	87.45±0.43
5	500	0	13.54±0.56	18.9±0.45	25.84±0.68	28.42±0.21
		30	59.72±0.66	48.42±0.65	44.74±0.50	62.43±0.32
		60	77.31±0.34	80.77±1.78	68.99±2.89	88.57±0.87
6	600	0	15.9±0.56	25.23±0.90	37.8±3.89	36.54±0.98
		30	62.37±0.89	56.84±0.30	62.99±0.87	71.16±0.98
		60	84.3±0.45	82.08±0.43	81.05±2.76	90.86±0.21
7	700	0	36.54±0.76	37.94±0.65	40.46±0.54	40.54±0.90
		30	69.16±0.75	65.92±0.34	67.89±0.32	71.61±0.23
		60	90.86±0.07	92.83±0.01	86.76±0.11	93.05±0.18

Antioxidant activity of Methanolic extract for different parts of the plant was shown in Table. 4. In methanolic extract, all the parts of the plant exhibited very high antioxidant activity which was closer to standard L - Ascorbic acid (94%). The activity of root was 90%, stem and inflorescences was 93% leaf 87%. The antioxidant activities of different parts in methanolic extract were above 90% except for leaves and the order followed was: Inflorescences = Stem > Root > leaf.

Comparison of the antioxidant activities of different parts of *Achyranthes aspera* in different organic solvents are in 700 µg / mL at 60th minute are given in figure 1. The result clearly indicates that as the concentration and time increases the antioxidant activity also increases. And the antioxidant activity was highest in methanolic extract at 700 µg/mL at 60th minute.

Figure. 1 Antioxidant Activities of Different Parts of the Plant in different solvents in 700µg /mL at 60 min



From regression analysis it was clear that there was a good linearity absorbed (R^2 =0.97) in antioxidant studies and the ANOVA showed that there is a significant difference among the extracts and the critical difference show that methanol extract of all the parts of the plant have high antioxidant activity in the concentration 700 µg/mL at 60th minute.

The antibacterial activity of plant extracts in Hexane, Chloroform, Ethyl acetate and Methanol was against S. aureus and *E. Coli.* The zone of inhibition recorded for the different parts of the plant in different extracts is presented in Table 5. The highest antibacterial activity against S. aureus was recorded as 22 mm by the chloroform extract of root at 25 mg concentration and 18 mm by the methanol extract of root at 25 mg concentration against *E. coli.*

Table.5 Antibacterial activities of the different part of the plant in different organic solvents

Micro-org.	Concentration (mg/mL)	Zone of Inhibition (mm)															
		Hexane				Chloroform				Ethyl acetate				Methanol			
		Rt	Sm	Lf	If	Rt	Sm	Lf	If	Rt	Sm	Lf	If	Rt	Sm	Lf	If
S.aureus	10.0	10	3	8	4	7	3	6	8	12	8	-	5	12	3	-	1
	12.5	13	4	8	6	12	10	10	10	17	9	-	8	16	6	8	3
	25.0	15	6	12	12	22	18	14	13	19	10	-	10	20	12	10	7
E. coli	10.0	5	-	-	2	10	5	4	4	-	-	-	-	3	6	-	5
	12.5	10	-	-	6	10	7	6	4	7	-	4	4	8	5	-	10
	25.0	13	-	-	10	14	8	8	6	15	-	11	5	18	14	10	13

Rt - Root , Sm – Stem, Lf – Leaves, If - Inflorescences

In Hexane extract, Root showed the highest antibacterial activity of 10mm even in low concentration (10mg) and 15 mm in 25mg concentration against S. aureus. The antibacterial activity against S. aureus decreased in the order of root > leaf = inflorescences > stem and against *E. Coli. the order is* root > inflorescences. The stem and Leaves extract doesn't seem to inhibit *E. coli.* In chloroform and methanolic extracts, the order shown is: root> stem >leaf> inflorescences against *S. aureus*. The chloroform extracts showed antibacterial activity against *E.Coli* in order of root > stem = leaf > inflorescences and methanolic extract showed in the order of root > stem =leaf > inflorescences. In Ethyl acetate, the order shown is: root> stem = inflorescences. However, Ethyl acetate extract of Leaves did not show any antimicrobial activity against *S. aureus* and stem does not show activity against *E.Coli*. Such behavior of the antibacterial action was also showed by Alam [23]. The inhibitory activities of all the extracts reported in Table 5 are comparable with standard antibiotic Gentamycin. Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional practitioners make use of water primarily as a solvent, but our studies showed that even organic solvents were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvent and flavonoids are least stable in water which is the primary polyphenolic compound in plants [24]. Literature survey revealed that chemical constituents like flavonoids, triterpenoids, polyphenolic compounds and steroids are responsible for antioxidant and antibacterial activity and these chemical constituents were reported in the methanolic extract of aerial parts of *Achyranthes aspera* [25, 26]. The preliminary Phytochemical screening of the extracts showed the presence of phenolic compounds, flavonoids, Alkaloids, Steroids, Tannins etc. These compounds may be responsible for antioxidant activity and antibacterial activity and may serve as a substitute for synthetic drugs.

