ISSN: 0975-8585

**Research Journal of Pharmaceutical, Biological and Chemical Sciences**

# Free Radical Scavenging Capacity and Antioxidant Activity of an Ayurvedic Medicinal Plant *Solanum rubrum Mill*.

**Santhosh Kumar S[1*], Subramanian A[2], SUJA SK[3], Sudarshan M[4], and Chakraborty A[5]**

[1]Department of Physics, Avvaiyar Government College for Women, Karaikal – 609 602, U.T of Puducherry, India.
[2]Department of Biotechnology, Government Arts College, Kumbakonam-612001, Tamil Nadu, India.
[3] Department of Chemistry , Lady Doak College, Tallakulam, Madurai - 625002, Tamilnadu, India.
[4]Trace Element Sciences Laboratory, UGC-DAE Consortium for Scientific Research, Kolkata - 700 098, India.
[5]Radiation Biology Laboratory, UGC-DAE Consortium for Scientific Research, Kolkata 700 098, India.

## ABSTRACT

For the first time, one of the traditionally used Ayurvedic medicinal plant *Solanum rubrum* Mill is subjected into investigation for its antioxidant potency. The difference between this plant and *Solanum nigrum* is identified with the help of topology, smell and taste. Preliminary phytochemical analysis of the pet-ether fraction of this plant leaves shows possession of alkaloids, flavanoids, saponins, phenols, steroids, protein, lignin, diterpene and glycosides. This plant leaf also exhibited significant antioxidant activity. The DPPH radical scavenging activity of S.rubrum showed $IC_{50}$=474.11 μg/ml, and the nitric oxide scavenging activity $IC_{50}$ = 229.11μg/ml. This study reveals the presence of new compounds in the plant leaf and which validates its potential usage in the traditional Ayurvedic system of medicine, to some extent.

**Keywords:** Ayurvedic medicinal plant, phytochemical, antioxidant activity, DPPH, Solanum rubrum

***Corresponding author***

## INTRODUCTION

In recent years, the use of natural antioxidants present in plants has attracted considerable interest due to their presumed safety and therapeutic value. The therapeutic use of plants is mainly for antioxidants, in reducing free radical induced tissue injury. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Even though synthetic antioxidants are available commercially, due to their toxicity, these have to be replaced with naturally available materials and hence plants are the only source.

The main characteristic of an antioxidant is its ability to trap free radicals and oxygen species, are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds scavenge free radicals and inhibit the oxidative mechanism that lead to degenerative diseases.

In the present day biological studies, identification of plants is by comparison with previously collected specimen or with the aid of books or identification manuals, more specifically, plant taxonomy. But in Ayurvedic system the procedure is little different, i.e., taste and odor of the plant leaves took leading role in identification and classification. This leads us the identification of *Solanum rubrum* which is different than the *Solanum nigrum* both in taxonomy and phytochemical aspects.

It is known that plants contain many bioactive chemical substances that produce definite physiological and biochemical actions in the human body. These bioactive constituents are alkaloids, tannin, flavonoids, phenolic compounds etc.[1,2]. Plant derived natural products have received considerable attention in recent years due to diverse pharmacological properties, including antioxidant and antitumor activity [3].

*In-vitro* tests are useful in comparing different plants for their antioxidant capacity but that the chemical antioxidant capacity of a plant part may not be relevant to what happens in the human body, in a form of drug or food. While consuming, some are absorbed, while others aren't or are otherwise modified. There are enzymes in cells and tissues in the body that protect the body from excess oxidation. In addition, other components from plants or vegetative drugs and their metabolites may influence oxidation/antioxidant status once absorbed. There must be interactions and synergies between antioxidants from consumed plant part or drug and antioxidant enzymes in the body that affect the existing antioxidant status of the body [4]. Hence it is a necessity to find the right plant which has a higher level of medicinal value.

