Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

FREE RADICAL SCAVENGING AND ANTIOXIDANT POTENTIAL OF *THESPESIA POPULNEA* (L.) FLOWER EXTRACT

PATIL P.S.*[1], VENKATNARAYANAN R.[1], ARGADE P.D.[1], SHINDE P.R.[2]

[1]R.V.S. College of Pharmaceutical Sciences, Sulur, Coimbatore, Tamilnadu, India. [2]K.B.H.S.S. Trust's Institute of Pharmacy, Malegaon camp, Malegaon, Maharashtra, India. Email: pankajh33patil@gmail.com

Received: 3 Feb 2012, Revised and Accepted: 28 March 2012

ABSTRACT

Background: Antioxidants are act as scavengers and play the housekeeper's role by mopping up free radicals before they possibly will get a chance to create havoc in a body. Thus they may well be defined as the substances that are capable of quenching or stabilizing free radicals. *Thespesia populnea* widely distributed throughout the India mostly found in Coast forests and evergreen forest of Western Ghats, largely grown as a roadside tree in tropical regions.

Objective: To evaluate the antioxidant potential of flower of *Thespesia populnea*; yet unreported.

Material and Method: Antioxidant potential of methanolic extract of flower (TPF) was evaluated by *in- vitro* antioxidant studies like free radical scavenging activity by DPPH method, nitric oxide method, anti- lipid peroxidation study, and reducing power assay and expressed as % scavenging and IC_{50}. Ascorbic acid was served as standard for the study.

Result: The phytochemical screening of the extract mainly revealed the presence of terpenoids and flavonoids mainly; whereas the HPTLC profile of extract confirmed the presence of β- sitosterol in the extract. The extract showed the significant antioxidant activity as compared to control; but comparatively less than the ascorbic acid. The antioxidant potential was increased in dose dependant manner.

Conclusion: From the present study it can be conclude that TPF exhibits better antioxidant activity due to prominent presence of vital bioactive constituents.

Keywords: Antioxidant, *Thespesia populnea*, Free radical, DPPH, Lipid peroxidation, Reducing power, Nitric oxide

INTRODUCTION

Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron-stealing reaction. The antioxidants do not themselves become free radicals by donating electrons because they are stable in either form. These act as scavengers and play the housekeeper's role by mopping up free radicals before they get a chance to create havoc in a body. Thus they may well be defined as the substances that are capable of quenching or stabilizing free radicals.[1]

The term free radical seems to appear recently, from vitamin brochures to cosmetic advertisements. When any stable molecule that has been attacked by free radicals and loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process is start, it can initiate lipid peroxidation which results in destabilization and disintegration of the cell membranes or oxidation of other cellular components like proteins and DNA, finally results in the disruption of cells.[2]

Terpenes with their long carbon side chain may portioning themselves into the fatty cellular membrane; thereby transfer an electron with free radicals and stabilize them. Phytosterols are another important terpene subclass and two sterol molecules that are synthesized by plants are β-sitosterol and its glycoside. In animals these two molecules exhibit anti-inflammatory, antineoplastic, anti-pyretic and immune modulating activity.[3-5]

Few studies on pharmacological activity of the plant such as wound healing activity[6], anti-inflammatory and antinociceptive activity[7], antifertility activity[8] and in alzheimer's disease[9] have been reported scientifically.

Many plants derived from nature possess antioxidant and antimicrobial activities. The interest in these plants is increasing because of finding safer antioxidant and microbicides; as synthetic molecule can produce therapeutic effect with wide range of side effects. Although the antioxidant potential of bark was investigated[10]; and very less work done on flower of the plant, with this concept the antioxidant activity of flower was selected for our study along with references to their traditional use was emphasized.

MATERIALS AND METHODS

Collection and identification of plant material

The flower of *Thespesia populnea* was obtained from Nasik district (M.S.) and authenticated by Dr. D. A. Patil, reader and the authorized plant identifier of Department of Botany, SSVPS College, North Maharashtra University, Dhule (M.S); a specimen is preserved in the college herbarium (KBHSS/PCG/2009/12).

Chemicals

All the chemicals were of analytical grades and obtained from various sources. 1, 1-Diphenyl-2-picrylhydrazyl radical (Sigma), Thiobarbituric acid (Sigma), Ascorbic acid (Lobe chem)

Extraction

The dried plant material was pulverized into a dry powder and extracted with methanol for 72 h using a soxhlet extractor. The extract was concentrated to obtain a dark brown colored semisolid mass (yield 8.7% w/w) under reduced pressure.

