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Original Article

In vitro anti-oxidant studies on ethanolic extracts of leaves and stems of *Nyctanthes arbor-tristis. L* (Night-flowering Jasmine)

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ABSTRACT

Under most pathological conditions there is generation of reactive oxygen species and other free radicals. An increase in the antioxidant reserves of the organism can reduce oxidative stress and some of the plant-derived agents may help to reduce it. *Nyctanthes arbor-tristis leaf* extracts are extensively used in Indian traditional medicine. In the present study we have examined the *in vitro* antioxidant activity of leaves and stem of the plant. The antioxidant activities of different concentrations of ethanol extracts of NAT-L and NAT-S were determined by DPPH radical scavenging assay, Reducing power ability, Hydrogen peroxide scavenging assay and Total antioxidant assay. The effective antioxidant activity of NAT-S and NAT-L has found increased with increasing concentration. Comparing NAT-S, there was an increased activity found in NAT-L extract. The results obtained in the present study indicate that the leaves and stem of *Nyctanthes arbor-tristis* are a potential source of natural antioxidants.

1. Introduction

In living systems, free radicals are generated as part of the body's normal metabolic process. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias [1].

The most commonly used synthetic antioxidants presently are butylated hydoxyanisole (BHA), butylated hydoxytoluene (BHT) Propylgallate (PG), and test butylated hydroquinone. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis [2, 3]. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicinal materials to replace synthetic antioxidant.

Several studies have demonstrated that plants produce potent antioxidants and represent an important source of natural antioxidants [4, 5, 6]. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, β-carotene, and α-tocopherol are known to possess antioxidant potential [7,8,9]. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue injury [10].

India, with its wealth and variety of medicinal plants has accumulated over generations, a great mass of popular remedies. Many of these plants are in common use even today. *Nyctanthes arbor-tristis* (NAT) commonly known as Night Jasmine is among them. The decoction of leaves is extensively used by Ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, intestinal worms and as a tonic, cholagogue and laxative [11-14]. In addition, analgesics, antipyretic along with ulcerogenic potency have also been observed [15]. This plant has also been found to possess anti-allergic [16], antimalarial [17,18], leishmanicidal [19,20], amoebicidal [21] and anthelminthic [22] activities.

In view of the diverse pharmacological activities present in this plant together with gross central effects and hypothermia [23] the present investigation was undertaken to explore the antioxidant properties of stem and leaf extracts of *Nyctanthes arbor-tristis*.

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2. Materials and Methods

2.1 Plant Materials: Collection

Leaves and Stem of *Nyctanthes arbor-tristis* were collected from Coimbatore district Tamilnadu (India) during the month of September 2009 and authenticated by Dr.V.S.Ramchandran, Reader, Department of Botany, Bharathiar University, Coimbatore.

2.2 Extraction and Phytochemical analysis

Collected materials were washed thoroughly; shade dried, powdered coarsely and named it as NAT-L (leaf) and NAT- S (stem). The powder obtained (250 g) were extracted successively with Petroleum ether, Chloroform, Ethyl acetate, Ethanol in a Soxlet extractor for 18-20 hrs. The extracts were concentrated using rotary flash evaporator and preserved at 4^0C in air tight container. All the extracts were subjected to qualitative chemical tests for the identification of various phytoconstituents followed by the method of Kokate [24]. The total phenol content was determined using Folin- Ciocalteu reagent by the method of Sadasivam and Manikam [25] and the total flavanoid content was estimated using the aluminium chloride method [26]

2.3 DPPH Radical Scavenging Activity [27, 28]

Different concentrations of the substrate, 1ml of (0.1mM) DPPH in ethanol, 550 µl of 50 mM Tris HCl buffer pH (7.4) were added and the mixture was incubated for 30 min at room temperature. After 30 min, absorbance of the mixture was measured using spectrophotometer (Genosys UV 10) at 517 nm. Mixture without substrate served as absolute control.

2.4 Scavenging of Hydrogen peroxide

A solution of Hydrogen peroxide (20mM) was prepared in Phosphate buffer saline (PBS, pH 7.4). Various concentrations of the extract or standard in ethanol (1 ml) were added to 2 ml of Hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230 nm [29]. The percentage inhibition of different concentrations of the extracts was determined and compared with the standard, ascorbic acid.