Thus the current focus is towards natural antioxidants, and it is interesting to investigate the antioxidant properties of plants, especially, which are in traditional medicinal practice. In this work we have taken the plant, *Solanum rubrum* Mill, which was identified as a synonym for *Solanum nigrum*. In fact, Ayurvedic system pronounces these two plants differently, such as

*Solanum nigrum* as '*Manithakkali'* and *Solanum rubrum* as '*Karunthakali*'. From the literatures available we understood that this plant has not been studied yet, even though it has potential application in traditional practice of medicine. Its phytochemical analysis yielded different results than other plants in the same family '*Solanaceae*'. A photograph of the plant is given in Fig.1., for identification.

**Fig.1 Solanum rubrum Mill**

*Solanum rubrum Mill* is a shrub, which is available in Western Ghats of Peninsular India which has much ecological significance and grows upto one feet to one meter height with dark green coloured leaves and the fruits are red or yellow in colour. Commonly, this plant is not in use nowadays for any purpose. In old palm leaves and manuscripts of Ayurvedic system it is mentioned as this plant can be used for skin diseases, and paralysis disease. With the structure and taste of the leaf we suspect that this plant has some more valuable medicinal characters. Hence for the first time, we report the antioxidant activity of this plant.

## MATERIAL AND METHODS

The Plant leaves of *Solanum rubrum Mill,* are collected from the southernmost part of India, Kanyakumari District of TamilNadu at coordinates 8°18'8"N 77°10'34"E. The fresh leaves of this plant are collected and washed well in pure water and shadow dried. This dried leaves are powdered using morter and pistle. The pet ether extract of the sample was obtained by adding the leaf powder with pet-ether in the ratio of 10gm for 100ml and extracting for 48 hours using Soxhlet apparatus. The solvent is concentrated at temperature below $40^{o}C$ and the resulting extracts are used for determination of DPPH radical scavenging activity, reducing power and Nitric oxide scavenging activity, at different concentrations. The experiment was repeated for three times.

### Determination of antioxidant activity

The DPPH radicals are widely used to investigate the scavenging activity of natural compounds. The antioxidant activity of the plant extracts and the standard was assessed on the

basis of the radical scavenging effect of the stable 1,1,-diphenyl-2-picryl- hydrazyl (DPPH)- free radical activity by modified method[5]. The diluted working solutions of the test extracts are prepared in pet-ether. Ascorbic acid is used as standard in 100-500 µg/ml solution. 0.002% of DPPH was prepared in pet-ether and 1ml of this solution was mixed with 1ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Amile photocalorimeter.

In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow coloured diphenyl- picrylhydrazine, resulting a colour change from purple to yellow. The absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH molecule. In the radical form, the molecule had an absorbance at 517nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule[6]. This method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogenating antioxidant due to the formation of the non- radical form (DPPH-H) in the reaction[7]. $IC_{50}$ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

The percentage inhibition of the DPPH radical by the sample was calculated as follows[8,9],

$$\% \text{ DPPH. Scavenging} = 100 \times \frac{\{\text{Absorbance of Control- Absorbance of Sample}\}}{\text{Absorbance of Control}}$$

where Absorbance of Control is the absorbance in absence of standards or extracts; Absorbance of Sample is the absorbance in presence of standards or extracts

**Reducing power determination**

Yen and Chen[10] method was followed to determine the reducing power. Different amounts of extracts (100-500 µg/ ml) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50$^o$C for 20 min. and the reaction was stopped by adding 2.5 ml of trichloro-acetic acid (10%) and then it was centrifuged at 3000 rpm for 10 min. 2.5ml of the upper layer solution was mixed with equal quantity of distilled water and 0.5ml of $FeCl_3$, and the absorbance was measured at 700nm[11]. Increased absorbance of the reaction mixture is said to be increased reducing power. Ascorbic acid was used as positive control.

**Nitric oxide-scavenging activity**

This procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different

concentrations of each extracts dissolved in water and incubated at room temperature for 60 minutes. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 540nm [11]. Ascorbic acid was used as positive control.

## RESULTS AND DISCUSSION

### Phytochemical Analysis

Knowledge of the chemical constituents of plant is desirable, not only for the discovery of therapeutic drugs but also because such information may be value disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folklore remedies [12].

Antioxidant compounds may be water-soluble, lipid-soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in identifying the antioxidant activity. Here we have used pet-ether extract as the solvent to prepare the leaf extract and its phytochemical constituents.

