CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Journal of Drug Delivery and Therapeutics (JDDT)

Available online on 15.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Open Access Research Article

An Ethno-Pharmacological Evaluation of *Catunaregam Spinosa* (Thumb.) Tirveng for Antioxidant Activity

Hansa Saini[1]*, Jayesh Dwivedi[2], Himanshu Paliwal[3], Udichi Kataria[4], Maya Sharma[2]

[1] Department of Pharmacy, Bhagwant University, Ajmer, Rajasthan (India)

[2] Pacific College of Pharmacy, Pacific University, Udaipur, Rajasthan (India)

[3] Sunrise College of Pharmacy, Udaipur, Rajasthan (India)

[4] Geetanjali Institute of Pharmacy, Udaipur, Rajasthan (India)

ABSTRACT

Catunaregam spinosa (Thumb.) Tirveng have been reported in studies to possess antioxidant property. Therefore, present study was undertaken to validate different extracts of *C. spinosa* for antioxidant activity along with safety margin, according to OECD guidelines for toxicity. The extracts of different solvents, such as, distilled water, ethanol, methanol, acetone, chloroform; petroleum ether and benzene were subjected to phytochemical screening and characterization was done by using UV-Visible spectrophotometer. The toxicity studies displayed considerable margin of safety and no adverse effects observed upto 2gm/kg of administration of extract. The antioxidant property of extract of *C. spinosa* was evaluated for DPPH and FRAP activity. The IC50 value for DPPH activity was found to be 85% and the FRAP was found to be 2.5 µg/ml. These results when compared to standard values indicate towards superior antioxidant potential of *C. spinosa* extract.

Keywords: *Catunaregam spinosa*, Antioxidant activity, Toxicity studies, Characterization

Article Info: Received 21 June 2019; Review Completed 26 July 2019; Accepted 30 July 2019; Available online 15 August 2019

Cite this article as:

Saini H, Dwivedi J, Paliwal H, Kataria U, Sharma M, An Ethno-Pharmacological Evaluation of *Catunaregam Spinosa* (Thumb.) Tirveng for Antioxidant Activity, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):280-284
http://dx.doi.org/10.22270/jddt.v9i4-s.3189

***Address for Correspondence:**

Ms. Hansa Saini (M. Pharm.), Department of Pharmacy, Bhagwant University, Ajmer, Rajasthan (India)

INTRODUCTION

The medicinal plants are getting more attention than ever throughout the world because they have countless benefits to society or indeed to all mankind, especially in the line of medication and pharmacological studies. The beneficial properties of plants lie in the presence of bioactive phytochemicals that produce specific physiological action on the human body[1]. A variety of plants contain ordinary antioxidants as bioactive constituents such as alkaloids, essential oils, saponins, flavonoids, phenolics, tannins, curcumin and terpenoids[2,3]. In recent times, the use of natural antioxidants such as phenolic substances (flavonoids, phenolic acids and tocopherols) in food along with preventive and therapeutic medicine is gaining much appreciation. Such natural substances are supposed to show evidence of anticarcinogenic activity and offer diverse health promoting effects because of their antioxidant attributes[4,5,6]. The increased intake of plant foods correlates with decreased rate of death from dreaded diseases. Approximately, 60% of the commercially available antitumor agents are of natural origin[7]. The free radical scavenging potential of polyphenols is primarily due to their redox properties that make them to play the role as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants of ferryl hemoglobin[8,9,10,11].

Catunaregam spinosa (Thumb.) Tirveng is a large deciduous thorny shrub belonging to family Rubiaceae. It is also known as a *Randia dumetorum*. It occurs in almost throughout India up to 4,000 ft attitude. Leaves simple, obovate, wrinkled, shiny and pubescent. Flowers white, fragrant, solitary, seen on at the end of short branches. Fruits globose, smooth berries with longitudinal ribs; yellow when ripe. Seeds many, compressed, embedded in the dark fetid pulp. *C. spinosa* contains glycosides, triterpenoid glycosides and randianin. Saponins named as dumentoronin A, B, C, D, E and F etc. It has rasa, guna, virya, vipaka like Ayurvedic property. There has been numerous publications displaying pharmacological potentials of *C. spinosa* for its Anti-bacterial,

Anti-allergic, Anti-inflammatory, Analgesic and Immunomodulatory activity[12]. The main purpose of the present work is to investigate the antioxidant activity of extract of *C. spinosa* and its ethnopharmacological potentials in treatment of various diseases.

