Free Radical Scavenging Action of Solanum Torvum Fruit Extracts using in Vitro Antioxidant Methods

A.K. Umera Begam[1] R. Senthilkumar[2]
[1,2]Department of Biochemistry
[1]Holy Cross College, Tiruchirappalli – 2 [2]Rajah Serfoji Government College, Thanjavur - 5

Abstract— A plant-based diet protects against chronic oxidative stress-related diseases. Dietary plants contain variable chemical families and amounts of antioxidants. Highly reactive free radicals can cause tissue damage. The extent of tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system. The free radical scavenging and hence the antioxidant activity of different extracts of S. torvum fruit was studied against DPPH and nitric oxide free radicals using ascorbic acid as standard equivalent. Percentage of inhibition was found to increase in a dose dependant manner. Aqueous extracts were found to be more effective antioxidant followed by methanol, ethanol, ethyl acetate, diethyl ether and hexane respectively in both DPPH and NO scavenging methods. A concentration of 400 µg/ml of the aqueous extract was found to have an activity equal to ascorbic acid, a potent antioxidant. This study indicate that S. torvum fruit is an excellent source of natural antioxidant and could be an effective nutritional food supplement, which inturn will have therapeutic applications.

Key words: free radicals, DPPH, NO, antioxidant, S. torvum.

I. INTRODUCTION

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. The primary site of free radical damage is the DNA found in the mitochondria. To prevent free radical damage the body has a defense system of antioxidants. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet. Cells generate energy by reducing molecular oxygen to water. During the process, small amounts of partially reduced reactive oxygen forms are produced as unavoidable byproducts which are referred to as Reactive Oxygen Species. An imbalance between free radical-generating and radical scavenging systems results in Oxidative Stress. Reactive nitrogen species and other non reactive derivatives are also involved. These processes lead to tissue damage and contribute to the pathogenesis of many disorders like hypertension, cancer, diabetes, neurodegenerative disorders and others. The human body has several mechanisms to counter the effects of these reactive species by the production of antioxidant enzymes like glutathione and catalase. Antioxidants can also be taken exogenously through the diet. Antioxidants are substances that may protect your cells against the effects of free radicals. Free radicals are molecules produced when your body breaks down food, or by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases. Studies suggest that a diet high in antioxidants from fruits and vegetables is associated with a lower risk of cancer, cardiovascular disease, Parkinson's disease and Alzheimer's disease. Plants are the basis of life on earth and are central to people's livelihoods [1]. In recent times, there is an increasing interest in the role of free radical mediated damage in the etiology of human diseases. In normal metabolism, the levels of oxidants (i.e. free radicals) and antioxidants in humans are maintained in balance, for sustaining optimal physiological conditions [2]. Plants are rich sources for natural antioxidants, the best known are tocopherols, flavonoids,vitamin C and other phenolic compounds[3]. Other contributors to the antioxidant activity include alkaloids, proteins, minerals and other vitamins such as the carotenoids and vitamin B6, B12 and K [4]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [5].There are a number of clinical studies confirming the powerful anti-cancerous and anti heart disease properties of polyphenols [6,7,8].

It has been hypothesized that plant antioxidants may contribute to the beneficial health effects of dietary plants. Free radicals can originate endogenously from normal metabolic reactions or exogenously as components of tobacco smoke and air pollutants as well as on exposure to radiations. There are evidences that free radical damage contributes to various chronic health problems such as emphysema, cardio vascular and inflammatory diseases, cataracts and cancer. Many dietary micronutrients contribute greatly to the protective system. Due to the lack of effective therapies for many of the chronic diseases, the usefulness of essential safe nutrients in protecting against the adverse effects of oxidative injury warrants the further study. If a reactive molecule contains one or more unpaired electrons, the molecule is termed a free radical. Most of the biological free radicals contain oxygen. Active forms of oxygen such as singlet oxygen (1O2) and H2O2 although not radicals themselves lead to free radical formation and can also cause damage., an

The body has mechanisms to produce the small amounts of oxidants normally formed during metabolic reaction. Reactive species such oxidants are formed in controlled amounts by neutrophil leucocytes on exposure to microbes are beneficial to the body in that they participate in destroying the microbes. Excess of oxidants, however, can be

harmful to the body. Liver is also under constant threat of oxidants and some of the free radical especially H2O2. Lipid peroxidation has been demostrated as one of the important feature after exposure to hepatotoxic substances and also is a measure of extent of hepatic damage. Several herbs and herbal formulations are available for the scavenging activity. In addition to this there is a global trend to revive the traditional systems of medicines and renewed interest in the natural remedies for treating human ailments. Antioxidants have important preventive roles, not only on undesirable changes in the flavor and nutritional quality of food, but also on tissue damage in various human diseases.