Phytochemical investigation

Phytochemical investigation was performed to ensure the presence of different phytoconstituents in the obtained extract.[11-12] *Test for Terpenoids:* plant extract + 2 ml of chloroform + concentrated H_2SO_4 add carefully to form a layer. Reddish brown color interface to show positive results. *Test for Flavonoids:* plant extract + Magnesium turnings + few drops of concentrated HCl. Observe for presence of pink color to show presence of flavonoids. *Test for Tannins:* plant extract + water, heat and filter, add ferric chloride in filtrate. Dark green solution indicate positive test. *Test for Saponins:* plant extract add water, heat and shake vigorously. Occurance of frothing (creamy small mass of small bubbles) confirms the presence of saponins. *Test for Alkaloids:* plant extract + 2% H_2SO_4 heat, add few drops of Dragendroff's reagent. Presence of orange red precipitate confirms the presence. *Test for reducing sugar:* extract + water, boiled with few drops of fehling's solution A and B for 2 min. Red precipitate which indicate presence of reducing sugar. *Test for steroids:* Plant

extract + 2 mL acetic anhydride + 2 mL concentrated H_2SO_4. Color changes from violet to blue green.

HPTLC analysis

HPTLC analysis was performed with modified solvent system such as petroleum ether: ethyl acetate (8.5:1.5).[13,14] The chromatograph was developed by using 50% ethanolic H_2SO_4 as spraying reagent and heated at 110^{0}C and analyzed with CAMAG-HPTLC system at 366 nm; equipment with sample application Linomat IV, Twin trough chamber, TLC scanner III, Integration Software CATS 4.05. TLC Aluminium pre-coated plates i.e. HPTLC plates (10 X 10), Silica gel 60 F, 254 (E Merck) were used for the study.

Free radical scavenging study by DPPH method

Scavenging of free radical potentialities were evaluated against a methanolic solution of 1, 1- diphenyl-2-picryl hydrazyl (DPPH), and a stable free radical. Antioxidant reacts with DPPH and converts it to 1, 1- diphenyl-2-picryl-hydrazine and the degree of discoloration indicates the scavenging activity of drug. 100 µl of various concentrations of TPF and 100 µl solution of DPPH (0.1 mM in methanol) were incubated at 37ºC for 30 minutes and change in absorbance of reaction mixture was read at 517 nm using UV-spectrophotometer. An equal amount of methanol and DPPH was served as control. The experiment was performed in triplicate and percentage radical scavenging activity was calculated. The IC_{50} value was calculated for each sample and standard, results were expressed as µg/ml. IC_{50} were calculated by using the following formula.[15,16]

$$IC_{50} = a + b\ (50)$$

Where, a = Mean of y – b (Mean of x) and b= $\Sigma xy / \Sigma x^2$

Anti- lipid peroxidation study

Percentage anti-lipid peroxidation was measured according to the method describe by Okhawa.[17] The mixtures containing 50 µl of homogenate, 100 µl of 0.15 M KCl and 50 µl of different concentrations of TPF (10- 80µg/ml) were prepared. Lipid peroxidation was initiated by adding 10µL ferric chloride (400 mM) and 10µL ascorbic acid (200 mM). After incubation for 1 h at 37˚C, the reaction was stopped by adding 2mL of 0.25N HCl containing 15% trichloroacetic acid and 0.375% thiobarbituric acid and the reaction mixture was boiled for 15 min then cooled, centrifuged and the absorbance of the supernatant was measured at 532 nm. The percentage of lipid peroxidation effect (% ALP) was calculated.

Nitric oxide scavenging activity

The nitric oxide scavenging activity was determined according to method reported by Ravishankara.[18] Nitric oxide radicals were generated from sodium nitroprusside solution at physiological pH. Sodium nitroprusside (1 ml, 10 mM) was mixed with 1ml of different concentrations of TPF (10-80µg/ml) in phosphate buffer (pH 7.4). The mixture was incubated at 25^{0}C for 150 min. To 1 ml of incubated solution, 1ml of Griess reagent was added and absorbance was read at 546 nm. The same reaction mixture without the TPF or sample but equivalent amount of distilled water was served as control.