METHODOLOGY

Collection of plant material and authentication

The aerial part of the catunaregum spinosa, belongs to the family rubiaceae was collected and authenticated by the botanist Dr. Dilip Gena, M.Sc., Ph.D. pteridophyte biology lab Department of Botony, Govt. college Ajmer. The collected plant materials were dried. The dried material was powdered in the coarse powder by mechanical grinder. The resulting coarse powder was used for the studies.

Extraction procedure for collected plant material

The leaves of the *Catunaregum spinosa* were taken for the extraction process using a series of polar to non-polar solvents like, Distilled water, Ethanol, Methanol, Acetone, Chloroform; Petroleum ether and Benzene. The leaves of *Catunaregum spinosa* were shade dried and powdered. These powdered plant materials were subjected to extraction (soxhlet and maceration method) where in, 8gm of powdered plant material is extracted by the maceration method using 250 ml of various solvents[13]. The macerated extracts were selected for further analysis. The macerated extract was kept on the shaker for 24 hours, later it was centrifuged and the supernatant was taken for the excess solvent evaporation in laboratory conditions. After evaporation of excess solvent the crude extract was stored in refrigerator till further analysis.

Phytochemical analysis of *Catunaregam spinosa*

Phytochemical tests for the identification of amino acids, carbohydrates, saponins, tannins, phytosterols, alkaloids, proteins, glycosides, flavanoids and phenolic compounds were carried out for all the extracts. The present investigation was planned with an objective to establish Pharmacognostic standards and to evaluate preliminary phytochemical data that can facilitate the authentication and the isolation of the desired constituent from the correct extract[14].

Characterization of *Catunaregam spinosa* by using UV visible spectroscopy

Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined by using UV-visible spectroscopy. Phenolic compounds including anthocyanins, tannins, polymer dyes, and phenols form complexes with iron that have been detected by the ultraviolet/visible (UV-Vis) spectroscopy. Moreover, spectroscopic UV-Vis techniques were found to be less selective and give information on the composition of the total polyphenol content.

Table 1: Concentration of Solvent extract for spectral analysis

Concentration	Methanol Extract	Ethanol Extract
20%	200µl/ml	200µl/ml
40%	400 µl/ml	400 µl/ml
60%	600 µl/ml	600 µl/ml
80%	800 µl/ml	800 µl/ml
100%	Pure extract	Pure extract

The UV-Vis spectroscopy was used to determine the total phenolic extract (280 nm), flavones (320 nm), phenolic acids (360 nm), and the total anthokyanids (520 nm).[15] Extraction of dry leaves was carried out by 5 g of powder for 24 cycle of soxhlet extract. Three solvent were used for extraction viz. Methanol, Ethanol and Chloroform. Different concentrations and dilutions were making for UV analysis in 300nm to 1000nm range for all three samples in replicate (Table 1).

Acute oral toxicity

The acute oral toxicity study was carried out for ethanolic extracts of *C. spinosa*, as per the limit dose test of up and down system mentioned in OECD test guidelines No. 423 at a limit dose of 2 g/kg body weight (*p.o.*). Three rats (one male and two female) were selected for each group, in such a way that the weight differences were not exceeding ±10% of the mean initial weight of the population. Rats were fasted for food but water was provided *ad libitum* overnight prior to extract administration (2000 mg/kg, *p.o.*), suspended in 1.0%, w/v, carboxymethyl cellulose (CMC) and the access to food was reinstated after 3n4 h. After dosing, individual rat was observed at least once during the first 30 min, periodically during the initial 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. The systemic and behavioral toxicity patterns were studied as described in OECD test guidelines. At the end of toxicity study, all surviving animals were sacrificed[16].