The Solanaceae family comprises about 90 genera and 3000 species which are widely distributed in the world. They are a rich source of active secondary metabolites [9]. Within this family, the genus Solanum is the largest and most complex with more than 1500 species [10]. Numerous species of Solanum are known to possess a variety of biological activities including anti-mycotic [11,12], antiviral [13], molluscicidal [14], teratogenic [15] and cytotoxic properties [16,17]. Solanum torvum belong to the family Solanaceae and generally recognized as Turkey Berry. It has antioxidant properties [18]. It is intensively used worldwide in the traditional medicine as poison anti-dote and for the treatment of fever, wounds, tooth decay, reproductive problem and arterial hypertension [19]. The valuable medicinal properties of different plants are due to presence of several constitutes i.e, saponins, tannins, alkaloids, alkyl phenol, glycoalkaloids, flavonoids, sesquiterpens lactones, terpenoids and phenol ether [20]. In the present study, the free radical scavenging activity and hence the antioxidant potency of the various extracts of S. torvum fruit extracts were compared to assess the extract with maximum antioxidant efficacy and hence the damage reversal property especially through the dietary intake.

II. Materials and Methods

In the present study, the free radical scavenging action of Solanum torvum fruits extracts were analyzed through in vitro studies like DPPH and Nitric oxide scavenging assays and thereby the antioxidant potentcy of the different extracts were assessed and the extract with high protective effect is identified through standard procedure.

A. Plant Material

Fresh fruits of Solanum torvum were collected locally in the month of November, and the voucher specimen was submitted in Rapinet Herbarium, St. Joseph's College, Tiruchirappalli, for authentication.

B. Chemicals

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemicals,Mumbai.

C. Sample Preparation

Solanum torvum fruits were washed under tap water and dried in hot air over at 35° C. The dried fruits were then ground to a coarse powder using a blender and then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place until investigation. The powdered plant material was successively extracted in a Soxhlet extractor at elevated temperature using n-hexane, followed by petroleum ether, chloroform, ethanol, methanol and water. All the extracts were filtered individually through filter paper and poured on petri dishes to evaporate the liquid solvents from the extracts to get dried residues. After drying, crude extracts were stored in stock vials and kept in refrigerator for further use.

D. Phytochemical Screening

Preliminary phytochemicals analysis was carried out for all the extracts as per standard methods described by Brain and Turner 1975 and Evans 1996. (Table 1).

1) 2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The free radical scavenging capacity of the extracts was determined using DPPH [21]. Freshly prepared DPPH solution was taken in test tubes and extracts were added followed by serial dilutions (15.625 to 250 µg/ml) to every test tube so that the final volume was 5 ml and after 30 min, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract and standard, and the absorbance was read at 517 nm using a spectrophotometer. Methanol was served as blank. Percentage inhibition of the DPPH free radical was measured using the following equation:

% inhibition = (1- Al / A0) X 100

Where, Al = Absorbance of the extract or standard and A0 = Absorbance of the control

IC50 value (a concentration at 50% inhibition) was determined from the curve between percentage inhibition and concentration.

2) Nitric Oxide Scavenging Capacity Assay

Nitric oxide scavenging assay was carried out using sodium nitroprusside [22]. This can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract/sub-fraction at various concentrations and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 0.5 ml was taken out and added into 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) and further incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 min. The absorbance was measured at 546 nm. A typical blank/control solution contained the same solution mixture without plant extract or standard. The absorbance of the blank/control solution was measured at 546 nm. The percentage inhibition was calculated according to the following equation:

% inhibition = (1- Al / A0) X 100

Where, Al = Absorbance of the extract or standard and A0 = Absorbance of the control.

III. Results and Discussion

The results of the phytochemical screening of different extracts are given in Table 1. Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present paper, we have evaluated the free radical scavenging

activity of the various extracts of S. torvum against deleterious DPPH and NO radicals.

A. DPPH Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorption at 517 mm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment. The free radical scavenging activity of different extracts of S. torvum was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maximal of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2- diphenyl-1-picryl hydrazine resulting in decrease in absorbance. The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidant. The various extracts of S. torvum are found to possess scavenging activity on DPPH radicals in a concentration dependant way. Maximum antioxidant activity is found at higher concentrations. Water extract showed highest percentage inhibition at 500 µg/ml followed by methanol and ethanol extracts (Table 2a and fig.1). Diethyl ether, petroleum ether and hexane extracts showed inhibition effect only at very high concentrations when compared to the above mentioned extracts (Table 3) Aqueous extract showed a very good scavenging activity on DPPH radicals with an IC50 value of 287.90 µg/ml followed by methanol (IC50 value – 341.29 µg/ml) and ethanol (460.41 µg/ml) respectively (Table 2b and fig. 1). Hexane extract was found to have least inhibition activity. The IC50 value of ascorbic acid against DPPH radical was found to be 85.67 µg/ml. (Table 4).