Reducing power assay

Reducing power of TPF was determined on the basis of the ability of antioxidants to form colored complex with potassium ferricyanide, TCA and $FeCl_3$. Different concentrations of TPF (10- 80µg/ml) were mixed with 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50ºC. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was mixed with 2.5 ml of water and 0.5 ml of $FeCl_3$ was added to it and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.[19,20]

RESULT

Phytochemical investigation

Phytochemical investigation of TPF revealed the presence of mainly flavonoids, terpenoids, also saponins, tannins, steroid; however reducing sugar, phlobatanins, alkaloids were absent.

HPTLC analysis

HPTLC pattern of TPF showed the presence of 9 peaks (fig 1) in which compound with R_f 0.26 matched with that of standard β-sitosterol (fig 2), in solvent system petroleum ether: ethyl acetate (8.5:1.5) v/v. The amount of β-sitosterol in TPF was found to be 34.15 % as shown in table 1.

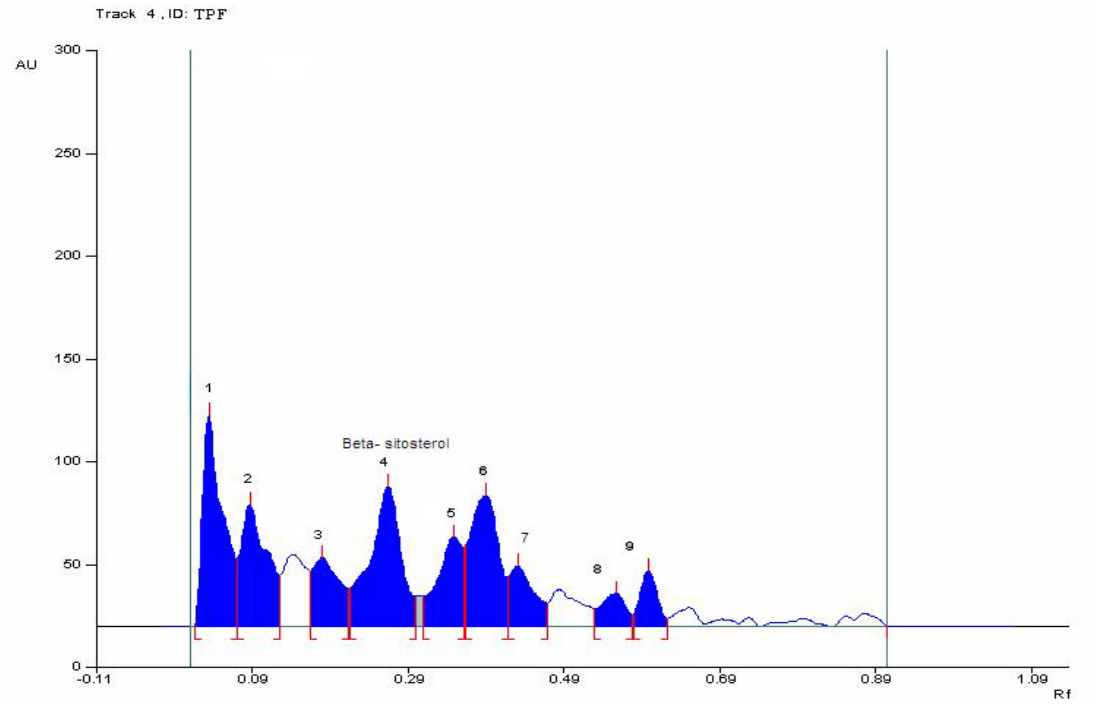

Fig. 1: It shows chromatogram of TPF for the presence of β-sitosterol scanned at 366 nm