2.5 Assay of Reducing Power [30, 31]

Different concentrations of plant extract solution (final concentration 100-500 µg /ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN_6)$] (10g/l), then mixture was incubated at 50o C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1g/l) and absorbance measured at 700 nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 117, India). Ascorbic acid was used as standard and phosphate buffer used as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power.

2.6 Total Antioxidant Capacity

For total antioxidant capacity assay [32], various concentrations of the substrate dissolved in water were combined in an eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

2.7 Rapid radical scavenging screening

The method of Mensor [33], Burtis [34], and Adebajo [35] was followed in screening for the antioxidant property of the extracts. With the aid of capillary tube, stock solutions (1 mg/ml) of extracts were spotted on silica gel thin layer chromatographic (TLC) plate and developed with a solvent system of ethanol: methanol (90:10). After development, the chromatograms were dried and sprayed with a 0.3 mM solution of the stable radical DPPH. Purple spot formed against white background were taken as positive results. The duration for the development of yellow colour indicated whether the antioxidant activity was strong or not. Ascorbic acid was used as a reference standard in all the above assays.

2.8. Statistical analysis

All the grouped data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± S.D. for five experiments in each.

3. Results

Table 1 shows the phytochemical constituents of NAT-S and NAT-L extracts of the plant. The preliminary phytochemical analysis indicated the presence of steroids and terpenoids in petroleum ether extract; alkaloids, flavanoids, phenols in the chloroform extract; flavanoids, phenols, saponins, glycosides and Tannins in ethyl acetate extract; alkaloids, phenols, saponins, Tannins and glycosides in ethanol extract.

Table 1. Phytochemical constituents of NAT-S and NAT-L extracts

Extract	Phytochemical analysis	
	NAT-S	NAT-L
Petroleum ether extract	Terpenoids, Steroids	Steroids, Terpenoids
Chloroform extract	Alkaloids, Phenols and Flavanoids	Alkaloids, Flavanoids and Phenols
Ethyl acetate extract	Flavanoids, Glycosides, saponins, Tannins and Phenols	Phenols, Saponins, Glycosides, Tannins and Flavanoids.
Ethanol extract	Alkaloids, Saponins , Tannins , Phenols and Glycosides	Alkaloids, Phenols, Saponins, Tannins and Glycosides

Table 2 exhibits the phenol and flavanoid content of NAT-L and NAT-S extract. The total phenol and flavanoid contents in the NAT-L extract were found to be higher than the NAT-S extract.

Table 2. Total phenol and flavanoid contents in the ethanolic extract NAT-L and NAT-S

Extract	Total phenol (mg/g)	Total flavanoid (mg/g)
NAT-L extract	98.56 ± .46	34.51 ± 0.45
NAT-S extract	90.73 ± 2.75	29.55 ± 0.49

The result of the rapid radical scavenging screening is shown in Table 3. NAT-L showed an immediate reaction from purple to yellow and hence more active than NAT-S.

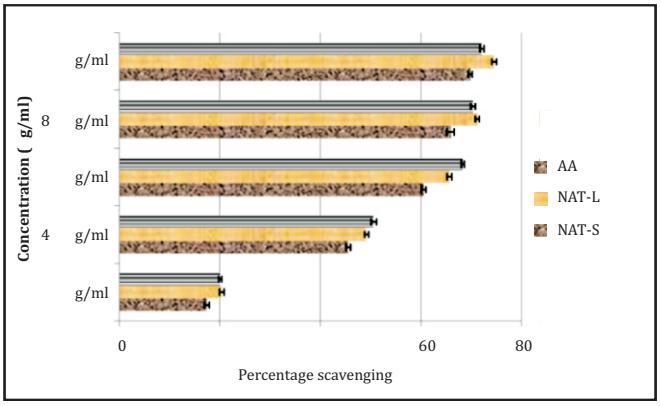
DPPH radical scavenging activity of stem and leaf extracts of Nyctanthes arbor-tristis are presented in Figure1. Both the extracts of Nyctanthes arbor-tristis have got profound antioxidant activity. The percentage of DPPH radical scavenging activity of NAT-S and NAT-L were increased with increasing concentration. Comparing NAT-S, there was an increased activity found in NAT-L extract.