Antioxidant activity

The antioxidant activity was carried by the evaluating the radical scavenging activity by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method and the total antioxidant activity by using the ferric ion reducing antioxidant power (FRAP) method.

DPPH radical scavenging assay

The effect of the extracts on DPPH radical was estimated using the method of Liyana- Pathiranan and Shahidi. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in ethanol containing 0.02–0.1 mg of the extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference.[17] The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control)] x 100

where *Abs control* is the absorbance of DPPH radical + methanol;

Abs sample is the absorbance of DPPH radical + sample extract /standard.

Reducing ability (FRAP assay)

The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ(2, 4, 6-tripyridyl-*s*-triazine) and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before use. Plant extracts (150 µL) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tri-pyridyl-triazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results are expressed in µM Fe (II)/g dry mass and compared with that of ascorbic acid[18].

The total antioxidant activity of FRAP is calculated by the following equation:

FRAP value of Sample (µM) = (Change in absorbance of sample from 0 to 4 minute /Change in absorbance of standard from 0 to 4 minute) X FRAP value of standard (1000 µM).

RESULTS AND DISCUSSION

The main aim of the present study is to study of antioxidant activity of the different extracts of leaves of *C. spinosa*. The preliminary phytochemical for the ethanolic leaf extract showed the presence of the triterpenoids in the extracts of solvents such as, Petroleum ether, Benzene, Chloroform, Acetone, Methanol, Ethanol except for the aqueous extract. The glycosides, carbohydrates, phenols/tannins and flavonoids were present in the ethanolic, methanolic and acetone extracts Proteins were present only in aqueous extract and alkaloids and Gum/mucilages were absent in all the extracts. Saponins were present in only ethanolic, methanolic and aqueous extracts. The presence of these phenolic compounds in this plant contributed to their anti-oxidative properties and thus the usefulness of these plants in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. This plant is used routinely among many tribes in Africa for the treatment of various diseases.

UV Visible Spectral Analysis:

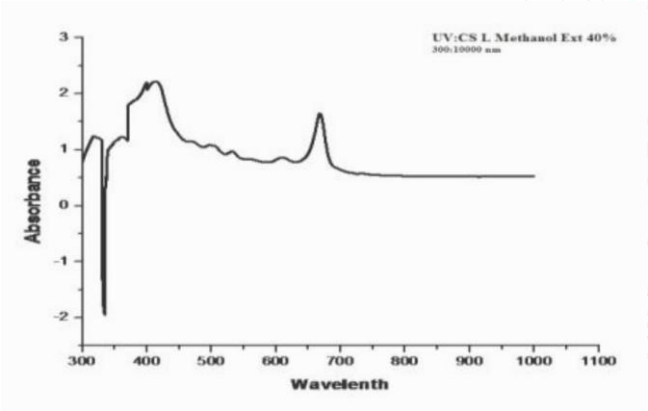

Figure 1: UV spectra 40% Conc. for Methanol

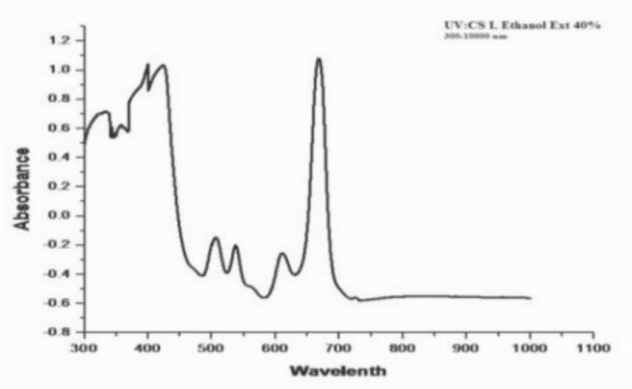

Figure 1: UV spectra 40% Conc. for Ethanol.