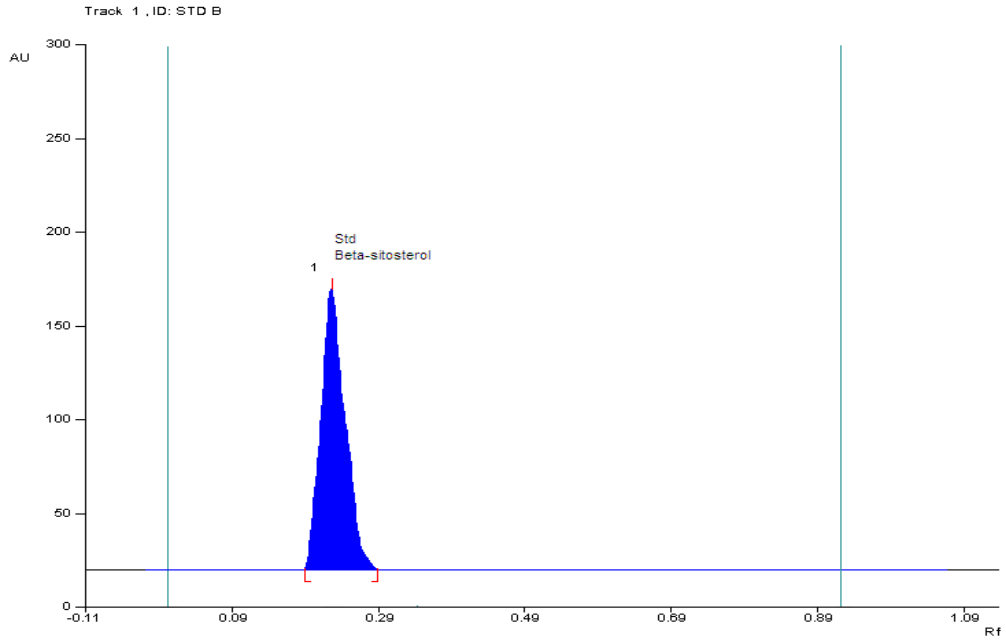

Fig. 2: It shows chromatogram of standard β-sitosterol scanned at 366 nm

Table 1: Table shows peak area and maximum R_f of TPF and β- sitosterol as scanned under 366 nm

Track No.	extract applied (µl)	Maximum R_f	Peak area	Amount of β-sitosterol found (µg)	% of β-sitosterol found
TPF	5	0.26	1242	1707.8	34.15
Std. 1	5	0.26	3231.0	5000	100

TPF: methanolic extract of flower of *T. populnea*, std 1: β- sitosterol.

Free radical scavenging by DPPH method

The results of the DPPH radical scavenging activity of TPF is shown in Table 2. The scavenging ability of the TPF was found to be significant as compared to control, however ascorbic acid was showed DPPH radical scavenging efficacy with IC_{50} 37.79.

Anti- lipid peroxidation study

Higher concentration of TPF shows grater anti- lipid peroxidation activity, as indicated by their % inhibition values (Table 3) respectively; but less as compared to ascorbic acid which shows % inhibition nearly 73.80. The level of lipid peroxidation was suppressed dose dependently by TPF.

Nitric oxide radical scavenging activity

TPF showed significant scavenging of nitric oxide radical. The % scavenging and IC_{50} vales for ascorbic acid and TPF were 41.32 and 43.02 respectively (Table 4). TPF also showed dose dependant inhibition of nitric oxide radical.

Reducing power assay

Reducing power of TPF was concentration dependant. It showed higher reducing power, which is also higher than ascorbic acid. The absorbance of higher concentration of TPF was **(**0.6794±0.004**)** and of ascorbic acid was **(**0.5493±0.001**)** (Table 5)

Table 2: Table shows percentage scavenging activity of TPF by DPPH radical scavenging method

Concentration (µg/ mL)	Standard (AA) Absorbance	% Scavenging	TPF Absorbance	% Scavenging
20	0.6411±0.002	14.63	0.4564±0.002	39.22
40	0.5618±0.001	25.19	0.4250±0.002	43.41
60	0.3915±0.001	47.86	0.3952±0.002	47.38
80	0.2851±0.001	62.03	0.2831±0.001	62.30
100	0.1676±0.004	77.68	0.2230±0.001	70.30
IC_{50}	37.79		44.62	

Vales are expressed in mean + SEM (n=3), AA: Ascorbic acid, TPF: methanolic extract of flower of *T. populnea*

Table 3: Table shows anti- lipid peroxidation activity of TPF

Concentration (µg/ mL)	Standard (AA) Absorbance	% Scavenging	TPF Absorbance	% Scavenging
20	0.613±0.010	22.41	0.4846±0.002	38.67
40	0.528±0.009	33.17	0.4335±0.003	45.13
60	0.437±0.013	44.69	0.3955±0.002	49.94
80	0.351±0.017	55.57	0.2834±0.002	64.13
100	0.207±0.025	73.80	0.2140±0.001	72.91
IC_{50}	38.52		45.97	