Table 3. Radical Scavenging activities of the ethanolic extracts of NAT-L and NAT-S using rapid DPPH TLC screening.

Plant parts	Reaction speed	Intensity of spots
NAT-L	Fast	+ + +
NAT-S	Fast	+ +

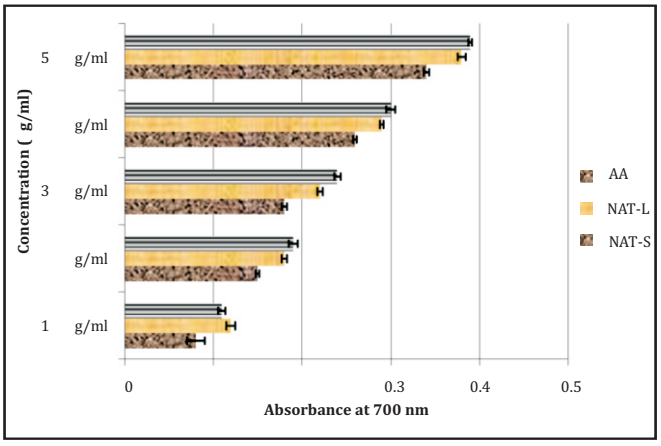
+ + + = Strong intensity (immediate reaction)

Figure 1. DPPH radical scavenging activity of various concentrations of ethanolic NAT-S and NAT-L extracts



Values are mean ±SD of five determinations

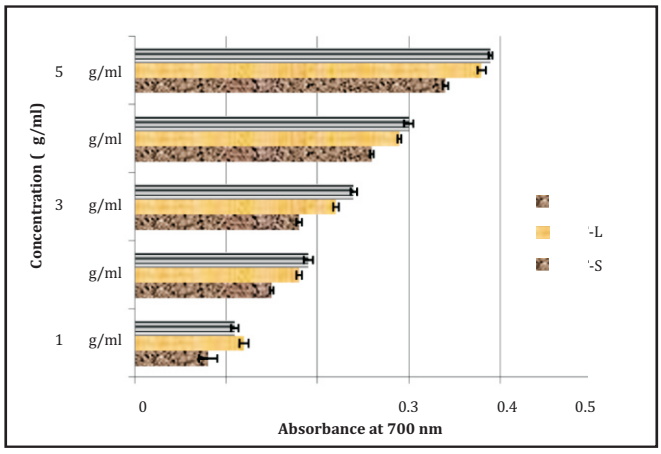
Figure 2. Hydrogen peroxide scavenging effect of different concentrations of NAT-S and NAT-L extracts.



Values are mean ±SD of five determinations

Ability of the investigated *Nyctanthes arbor-tristis* extracts to scavenge hydrogen peroxide is shown Figure 2. The H_2O_2 scavenging activity ratio of the extracts to the standard molecule (Ascorbic Acid) showed that NAT-L extracts possessed the highest relative ration than NAT-S.

Figure 3. Reducing power of ethanolic NAT-S and NAT-L extracts.

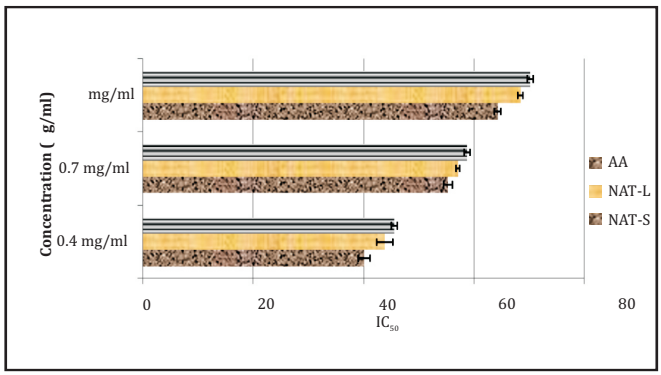


Values are mean ±SD of five determinations

Figure 3 reveals the reductive capabilities of NAT-L and NAT-S compared to ascorbic acid. The reducing power of NAT-L and NAT-S were potent and the powers were increased by increasing the concentration. From this NAT-L has shown more potent activity than NAT-S.