Acute oral toxicity studies

Acute toxicity studies of ethanolic extracts of *C. spinosa* were performed according to OECD guidelines (No. 423), where it exhibited significant safety margin as indicated by a lack of systemic and behavioral toxicity up to 2000 mg/kg. No adverse effects were observed at 2000 mg/kg during first 30 min, 24 h and even up to 14 days after administration of the extract. Therefore, randomly doses of the extract were selected from 25 and 500 mg/kg for the further studies.

Antioxidant activity

Relative antioxidant content provides an indication of importance of each of the extracts. Antioxidant activity will aid in the interpretation of clinical results obtained as various products which are tested in biological models for chronic disease. It is reasonable to expect that high antioxidant activity has greater potential to reduce free radicals in the body than do low antioxidant activity.

The antioxidant activity of various plant extracts can be determined accurately, conveniently, and rapidly using DPPH. The trend in antioxidant activity obtained by using the DPPH method is comparable to trends found using other methods reported in the literature. The reaction time of four hours and a temperature of 35ºC facilitate the extraction and reaction of antioxidant compounds with DPPH. Antioxidant activity measured using DPPH accounts partially for the bound and insoluble antioxidants. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593nm.

DPPH Activity

Table 2: Absorbance and evaluation of % inhibition of DPPH by ascorbic acid

Concentration of Ascorbic acid (ug/ml)	Absorbance (517 nm)			Absorbance of blank solution (nm)			% Inhibition
	1	2	Average	1	2	Average	
1	0.651	0.648	0.649	0.664	0.670	0.667	2.6
5	0.621	0.624	0.622				6.7
10	0.525	0.530	0.527				14
50	0.238	0.242	0.240				64
100	0.056	0.059	0.057				91
500	0.041	0.043	0.042				93.7

Table 3: Absorbance and evaluation of % inhibition of DPPH by the ethanolic leaf extract of *C. Spinosa*

Conc. of *C. spinosa* (ug/ml)	Absorbance (517 nm)			Absorbance of blank solution (nm)			% Inhibition
	1	2	Average	1	2	Average	
1	0.651	0.655	0.653	0.664	0.670	0.667	2.09
5	0.600	0.598	0.599				10.19
10	0.542	0.546	0.544				18.44
50	0.496	0.500	0.498				25.34
100	0.264	0.266	0.265				60.27
500	0.131	0.135	0.133				80.06