B. Nitric Oxide (NO) Radical Scavenging Assay

NO is a very unstable species and reacting with oxygen molecule produce stable nitrate and nitrite which can be estimated by using Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid will decrease which can be measured at 546 nm. NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. Suppression of released NO may be partially attributed to direct NO scavenging action of compounds. The NO scavenging activity of the extracts increased in a dose dependant manner. Highest percentage inhibition was seen for aqueous extract, followed by methanol, ethanol, ethyl acetate,diethyl ether and hexane (Table 3a and 3b; fig 2). Maximum NO free radical scavenging activity was reported by aqueous extract with an IC50 value of 336.97µg/ml compared to other extracts. The IC50 value of ascorbic acid against NO radical was found to be 97.77 µg/ml (Table 4)

From the above results it is found that aqueous extract of S. torvum has a very good radical scavenging activity against both DPPH and NO free radicals, followed by methanol and ethanol. Diethyl ether, ethyl acetate and hexane extracts have low scavenging activity with hexane extract being the least effective. A concentration of 400 µg/ml of the aqueous extract was found to have an activity equal to ascorbic acid, an effective antioxidant. So, a higher concentration of our plant extract can have a better antioxidant activity. From this it is understood that of all the extracts, aqueous extract of S. torvum is said to possess a significant antioxidant potential and hence can act as a good dietary antioxidant and protect from damages especially at higher concentrations. Hence, a higher concentration would be required to achieve maximal and a significantly higher content of phenolic compounds compared to other extracts. The high antioxidant potency may be contributed by the phytoconstituents like flavonoids, alkaloids, carbohydrates, saponins etc. present in it. Phenolic compounds, ubiquitous to the plant kingdom are composed of several classes of compounds including flavonoids (flavones, isoflavones and flavonones), anthocyanins and catechins. They possess an ideal structural chemistry for free radical scavenging activity. [23] Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors which can stabilize and delocalize the unpaired electron [24]. Flavonoids play an important role in antioxidant system in plants. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation [25]. Depending on their structure, flavonoids are able to scavenge practically all known ROS [26].

IV. CONCLUSION

The findings of this study supports the view, that the aqueous extract of S. torvum can be promising source of potential antioxidant and may be efficient as preventive agents in some diseases and can be considered as a natural dietary herbal medicine and also as a herbal source in pharmaceutical industry. Further detailed studies on isolation of phytoconstituents of the plant extracts are essential to characterize them as biological antioxidants. This study indicate that S. torvum fruit is an excellent source of natural antioxidant and could be an effective nutritional food supplement, which inturn will have therapeutic applications. In siddha medicine on the traditional systems of India the, ripened fruits are used in the preparation of tonic named as a "sundaivattaral choornam" is used to improve the health and prevent several diseases. This study has given an experimental evidence that S. torvum fruit is an excellent source of natural antioxidants.

S. No.	**Phytochemicals**	**Extraction of different extracts**					
		Ethanol	**Hexane**	**Ethyl acetate**	**Methanol**	**Diethyl ether**	**Water**
1.	Alkaloids	+	+	-	+	+	+
2.	Flavonoids	-	-	_	_	_	+
3.	Steroids	-	+	+	_	+	-
4.	Terpenoids	_	_	-	_	_	+
5.	Anthroquinone	_	_	_	_	_	_

6.	Phenols	+	+	+	+	+	+
7.	Saponin	_	+	_	+	_	+
8.	Tannins	_	_	_	_	_	_
9.	Carbohydrates	_	_	_	_	_	+
10.	Oils & resins	_	+	+	_	+	-
11.	Coumarin	+	_	_	+	+	+
12.	Quinone	+	+	+	+	+	+
13.	Lignin	_	_	_	_	_	_
14.	Glycosides	_	_	_	_	_	_

Table 1: Preliminary phytochemical screening of various extracts

Concentration of extracts (µg/ml).	% of DPPH Scavenging			
	Diethyl ether	Ethyl acetate	Hexane	Std Ascorbic acid at 100 µg/ml
500	3.04	22.7	4.8	58.36
1000	7.57	24.3	11.2	
1500	16.60	27.2	17.7	
2000	22.72	32.5	20.9	
2500	39.31	34.8	27.4	