The total antioxidant activity of different concentrations of NAT-L and NAT-S were depicted in Figure 4. NAT-L and NAT-S exhibited effective antioxidant activity in this system. The total antioxidant activity was found in the higher concentration.

Figure 4. Total Antioxidant activity of different concentrations of NAT-L and NAT-S



Values are mean ± SD of five determinations

4. Discussion

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defenses. Potential antioxidant therapy therefore should include either natural free radical scavenging antioxidant enzymes or agents, which are capable of augmenting the activity of these enzymes. Reactive oxygen species (ROS) has received considerable attention in the recent past because of its role in several pathological conditions including cancer, diabetes, arthritis, aging, and atherosclerosis [36]. If human diseases are believed to be due to the imbalance between oxidative stress and antioxidative defense, it is possible to limit oxidative tissue damage and hence prevent disease progression by antioxidant defense supplements [37]. Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy [38-40].

DPPH. is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The methodology involves reaction of specific compounds or extracts with DPPH. in ethanol solution. In the presence of hydrogen donors, DPPH is reduced and a free radical is formed from the scavenger. The TLC based qualitative DPPH spray revealed the presence of significant antioxidant activity in NAT extracts indicated by the intensity of the spot. The reaction of DPPH is monitored by the decrease of the absorbance of its radical at 517 nm, but upon reduction by an antioxidant, the absorption disappears [41]. NAT extracts strongly scavenged DPPH radical and a dose-response relationship was found in DPPH radical scavenging activity, the activity increased with an increase in the concentration of NAT-L and NAT-S.

The measurement of H_2O_2 scavenging activity is one of the useful methods of determining the ability of antioxidants to decrease the level of pro-oxidants such as H_2O_2 [42]. It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells [43]. In our study the results indicated that NAT-L and NAT-S showed more potent activity in par with standard ascorbic acid, the H_2O_2 scavenging activity was associated with corresponding increase in the concentration of NAT-L and NAT-S respectively. Based on previous studies phenols and flavanoids present in the plant materials has the potential to scavenge free radicals, phytochemical screening of NAT-L and NAT-S possess phenols and flavanoids, this may be attributed due to the presence of phenols in the extracts.

Different studies have indicated that the electron donation capacity, reflecting the reducing power of bioactive compounds is associated with antioxidant activity [44]. The reducing ability of a compound generally depends on the presence of reductants, which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants (i.e. antioxidants) in Nyctanthes arbor tristis leaves and stem extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. In this assay yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples NAT-L and NAT-S.

The total antioxidant capacity of the extract was calculated based on the formation of the phosphomolybdenum complex, which was measured spectrophotometrically at 695 nm [36]. The total antioxidant activity of the extract increases with increasing concentration.

It has been reported that compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants [45]. The mechanisms of action of flavonoids are through scavenging or chelating process [46, 47]. Phytochemical screening of the extract of the leaves and stems of *Nyctanthes arbor-tristis* revealed the presence of flavonoids, tannins, saponins, glycosides, alkaloids, steroids, and phenolic compounds. Phenolic compounds have been recognized as antioxidant agents, which act as free radical terminators [48] and have been known to show medicinal activity as well as exhibiting physiological functions [49].

5. Conclusion

In conclusion, the results of the study clearly indicate that ethanolic extract of Nyctanthes arbor tristis possess powerful in vitro antioxidant activity. The encouraging results of *Nyctanthes arbor tristis* with the various in vitro antioxidant tests proved the plant as a reducing agent and effectiveness as scavengers of hydrogen peroxide and free radicals. The overall antioxidant activity of *Nyctanthes arbor tristis* might be attributed to its polyphenolic content and other phytochemical constituents. The plant merits further investigation in animal models to confirm its antioxidant activity in vivo and to isolate the active constituents, which may result in a modern drug from this plant.

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6. References

[1] Ara N, Nur H. In vitro Antioxidant activity of methanolic leaves and flowers extracts of lippia alba. Res J Medicine & Med Sci.2009; 4(1): 107-110.