The phytochemical analysis of pet-ether extract of *Solanum rubrum* leaves showed the presence of alkaloids, flavanoids, saponins, phenols, steroids, protein, lignin, diterpene and glycosides. Tannin, Anthraquinone, cardiac glycosides and carotinoids are not found in the plant leaves[4]. Irrespective of the extract, the phytochemical composition of this plant is compared with few plants in the Solanaceae family and are tabulated in Table 1. From the table, it is clear that this plant has more compounds than other plants of this family.

The observed alkaloid content in *Solanum rubrum* Mill could be responsible for its medicinal values. Saponins are a special class of glycosides which have soapy characteristics [17], which have the property of precipitating and coagulating red blood cells, hemolytic activity, cholesterol binding properties and bitterness [18,19], which shows the high medicinal value of the leaf extract of *Solanum rubrum*. Tannins are water soluble plant polyphenols that precipitate proteins and are also antimicrobial agents and which have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them[20].The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins[21]. Flavonoids are potent water soluble antioxidants and free radical scanvengers, which prevent oxidant cell damage, have strong anticancer activity[19,22]. Flavonoids in intestinal tract lower the risk of heart disease and provide anti-inflammatory activity[19].

**Table 1. Qualitative phytochemical screening of *Solanum rubrum* in comparison with few other plants of the *Solanaceae* family.**

| Compounds | *Solanum nigrum* Ref(13) | *Solanum nigrum* Ref(14) | *Solanum myriacanthus* Ref(13) | *Solanum surattence* Ref(15) | *Solanum trilobatum* Ref(16) | *Solanum rubrum* |
|---|---|---|---|---|---|---|
| Alkaloids | ++ | ++ | ++ | -- | ++ | ++ |
| Flavanoids | ++ | ++ | ++ | -- | ++ | ++ |
| Tannins | ++ | ++ | ++ | ++ | ++ | -- |
| Saponins | ++ | ++ | ++ | ++ | ++ | ++ |
| Phenols | | | | | | ++ |
| Steroids | | | | | | ++ |
| Protein | ++ | | ++ | | ++ | ++ |
| Lignin | | | | | | ++ |
| Diterpene | | | | -- | | ++ |
| Anthraquinone | | | | | | -- |
| Glycosides | | -- | | | | ++ |
| Cardiac glycosides | | | | | -- | -- |
| Carotinoids | | | | | | -- |

++ - present; -- absent; blank means not tested/reported.

## *In vitro* antioxidant activity

### DPPH- radical scavenging activity

DPPH is a stable nitrogen-centered free radical, the color of which changes from purple to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers[23]. It is found that the radical-scavenging activities of the plant extract increased with increasing concentration (Fig.2). The $IC_{50}$ value is calculated by following the linear fitting procedure and for S.rubrum, $IC_{50}$ = 474.11 μg/ml, and for Ascorbic acid, $IC_{50}$ = 200 μg/ml. It has been shown that the scavenging effects on the DPPH radical increases sharply with the increasing concentration of the samples and standards to a certain extent[24], and hence are said to be strongly dependent on the phenols and antioxidant activity. Since the plant extracts are quite safe and their toxicity is a not a problem of concern unlike those of BHT, they could be exploited as antioxidant additives but not as nutritional supplements, since in traditional practice, it is suggested that high intake of this plant lead to poisoning.