CONCLUSION

The present study showed that there is a higher antioxidant and antimicrobial activity for the plant *Achyranthes aspera*. So this study supports the traditional usage as antiulcer and antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the active compound and carry out further pharmacological evaluation. This will surely complement to the previously known therapeutic values and improve the popularization of this plant.

Acknowledgments
The authors are grateful to the College management and Ms. B.V. Gowri, Assistant Professor of Microbiology department for providing facilities and support to pursue antibacterial activity analysis.

REFERENCES

[1] Naznin Ara, Hasan Nur, *Research Journal of Medicine and Medical Sciences.*, **2009**, 4(1): 107-110.
[2] M.N. Saha, M.A. Alam, R. Aktar, R. Jahangir, *Bangladesh Journal Pharmacology.*, **2008**, 3: 90-96.
[3] N. Ozsoy, A. Can, R. Yanardag, N. Akev, *Food Chemistry*, **2008**, 110, 571–583.
[4] B. Halliwell, J.M.C. Gutteridge, *Free Radicals in Biology and Medicine. Oxford University Press, Oxford.* **1999.**
[5] R.L. Prior, *American Journal of Clinical Nutrition*, **2003,** 78, 570S-578S.
[6] T.C. Emori, R. Gaynes, *Clinical Microbial Review*, **1993**, 6, 428-442.
[7] J. Srivastava, J. Lambert, N. Vietmeyer, *J. W.k Technical Paper*. **1996**, No. 320,.
[8] S.K. Uniyal, K.N. Singh, P. Jamwal, B. Lal, *J. Ethnobiol*., **2006**, 2, 1-14.
[9] A.M. Clark, C.D. Hufford, *Human Medical Agents from Plants. American Chemical Society:534*, ***1993***, 228-241.
[10] H.S. Lee, *Food Sci. Biotech*, **2000**, 9: 52-56.
[11]H.K. Makari, N. Haraprasad, H.S. Patil, Ravikumar, *The Internet J. Aesthetic and Antiaging Medicine*, **2008**, 1, 1-10.
[12] P. Suresh Kumar, S. Sucheta, V. Sudarshana Deepa, P. Selvamani, S. Latha, *African Journal of Biotechnology*, **2008**, 7 (12), 1826-1828.
[13] K.M. Nadkarni, A.K. Nadkarni, *Indian Materia Medica, vol. 1. Popular Prakashan, Bombay*, **1976**, 21.
[14]K. Shimada , K. Fujikawa, K. Yahara, T. Nakamura, *J Agric Food Chem*, **1992**, 40, 945 – 948.
[15]A. Cakir, A, Maci, A. Yyldyrym , M.E Durub, M. Harmandar, V. Kazaz, *J. Ethanopharmacol*, **2003**, 87, 73-83.
[16] B.A Adeniyi, H.A. Odelola, B.A. Oso, Afr. *J. Med. Sci.*, **1996**, 25, 221–224.
[17]D. JayaKumar, S. JhancyMary, R. Jaya Santhi, *Indian Journal of Science and Technology*, **2010**, 3(7), 720-723.
[18] O.I. Aruoma, B. Halliwell, *Biochem. J*., **1987**, 241, 273-278.
[19] R.C. Evans, V. Sampson , P.M. Bramley , D.E. Holloway, *Free Radical Res*, **1997**, 26, 381–398.
[20] L.V. Jorgensen, H.L. Madsen, M.K. Thomsen, L.O. Dragsted, L.H. Skibsted, *Free Radical Res*, **1999**, 30, 207-220.
[21] J.R. Soares, T.C.P. Dinis, A.P. Cunha, L.M. Almeida, *Free Radical Research*, **1997**, 26, 469–478.
[22] M. Oktay, I. Gulein, I. Kufreviolglu, Labenson-Wiss U. *Technol*, **2003**, 36, 263-71.
[23] M. T. Alam, M. M. Karim, Shakila N. Khan, *Journal of scientific research*, **2009**, 1(2), 393-398.
[24] H.J. De Boer, A. Kool, A. Broberg, W.R. Mziray, I. Hedberg, J.J. Levenfors, *J. Ethnopharmacol*, **2005**, 96, 461-469.
[25] A. Chakrabortya, A. Brantnera, T. Mukainakab, Y. Tobukuni, M. Kuchideb, T. Konoshimae, H. Tokubab, H. Nishino, *cancer Lett.*, **2002**, 177, 1-5.
[26] P. Tahiliani, A. Kar, *J.Ethnopharmacol* ., , **2000**, 71527-532.