[2] Wichi HP. Enhanced tumor development by butylated hydroxyanisole (BHA) from the properties of effect on fure stomach and oesophagel aquamoua epithelium. Food Chem Toxicol.1988; 26: 727-723.

[3] Sherwin ER, Brancn AL, Davidson PM, Salmincn S. Food Additives, Marvel Dekker Inc, New York, 1990, pp. 139-193.

[4] Es-Safi NE, hlifi SK, Kerhoas L, Kollmann A, Abbouyi A, Ducrot PH. Antioxidant constituents of aerial parts of *Globularia alypum* growing in Morocco. J Nat Prod.2005; 68: 1293-1296.

[5] Santos-Gomes PC, Seabra RM, Andrade PB, Fernandes-Ferreira M. Determination of phenolic antioxidant compounds produced by calli and cell suspensions of sage (*Salvia officinalis L.*). J Plant Physiol.2003; 160: 1025-12.

[6] Couladis M, Tzakou O, Verykokidou E, Harvala C. Screening of same Greek aromatic plants for antioxidant activity. Phytother Res.2003; 17:194-195.

[7] Prior RL. Fruit and vegetables in the prevention of cellular oxidative damage. Am J Clin Nutr.2003; 78: 570S-578S.

[8] Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds o f 112 traditional Chinese medicinal plants associated with anticancer. Life Sci.2004; 74: 2157-2184.

[9] Kaur C, Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol. 2002; 37: 153-161.

[10] Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and lavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol. 2006; 5(11): 1142-1145.

[11] Kirtikar KR, Basu BD. Indian Medicinal Plants, Lolit Mohan Basu, Allahabad, India, pp1526-1528.

[12] Samhita C. Shri Gulabkunverba Ayurvedic Society, Jamnagar, vol. V, Ayurvedic Mudranalaya, Jamnagar,1949; pp23-24.

[13] hopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous drugs of India, second ed. UN Dhar and Sons, Calcutta, India, 1958; pp 408.

[14] Nadkarni KM. Indian Materia Medica. vol. 1. Bombay Popular Prakashan, Bombay, 1976; pp 857.

[15] Saxena RS, Gupta B, Saxena KK, Srivastava VK, Prasad DN. Analgesic, Antipyretic and ulcerogenic activity of Nyctanthes arbor tristis leaf extracts. J Ethnopharmacol.1987; 19: 193-200.

[16] Gupta PP, Srimal RC, Tandon JS. Antiallergic activity of some traditional Indian medicinal plants. Int J Pharmacog.1993; 31: 15-, 18.

[17] Badam L, Deolankar RP, Rojatkar SR, Nagsampgi BA, Wagh UV. In vitro antimalarial activity of medicinal plants of India. J Med Res.1988; 87:379-383.

[18] Misra P, Pal NL, Guru PY, Katiyar JC, Tandon JS. Antimalarial activity of traditional plants against erythrocytic stages of Plasmodium berghei. Int J Pharmacog.1991; 29: 23.

[19] Singh UK, Guru PY, Sen AB, Tandon JS. Antileishmanial activity of traditional plants against Leishmania donovani in golden hamsters. Int J Pharmacog.1992; 30:289-295.

[20] Tandon JS, Srivastava V, Guru PY. Iridoids: a new class of leishmanicidal agents from Nyctanthes arbor-tristis. J Nat Prod.1991; 54: 1102-1104.

[21] Chitravanshi VC, Singh AP, Ghoshal S, Prasad BNK, Srivastava V, Tandon JS. Therapeutic action of Nyctanthes arbor tristis against caecal amoebiasis of rat. Int J Pharmacog.1992; 30:71-75.

[22] Lal J, Chandra S, Raviprakash V, Sabir M. In vitro anthelmintic action of some indigenous medicinal plants on Ascaridia Galli worms. Ind J Phy Pharmacol.1976; 20: 64-68.

[23] Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. Screening of Indian plants for biological activity. Part II. Ind J Exp Biol.1969; 7: 250-
, 262.

[24] Kokate CK, Purohit AP, SB Gokhale. Pharmacognosy,7th edition, Nirali Prakashan, Pune. 2003, pp105.