ISSN: 0975-8585

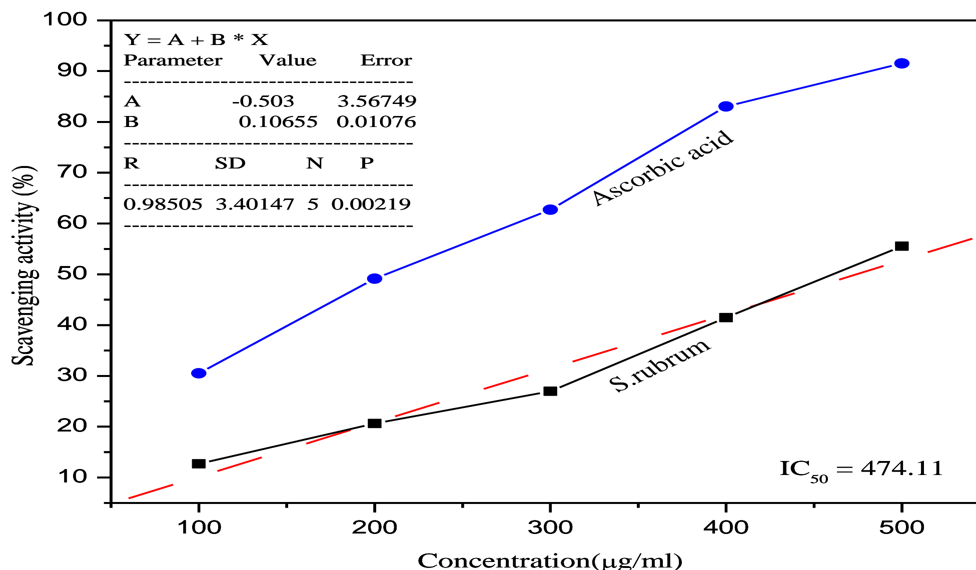


**Fig.2. Free radical scavenging activity of pet-ether extract of *Solanum rubrum* leaves measured using the DPPH assay. Ascorbic acid was used as reference control.**

## Reducing power

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action[25]. In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of $Fe^{3+}$ to $Fe^{2+}$ by donating an electron. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig.3 shows dose-response curves for the reducing powers of the samples. It is found that the reducing power of the sample increased with concentration, but is weak.

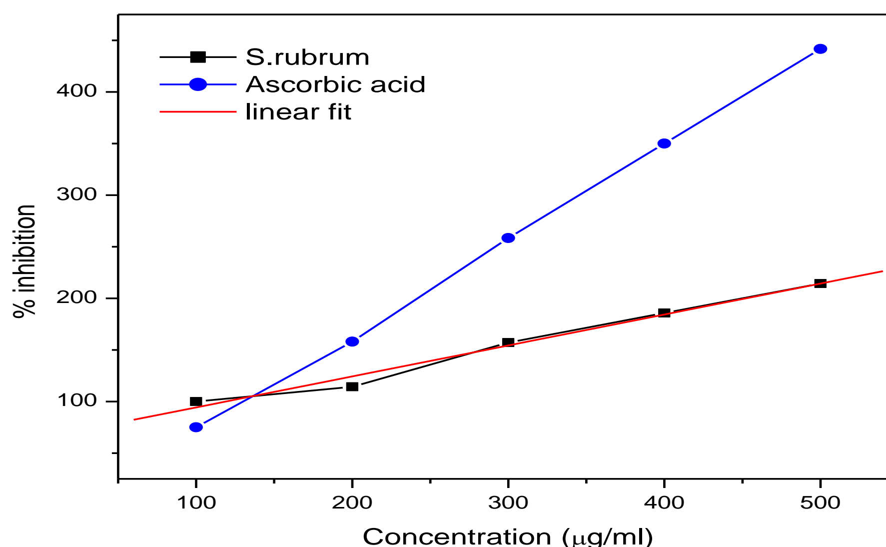


**Fig.3. Reducing power of pet-ether extract of *Solanum rubrum* leaves.**

**Nitric oxide-scavenging activity**

From the Fig.4, it is found that the % inhibition was increased with increasing concentration. The $IC_{50}$ value is calculated by fitting it with polynomial fit and the value is, $IC_{50}$=229.11. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions [25].