Vales are expressed in mean + SEM (n=3), AA: Ascorbic acid, TPF: methanolic extract of flower of *T. populnea*

Table 4: Table shows percentage scavenging activity of TPF by nitric oxide radical scavenging method

Concentration (µg/ mL)	Standard (AA) Absorbance	% Scavenging	TPF Absorbance	% Scavenging
20	0.6412±0.001	24.23	0.5275±0.001	37.67
40	0.5556±0.001	34.34	0.4847±0.002	42.72
60	0.4836±0.002	42.85	0.4050±0.002	52.14
80	0.3161±0.001	62.64	0.3668±0.002	56.66
100	0.1454±0.001	82.81	0.3106±0.006	63.29
IC_{50}	41.32		43.02	

Vales are expressed in mean + SEM (n=3), AA: Ascorbic acid, TPF: methanolic extract of flower of *T. populnea*

Table 5: Table shows reducing power capacity of TPF

Concentration (µg/mL)	Absorbance Standard (AA)	TPF
20	0.1909±0.002	0.2550±0.002
40	0.2211±0.001	0.3214±0.001
60	0.2651±0.002	0.3899±0.003
80	0.3432±0.006	0.6079±0.006
100	0.4558±0.001	0.6794±0.004

Vales are expressed in mean + SEM (n=3), AA: Ascorbic acid, TPF: methanolic extract of flower of *T. populnea*,

DISCUSSION

In recent times, there is an increasing interest in the role of free radical- mediated damage in the etiology of human disease. In the status of normal metabolism, the level of oxidant and antioxidant in human are maintained in balance, which is important for sustaining optimal physiological conditions.[21]

In present study first time, the attempt is made to evaluate antioxidant effect of flower extract of *T. populnea*. The alcoholic and aqueous extract of bark of the plant was extensively studied for their antioxidant effects in CCl_4 induce liver injury in animals; [10] but flowers are lacking such kind of evaluation. The TPF showed significant antioxidant effect in various *in- vitro* models.

The TPF showed presence of several bioactive compound viz. flavonoids, terpenoids, tannins, saponins and to lesser extent steroids etc. which encourages antioxidant studies. β- sitosterol (34.15 %) as quantified by HPTLC analysis and other bioactive compounds may confer the antioxidant and free radical scavenging properties to TPF.

The DPPH test provided information on the reactivity of test compound with a stable free radical. The antioxidant effect is proportional to the disappearance of DPPH• in the test samples. A freshly prepared DPPH• solution exhibit a deep purple color with absorption maximum at 517 nm.[22] By providing hydrogen atoms or by electron donation, possibly via free radical attack on the DPPH molecule and converted them to a colorless stable molecule 2,2-diphenyl- 1- hydrazine or substituted analogues. Hence absorbance decrease and antioxidant potency is increased.

Liver mitochondria are organelles in which the reactive oxygen species production much higher than other organelles. Lipid peroxidation is may be play an important role in tissue damage which might be induced by greater incidence of free radicals in vivo.[23] The inhibitory effect of TPF on lipid peroxidation suggests the ability to interrupt the free radical chain reaction. The higher lipid solubility of the terpenoids and flavonoids may contribute to the antioxidant potential of *Thespesia populnea*.

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation, antimicrobial and antitumor activities.[24] *T. populnea* extract moderately inhibited nitric oxide in dose dependent manner. (Table 4) The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power might be due to hydrogen-donating ability and is generally associated with the presence of reductones.[25]

In conclusion, the results of the present study show that the extract of *T. populnea* flower which contains flavonoids, terpenoids, tannins etc. exhibits the greatest antioxidant activity through the scavenging of free radicals such as nitric oxide, DPPH radical, and lipid peroxidation which participate in various pathophysiology of diseases including ageing. Overall, the plant extract is a source of natural antioxidants that can be important in disease prevention, health preservation and promotion of longevity promoter.