Table 2a: Percentage of DPPH Scavenging Activity of varying concentrations of Solanum torvum extracts

Concentration of extracts (µg/ml).	% of DPPH Scavenging			
	Methanol	Ethanol	Aqueous	Std Ascorbic acid at 100 µg/ml
100	15.2	6.5	19.5	58.36
200	41.3	15.2	36.9	
300	54.3	23.9	52.1	
400	58.6	36.9	60.8	
500	73.9	54.3	78.2	

Table 2b: Percentage of DPPH Scavenging Activity of varying concentrations of *Solanum torvum* extracts

Concentration of extracts (µg/ml).	% of NO Scavenging			
	Diethyl ether	Ethyl acetate	Hexane	Std Ascorbic acid at 100 µg/ml
500	12.90	19.35	9.67	51.14
1000	19.35	32.25	16.12	
1500	25.80	38.70	19.35	
2000	38.70	45.16	32.25	
2500	41.93	54.83	35.48	

Table 3a: Percentage of Nitric Oxide Scavenging Activity of varying concentrations of Solanum torvum extracts

Concentration of extracts (µg/ml).	% of NO Scavenging			
	Methanol	Ethanol	Aqueous	Std Ascorbic acid at 100 µg/ml
100	16.12	6.45	12.90	51.14
200	29.0	22.58	25.80	
300	41.93	32.25	38.70	
400	58.06	41.93	61.29	
500	67.74	51.61	74.19	

Table 3b: Percentage of Nitric Oxide Scavenging Activity of varying concentrations of Solanum torvum extracts

Antioxidant Radical	50 % Inhibition concentration* (IC_{50})						
	Aqueous Extract	Methanol Extract	Ethanol Extract	Ethyl acetate Extract	Diethyl ether Extract	Hexane Extract	Ascorbic acid (std)
DPPH radical	287.90	341.29	460.41	3691.95	3179.85	4562.04	85.67
Nitric Oxide radical	336.97	369.05	484.40	2279.77	2981.15	3523.11	97.77

Table 4: 50% Inhibition concentrations (IC50) of different extracts of S. torvum fruits against DPPH and NO radicals

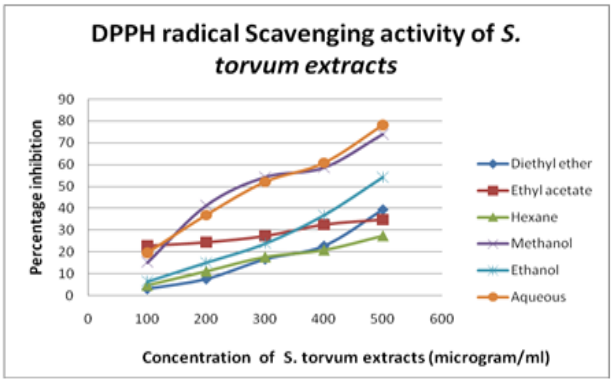

Fig. 1: DPPH radical scavenging activity of various Solanum torvum fruits

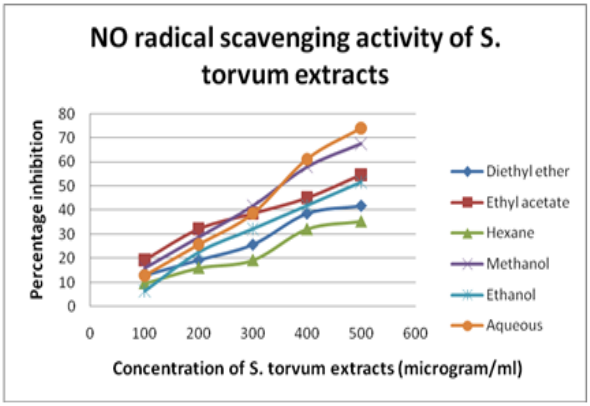

Fig. 2: Nitric oxide radical scavenging activity of various Solanum torvum fruits