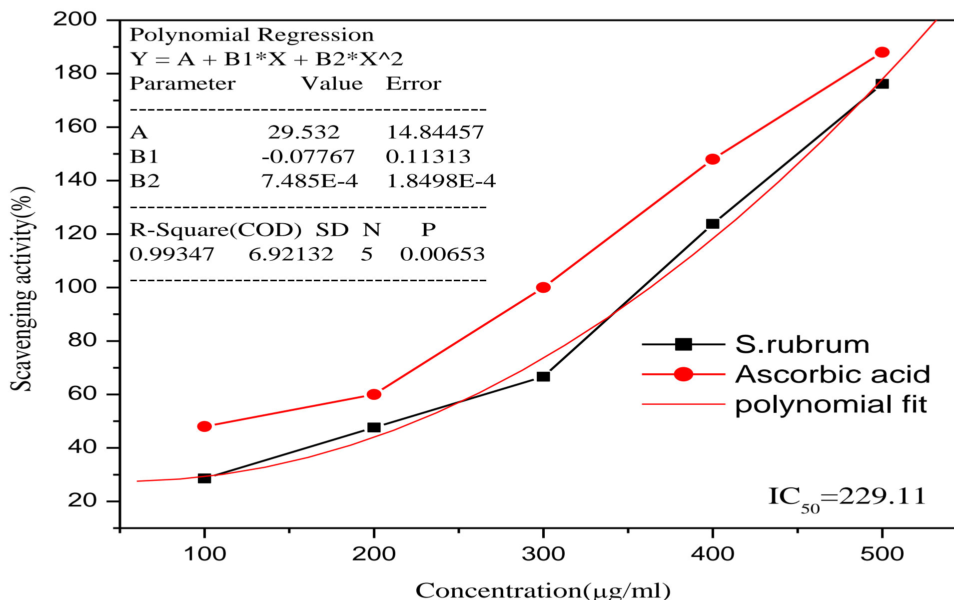


**Fig.4. Nitric oxide scavenging activity of pet-ether extract of *Solanum rubrum* leaves.**

Antioxidants, scavenge for free radicals and consequently are a very special group of nutritional supplements. The free radicals are all along present in the body, due to the occurrences of biochemical process, but it is harmful if it exceeds the normal level. Antioxidants are substances with free radical chain breaking properties. Among the numerous antioxidants available, flavonoids are known to inhibit lipid peroxidation to scavenge free radicals and active oxygen to chelate iron ion and to inactive lipoxygenase [26].

Thus it is important to characterize different types of medicinal plants for their antioxidant and anti-infections, arthritis potential according to Mothana et al. [27], Bajpai et al.[28] and Wajdylo et al.[29]. Aromatic and medicinal plants are known to produce certain, bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (anti infectious arthritis activity) [30,31]. Therefore these plant drugs deserve detailed studies in the light of modern science.

In the present paper we have evaluated the free radical scavenging activity, of *Solanum rubrum*. The crude extracts of the plant leaf showed considerable antioxidant activity. The chemical constituents present in the extract, which are responsible for this activity, need to be investigated, and it is obvious that the constituents like alkaloids, tannins, saponins, and

flavanoids present in the extract may be responsible for such activity. Several of such compounds are known to possess potent antioxidant activity [32]. This first observation of antioxidant activity of this plant may be due to the presence of these constituents. Hence, this plant may exhibit strong anticancer, hepato protective, antiviral and several other activities. The crude pet ether extract may merits further experiments in vivo.

## CONCLUSION

Eventhough different extracts exhibited different levels of antioxidant activities in different models studied, pet-ether extract, in general shows a good result of antioxidant activity, which is evidenced from the phytochemical analysis. It is reasonable to expect that high antioxidant extracts have greater potential to reduce free radicals in the body than do low antioxidant extracts. Further studies are needed to identify the unknown antioxidant components to establish their pharmacological properties. However, it is said to be that Cellular Antioxidant Activity assay is more biologically relevant than the chemical assays, since it takes into account some aspects of cell uptake, distribution, and metabolism of antioxidant compounds, it is good to have such studies in future on this plant for better knowledge of its antioxidant activity.

## ACKNOWLEDGEMENTS

The author (SK) wishes to express his gratitude to Shri. C.H. Sridaran Gurukkal(Late) of Kerala Martial Arts and Shri Dhamodaran Vaidhyar, RMP for their help in identification of the plant through palm leaves, manuscripts and their expertise in Ayurvedic system. The author thanks Rajesh Kappakadavath, Rathinesh, and Sathish Kumar of Udaya Kalari Sangam, Mahe for their support in obtaining the knowledge of traditional usage of this plant from various sources.