[25] Sadasivam S, Manikam A. Biochemical methods for agricultural sciences, Wiley Eastern Limited, New Delhi.1992, pp187.

[26] Woisky R, Salatino. Screening of Indian plants for biological activity. Part II. A. J Agric Res.1998; 37: 99-105.

[27] Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH , Kim SK.. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavanoids by assay guided comparison. Plant sci.2002;163:1161-1168.

[28] Tagashira M, Ohtake Y. A new antioxidative 1,3-benzodioxole from Melissa officinalis, Planta Med.1998; 64: 555-558.

[29] Jayaprakasha GK, Rao LJ, Sakariah KK. Antioxident activities of flavidin in different in vitro model systems. Bioorg Med Chem.2004; 12: 5141-5146.

[30] Makari HK, Haraprasad N, Patil H, Ravikumar S. In Vitro Antioxidant Activity of the Hexane And Methanolic Extracts Of Cordia Wallichii And Celastrus Paniculata. Internet J Aesthetic Antiaging Med. 2008;1:1-10.

[31] Lu Y, Foo Y. Antioxidant activities of polyphenols from sage (Salvia officinalis.). Food Chem. 2000; 75: 197-202.

[32] Preito P, Pineda M, Aguilar M. Spectrophotometric quanification of antioxidant capacity through the formation of phosphomolybdnum complex: specific application of vitamin E. Anal Biochem.1999; 269: 337-341

.[33] Mensor LL, Fabio SM, Gildor GL, Alexander SR, Tereza CD, Cintia SC, Suzane GL. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical methods. Phytother Res.2001; 15: 127-130.

[34] Burit M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res. 2001; 14:323-328.

[35] Adebajo AC, Aladesanmi AJ, Akinkimi EO, Taiwo BJ, Olorunmola FO, Lamikanra A. Antimicrobial and antioxidant activities of some Nigerian Medicinal Plants. Afr J Trad CAM. 2007; 4(2): 173-184.

[36] Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AK, Mehrotra S, Pushpangadan P. Studies on the Antioxidant Activities of Desmodium gangeticum. Biol Pharm Bull.2003; 26(10):1424-1427.

[37] Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK. Antioxidant activity of active tannoid principles of Emblica officinalis (amla). Indian J Exp Biol. 1999; 37:676-680.

[38] Singh G, Rao GP, Kapoor PS, Singh OP. Chemical constituents and antifungal activity of Lippia alba Mill leaf essential oil. J Med Arom Pl Sci. 2000; 22: 701-708.

[39] Hasan MS, Ahmed MI, Mondal S, Uddin SJ, Masud MM, Sadhu SK, Ishibashi M. Antioxidant, antino-ciceptive activity and general toxicity study of Dendrophthoe falcata and isolation of quercitrin as the major component. OPEM.2006; 6: 355-360.

[40] Erdemoglu N, Turan NN, Cakici I, Sener BB, Aydin A. Antioxidant Activities of Some Lamiaceae Plant extracts. Phytother Res. 2006; 20: 9-13.

[41] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity.LWT Food Sci Technol.1995; 28: 2530.

[42] MP Czochra, Widensk A. Spectrophotometric determination of hydrogen peroxide scavenging activity. J Anl Chemic ACTA. 2002; 452: 177-184.

[43] Gulcin I. Antioxidant and antiradical activities of L- Carnitine. Life Sci. 2006; 78: 803-811.

[44] Siddhuraju P, Mohan PS, Becker K. Studies on the antioxidant activity of Indian laburnum (Cassia fistula L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chem. 2002; 79: 61-67.

[45] Das NP, Pereira TA. Effect of flavonoids on thermal Autoxidation of palm oil: Structure- activity relationship. J Am Oil Chem Soc. 1990; 67:255-258.

[46] Kessler M, Ubeand G, Jung L. Anti and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol. 2003; 55:131-142.

[47] Cook NC, Samman S. Flavonoids Chemistry, metabolism, Cardioprotective effects, and dietary sources. Nutr Biochem. 1996; 7:66-76.

[48] Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Crit Rev Food Sci. Nutr. 1992; 32: 67-103.

[49] Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum books ltd, Ibadan, Nigeria, 1993 pp 289.