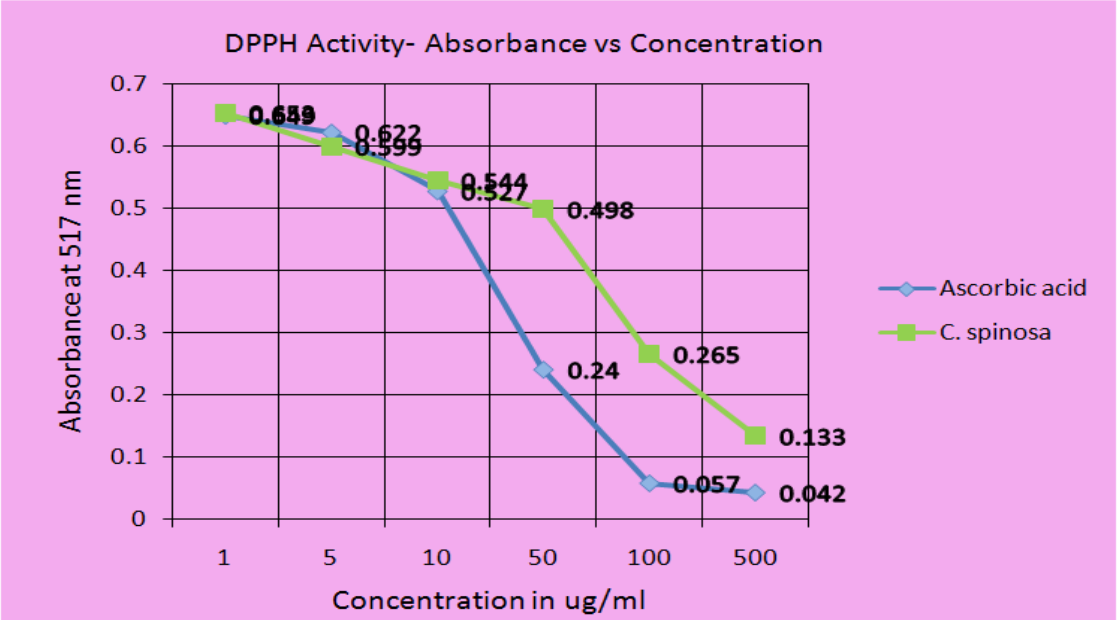

Figure 2: Graphical representation of Absorption v/s Concentration for DPPH activity for ascorbic acid and C. Spinosa.

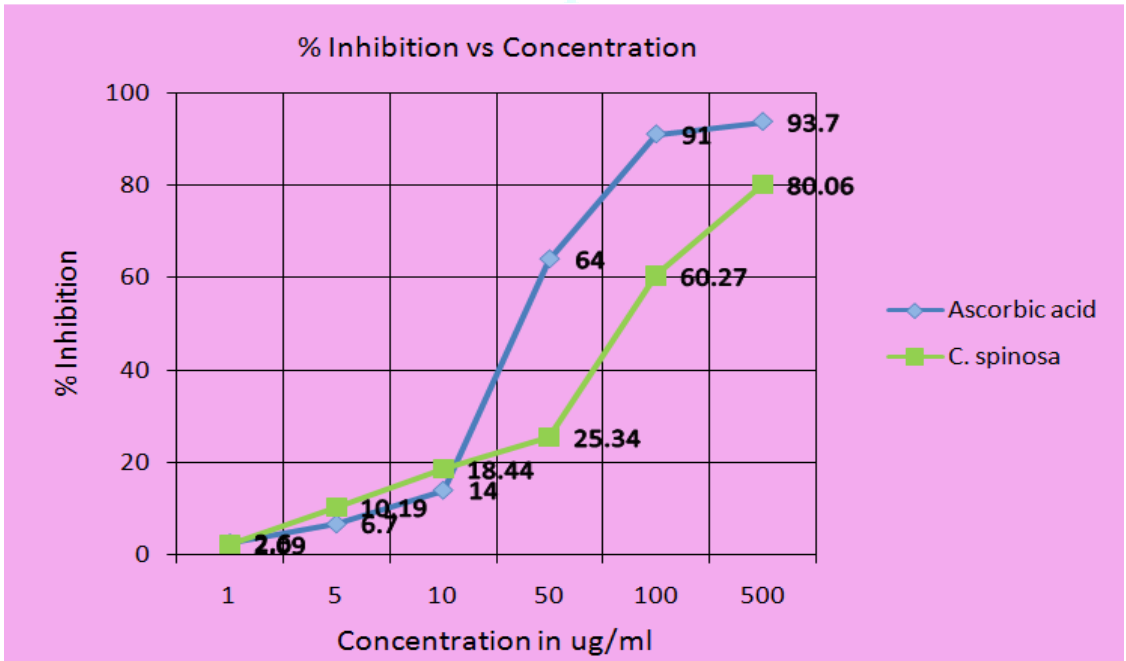

Figure 3*:* Graphical representation of % inhibition v/s Concentration for DPPH activity for ascorbic acid and *C. spinosa.*

FRAP VALUES

Table 4: The FRAP activity for ascorbic acid and *C. spinosa*

Test samples	FRAP activity µmol TE/100g of Fe2+ (dry weight)
Ascorbic acid	2.0 (1.9–2.1) measured range
Catunaregum spinosa (Ethanolic leaf extract)	2.5

The reaction is non-specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe III to Fe II) ion formation. The change in absorbance is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture.

The botanical species collected were separated into fruits and leaves. After shade drying the crude extracts were prepared using ethanol separately. The *Catunaregum spinosa* leaf extract were tested for DPPH and FRAP activity. The results observed showed that the *C. spinosa* had the potential antioxidant property for DPPH and FRAP activity. The DPPH activity of *C.spinosa* and ascorbic acid graphically where in the IC50 value is found to be 85% and 37% respectively (Table 2 and 3) and the FRAP was found to be 2.5 and 2µg/ml respectively(Table 4).