REFERENCES

1. Charanjit Kaur, & Kapoor. Antioxidants in fruits and vegetables - the millennium's health. International Journal of Food Science and Technology. 2001; 36: 703-725.
2. Halliwell B., Murcia MA., Chirico S., Auroma OI. Free radicals and antioxidants in food and in vivo: what they do and how they work?, Critical Review in Food Science and Nutrition. 1995; 35: 7- 20.
3. Hertog MGL., Feskens EJM, Hollman PCH, Katan JB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. 1993; 342: 1007- 1011.
4. Mimaki Y., Kuroda M., Kameyama A., Yokosuka A., Sahida Y. Steriodal saponins from the under- ground parts of *Ruscus aculeatus* and their cytostatic activity on HL60 cells. Phytochemistry. 1998; 48: 485- 493.
5. Bouic PJ., Lamprecht JH. Plant sterols and sterolins: a review of their immune-modulating properties. Alternative Medical Review. 1999; 4: 170- 179.
6. Nagappa AN., Cheriyan Binu. Wound healing activity of the aqueous extract of *Thespesia populnea* fruit. Fitoterapia. 2001; 5: 503- 506.
7. Mani Vasudevan, Kumar KG., Milind Parle. Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract. Journal of Ethnopharmacology. 2007; 109: 264- 270.
8. Waller DP., Bunyapraphatsara N., Martin A., Vournazos CJ., Ahmed MS. Effect of (+)-gossypol on fertility in male hamsters. Journal of Andrology. 1983; 4: 276–279.
9. Mani Vasudevan, Milind Parle. Pharmacological actions of *Thespesia populnea* relevant to Alzheimer's disease. Phytomedicine. 2006; 13: 677- 687.
10. Ilavarasan R., Vasudevan M., Anbazhagan S., Venkataraman S. Antioxidant activity of *Thespesia populnea* bark extracts against carbon tetrachloride-induced liver injury in rats. Journal of Ethnopharmacology 2003; 87: 227-230.
11. Trease GE., and Evans MC. Textbook of Pharmacognosy. 12th ed. Balliere Tindal, London. 1979, pp 343.
12. Khandelwal KR. Practical Pharmacognosy-techniques and experiments. 10th ed. Nirali Prakashan, Pune, India. 1996, pp 31-40.
13. Egon Stahl, Thin layer Chromatography a laboratory handbook, 2nd ed. Springer, pp 240-245.

14. Quality Standards of Indian Medicinal Plants. New Delhi: Indian Council of Medical Research, Vol IV, 119; 2005.
15. Shah R., Kathad H., Sheth R., Sheth N. In vitro antioxidant activity of roots of *Tephrosia Purpurea* Linn. International Journal of Pharmacy and Pharmaceutical Sciences.2010; 2(3): 30-33.
16. Tatiya AU., Ushir YV., Surana SJ. Antioxidant, analgesic and antipyretic activity of essential oil from *Anisomeles indica* (Labiateae) on experimental animal model. The Journal of Research and Education of Indian Medicine. 2007; 4: 39-48.
17. Ohkowa H., Ohishi W., Yagi K. Assay formulation lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979; 95: 351-358.
18. Ravishankara MN., Neeta Shrivastava, Harish Padh and Rajani M. Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* R. Br. (Anantmul). Phytomedicine. 2002; 9: 153–160.
19. Oyaizu M. Studies on product of browning reaction prepared from glues amine. Japanese Journal of Nutrition. 1986; 44: 307-314.
20. Nagore DH., Ghosh VK., Patil MJ., and Wahile AM. In v*itro* antioxidant and *in vivo*-anti-inflammatory activity of *Cassia Sophera* Linn. International Journal of Pharmacy and Pharmaceutical Sciences.2010; 2(1): 113-121.
21. Gutteridge JM., Rowley DA., Halliwell B. Superoxide dependent formation of hydroxyl radicals in the presence of iron salts. Biochemistry Journal. 1981; 199: 263–265.
22. Wang M., Jin Y., Ho CT. Evaluation of resveratrol derivatives as potential antioxidants and identification of a reaction product of resveratrol and 2, 2-diphenyl-1-picryhydrazyl radical. Journal of Agricultural and Food Chemistry. 1999; 47: 3974-3977.
23. Min Zhu, Qi Chang, Leone K Wong, Finny S. Chong and Ronald C Li. Triterpene Antioxidants from *Ganoderma lucidum*. Phytotherapy Research. 1999; 13: 529–531.
24. Hagerman AE, Riedl KM., Jones GA., Sovik KN., Ritchard NT., Hartzfeld PW. High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry. 1998; 46: 1887-1892.
25. Shimada K., Fujikawa K., Yahara K., Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 1992; 40: 945–948.