## REFERENCES

[1] Hill AF, Economic Botany(Ed). A textbook of useful plants and plant products,2,(New Garw: Mc Garw. Hill Book Company Inc-1952).
[2] Edeoga HO, Okwu DE, and Mbaebie BO. African J Biotechnol 2005; ,4(7): 685-688.
[3] Karthikumar S, Vigneswari K, and Jegatheesan K. Sci Res Essay 2007; 2(4): 101-104.
[4] Wolfe KL and Liu RH. J Agric Food Chem 2008; *56*(18): 8404–8411.
[5] Braca A, Sortino C, Politi M, Orelli I, and Mendez J. J Ethnopharmacol 2002; 79: 379-381.
[6] Matthaus B. J Agri Food Chem 2002; 50: 3444-52.
[7] Blois MS. Nature 1958; 26: 1199-1200.
[8] Chu YH, Chang CL and Hsu HF. J Sci Food Agri 2000; 80: 561-566.
[9] Yildirim A, Mavi A, Kara AA. J Sci Food Agri 2003; 83: 64-69.
[10] Yen GC and Chen HY. J Agri Food Chem 1995; 43(1): 27-32.
[11] Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, and Bekhradnia AR. Pak J Pharm Sci 2010; 23: 29-34.
[12] Mojab F, Kamalinijad M, Ghaderi N, and Vahidipour H. Iranian J Pharma Res 2003,77-82.
[13] Pronob Gogoi and M. Islam, IOSR J Pharm 2012; 2: 455-459.

ISSN: 0975-8585


[14] Ravi V, Saleem TSM, Maiti PP, Gauthaman K, and Ramamurthy J. Afr J Pharm Pharmacol 2009; 3(9):454-457.
[15] Sudhanshu, Nidhi Rao, Santhya Mittal and Ekta Menghani. J Adv Pharm Res 2012; 3(1): 9-13.
[16] Doss A, and Dhanabalan R. Ethnobotanical Leaflets 2008; 12: 638-642.
[17] Fluck H, Medicinal plants and their uses, (New York:W.Feulshom and Com Ltd-1973)7-15.
[18] Sodipo OA, Akiniji JA, and Ogunbamosu JU. Global J Pure Appl Sci 2000; 6:83-87.
[19] Okwu DE. J Sustain Agri Environ 2004; 6:3-34.
[20] Sodipo OA, Akanji MA, Kolawole FB, and Odutuga AA. Biosci Res Commun 1991; 3: 171.
[21] Chung KT, Wong TY, Wei CI, Hung YW, and Lin Y. Crit Rev Food Sci Nutr 1998; 38(6):421-464.
[22] Salah N, Miller NJ, Pagangeg G, Tijburg L, Bolwellg P, Rice E, and Evans C. Arch Biochem Broph 1995; 2:339-34.
[23] Dehpour AA, Ebrahimzadeh MA, Nabavi SF and Nabavi SM. Grases Aceites 2009; 60: 405-412.
[24] Motalleb G, Hanachi P, Kua SH , Fauziah O, and Asmah R. J Biol Sci 2005; 5: 648-653.
[25] Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M and Eslami B Iran. Pharmacog Mag 2009; 4(18): 123-127.
[26] Saxena P, Arora A, Dey S, Malhotra Y, Nagarajan K, and Singh PK. J Drug Deliv Ther 2011; 1: 36-39.
[27] Mothana RAA and Lindequist U. J. Ethnopharmacol 2005; 96: 177-181.
[28] Bajpai M, Pande A, Tewari SK and Prakash D, Int J Food Sci Nutr 2005; 56(4): 287-291.
[29] Wajdylo A, Oszmianski J and Czemerys R. Food Chem 2007; 105: 940-949.
[30] Bruneton J, Pharmacognosy, phytochemistry, medicinal plants. France: Lavoisiler publishing Co., 1995; pp 265-380.
[31] Chopra RN, Nayer SL and chopra IC, Glossary of Indian medicinal plants, 3rd Edn. New Delhi Council of Scientific and Industrial Research, 1992; pp.7-246.
[32] Lee J, Koo N, Min DB. Comp Rev Food Sci Food Safety 2004; 3: 21-33.