The DPPH activity and high IC50 value showed that the antioxidant property of *C.spinosa* is higher when compared with the standard.

CONCLUSION

The present investigation is on the ethno-pharmacologically important plant *Catunaregum spinosa* Thunb. for antioxidant has lead us to explore these plants thoroughly to get a novel lead molecule for various diseases. Extractive values were also determined which determined whether it's an exhausted or adulterated drug. The percentage yield w/w of the extract was also analyzed wherein the highest yield was found to be in the ethanolic extract. The ethanolic extract of *C.spinosa* showed the presence of the triterpenoids was seen in all the solvent extracts except for the aqueous extract. The glycosides, carbohydrates, phenols/tannins and flavonoids were present in the ethanolic, methanolic and acetone extracts. The antioxidant activity by DPPH and FRAP assays showed very high IC50 value in the *C. spinosa* when compared with the standard. It is thereby apparent and promising, to state the obtained research findings of phytochemistry, antioxidant, anti-inflammatory and analgesic activity for the selected plant species clearly reveals that, the plant is potential and challenging enough to be worked out further.

REFERENCES

1. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO (2007). Pytochemical constituents and antioxidant activity of extract from the leaves of the Ocimum graticcimum. Sci. Res. Essay, 2: 163-166.
2. Prakash, D., Singh, B.N. and Upadhyay, G. 2007a. Antioxidant and free radical scavenging activities of phenols from onion (Allium cepa). Food Chemistry 102: 1389-1393.
3. Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. 4(7): 685-688.
4. Eloff JN (1999) The antibacterial activity of 27 southern African members of the Combretaceae. South African Journal of Science 95: 148–152.
5. Sultana, B., & Anwar, F. (2008). Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chemistry, 108(3), 879-884. doi: https://doi.org/10.1016/j.foodchem.2007.11.053.
6. Sultana, B., F. Anwar and R. Przybylski. 2007. Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna,Acacia nilotica and Eugenia jambolana. Food Chem., 104: 1106-1114.
7. Gordon M. Cragg,David J. Newman,* and, and Kenneth M. Snader *Journal of Natural Products* 1997 *60* (1), 52-60. DOI: 10.1021/np9604893
8. Rice-Evans, C. A.; Miller, N. J. Antioxidants—the case for fruit and vegetables in the diet. *Brit. Food J.* 97:35–40; 1995.
9. Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* 2005, *53*, 4290-4302.
10. Ciz M, Pavelkova M, Gallova L, Kralova J, Kubala L, Lojek A (2008) The in?uence of wine polyphenols on reactive oxygen and nitrogen species production by murine macrophages RAW 264.7. Physiol Res 57: 393–402.
11. Gebicka L, Banasiak E (2009) Flavonoids as reductants of ferryl hemo-globin. Acta Biochim Pol 56: 509–513.
12. Kirtikar K.R., Basu B.D. Indian Medicinal Plants. Panni office,Bhuwaneswari Ashrama, Bahadurganj, Allahabad. (1991) 648-652.
13. World Health Organisation. 1998; Quality Control Methods for Medicinal Plant Materials, WHO, Geneva.
14. Harborne JB. Phytochemical methods, *Chapman and Hall*, London (1998), Edn 3, 90 - 203.
15. Martín JA, Solla A, Woodward S, Gil L, Fourier transform-infrared spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex, Tree Physiology, 25, 2005, 1331–1338.
16. OECD/OCDE Guidelines for the testing of chemicals. Guideline 423: repeated dose oral toxicity study in rodents. Adopted 21 September, Paris 1998.
17. Chu Y.H., Chang, C.L., Hsu, H.F.: J. Sci. Food Agric. 80, 561 (2000).
18. Oyaizu M.: Jap. J. Nutr. 44, 307 (1986).