REFERENCES

[1] Albert LS and Kuldip G (2006).Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India. Journal of Ethnobiology and Ethnomedicine. 2:33-39.
[2] Temple NJ (2000). Antioxidants and disease: More questions than answers. Nutr Res. 20: 449-459.
[3] Landrault, N, Poucheret P, Ravel P, Gasc F, Cros G, Teissedre PL(2001). Antioxidant Capacities and Phenolics Levels of French Wines from Different Varieties and Vintages. J. Agric. Food Chem. 49(7):3341-3343.
[4] Smolin LA, Grosvenor MB (2007). Nutrition: Sci. Appl., Fourth edition.
[5] Subramanion LJ, Zuraini Z, Sasidharan S (2011). Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitiory activities of Cassia fistula seeds extract. J. Med. Plants Res. 5(10):1941-1947.
[6] Prakash D, Suri S, Upadhyay G, Singh BN (2007). Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. Int. J. Food Sci. Nutr. 58(1):18-28.
[7] Bajpai M, Pande A, Tewari SK, Prakash D (2005). Phenolic contents and antioxidant activity of some food medicinal plants. Int. J. Food Sci. Nutr., 56(4):287- 291.
[8] Siddhuraju P, Becker K (2003). Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (Moringa oleifera Lam.) Leaves. J. Agric. Food Chem. 51(8):2144-2155.
[9] Coletto da Silva A, Kinupp V, Absy M, and Kerr W. (2004). Pollen Morphology and Study of the Visitor (Hymenoptera, Apidae) of Solanum stramoniifolium Jacq. (Solanaceae) in Central Amazon. Acta Bot Bras. 18: 653-657.
[10] Chowdhury N, Bhattacharjee I, Laskar S, Chandra G. (2007). Efficacy of Solanum villosum Mill. (Solanaceae: Solanales) as a Biocontrol Agent against Fourth Instar Larvae of Culex quinquefasciatus Say. Turk J. Zool. 31: 365-370.
[11] Gonzales, M., Zamilpa, A., Marquina, S., Navarro, E., and Alvarez, L. (2004). Antimycotic Spirostanol Saponins from Solanum hispidum Leaves and their Structure Activity Relationships. J Nat Prod. 67: 938-941
[12] Singh O, Subharani K, Singh N, Devi N, Nevidita L (2007). Isolation of Steroidal Glycosides from Solanum xanthocarpum and Studies on their Antifungal Activities. Nat Prod Res. 21: 585-590.
[13] Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M, Thebtaranonth Y. (2002).Antiviral Isoflavonoid Sulfate and Steroidal Glycosides from the Fruits of Solanum torvum. Phytochemistry. 59: 459-463.
[14] Silva T, Camara C, Agra F, de Carvalho M, Frana M, Brandoline, S, da Silva L, Braz-Filho R. (2006). Molluscicidal Activity of Solanum Species of the Northeast of Brazil on Biomphalaria glabrata Fitoterapia. 77: 449-452
[15] Keeler R, Baker D, Gaffield W. (1990). Spirosolane-Containing Solanum Species and Induction of Congenital Craniofacial Malformations. Toxicon. 28: 873-884
[16] Nakamura T, Komori A, Lee Y, Hashimoto F, Yahara S, Nohara T, Ejima A.(1996). Cytotoxic Activities of Solanum Steroidal Glycosides. Biol Pharm Bull. 19: 554-566
[17] Lu Y, Luo J, Huang X, Kong L. (2009). Four New Steroidal Glycosides from Solanum torvum and their Cytotoxic Activities. Steroids. 74: 95-1
[18] Sivapriya M.and Srinivas .L. (2007). Isolation and purification of a Noval Antioxidant Protein from the water extract of Sundakai (Solanum torvum) seed, Food Chemistry 104: 510-517
[19] Ndebia E.J, Kamga R. and Nchunga-Anye Nkeh.B, (2007). Analgeric and anti-inflammatory properties of aqueous extract from leaves of Solanum torvum (Solanaceae), AJTCAM, 4:240-244
[20] Cox. P, Balick M (1994). The Ethnobotanical Approach to Drug Discovery. Sci American, 82
[21] Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I (2001). Antioxidant principles from Bauhinia tarapotensis . J. Nat. Prod., 64(7):892-895.
[22] Sreejayan N, Rao MNA (1997). Nitric Oxide Scavenging by Curcuminoids. J. Pharm. Pharmacol. 49(1):105-107.
[23] Rana MG, Katbamna RV, Padhya AA., Dudhrejiya AD, Jivani NP, Sheth NR (2010). In vitro antioxidant and free radical scavenging studies of alcoholic extract of Medicago sativa L. Rom. J. Biol. – plant biol., volume 55, no 1, p. 15–22.
[24] Rice-Evans C, Miller N, Paganga G (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci. 2(4):152-159.
[25] Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del-Rio JA (1997). Uses and properties of Citrus flavonoids. J. Agric. Food Chem. 45(12):4505-4515.
[26] Mohammad Shahriar*, Sadika Akhter, Md. Ismail Hossain, Md. Aminul Haque and Mohiuddin Ahmed Bhuiyan 2012, Evaluation of in vitro antioxidant activity of bark extracts of Terminalia arjuna J. Med. Plan Res. Vol. 6(39), pp. 5286-5298

