

Antioxidant and Hepatoprotective Potential of Ethanolic Leaves Extract of *Jatropha gossypifolia*

Sachin Jain^{1, *}, G. P. Choudhary², D. K. Jain¹

¹College of Pharmacy, IPS Academy, Indore, India

²School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore, India

Abstract

Jatropha gossypifolia Linn. (JG) has been traditionally used in the Ayurvedic system of medicine as a chief ingredient of many polyherbal formulations as antioxidant. To evaluate the antioxidant and hepatoprotective activity of ethanolic extract of *Jatropha gossypifolia* leaves. Animals were pre-treated with ethanolic plant extract for 7 days and then toxicity was induced with a single CCl₄ intraperitoneal injection. Pre-treatment with 500 mg/kg (p.o.) of ethanolic extract of *Jatropha gossypifolia* improved the glutathione (oxidized), SOD, catalase, and peroxidase lipid peroxidation levels significantly as compared to control group. Liv 52 was used as standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. The biochemical observations were supplemented with histopathological examination of rat liver sections. The results of this study strongly indicate that *Jatropha gossypifolia* leaves have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. Ethanolic extract was found more potent hepatoprotective. Meanwhile, in vivo antioxidant activities were also screened which were positive for ethanolic extracts. On the basis of this study we conclude that ethanolic extract of *Jatropha gossypifolia* possesses in vivo antioxidant activity and can be employed in protecting tissue from oxidative damage and stress.

Keywords

Jatropha gossypifolia, Liv 52, CCl₄, Antioxidant Activity, Superoxide Dismutase, Hepatoprotective

Received: May 26, 2015 / Accepted: June 20, 2015 / Published online: July 23, 2015

© 2015 The Authors. Published by American Institute of Science. This Open Access article is under the CC BY-NC license.

<http://creativecommons.org/licenses/by-nc/4.0/>

1. Introduction

Jatropha gossypifolia (Euphorbeacea) a common garden plant in tropical countries has been used as a traditional medicine. Plants are well known as a major source of modern medicines. From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine. *Jatropha gossypifolia* is one of the genera that are used in Chinese, Ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery [1].

Literature reveals that, the carbonyl groups are responsible for free radical scavenging activity. Free radicals are atoms or groups of atoms with an odd number of electrons and can be

formed when oxygen interacts with certain molecules. To prevent free radical damage, the body has a defense system of antioxidants [2, 3]. Antioxidants are able to give free radicals, which becomes a companion to their unpaired electron, thus eliminating the threat of gene alteration leading to cancer [2, 3]. Medicinal plants have attracted attention of not only professionals from various systems of medicines, but also the scientific community belonging to different disciplines [4, 5]. In recent years, these have been a great interest in herbal remedies for the treatment of number of ailments. In continuation of search in potential free radical, scavenging agents [6] the present investigation was aimed to determine antioxidant activity of *Jatropha gossypifolia* leave (Linn.) properties to strengthening the immune system of the body which helps to overcome cancer.

* Corresponding author

E-mail address: sachin225819@rediffmail.com (S. Jain), sachin9893459698@gmail.com (S. Jain)

The antioxidant activity of Du-Zhong (*Eucommia ulmoides*) [7], ear mushrooms and anise (*Pimpinella anisum* L.) seed were found to correlate with the phenolic compounds. Studies on local plants such as turmeric (*Curcuma domestica*), betel leaf (*Piper betel*), pandan leaf (*Pandanus odoratus*), asam gelugur (*Garcinia atroviridis*), mengkudu (*Morinda citrifolia*), pegaga (*Centella asiatica*), ginger (*Zingiber officinale*), cassava shoot (*Manihot asculenta*), kesum (*Polygonum minus*) and selom (*Oenathe javanica*) also exhibit good antioxidant activity. The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds which are known to be potent as antioxidants [8].

2. Materials and Methods

2.1. Collection of Plant

Leaves of *Jatropha gossypifolia* (Linn.) were collected from railway track and road side places of Indore MP and voucher specimen (COPIPS/T/098/2010) has been deposited. The authentication was done by Prof. S. R. Upadhyaya Professor, Govt. Post graduate College, Indore (M.P.) India.

2.2. Chemicals and Drugs

Carbon tetra chloride, Potassium chloride, Ellman's reagent, hydroxylamine.

2.3. Preparation of Extracts

The leaves of *Jatropha gossypifolia* were collected and shade dried. The dried leaves were coarse powdered and the powder was packed in to Soxhlet apparatus and extracted with ethanol (64.5 – 65.5°C). The extract was concentrated under reduced pressure (bath temp 50°C). The dried extracts were stored in airtight container.

2.4. Preliminary Phytochemical Screening

Tests for common phytochemicals were carried out by standard methods [9, 10].

2.5. Animals

Albino rats of Wister strain, weighing 100–150 g, kept on normal diet (Ashirwad Industries, Punjab) and water *ad libitum*, were divided into six groups of six animals each. Before starting the experiment, permission from the Institutional Animal Ethics Committee was obtained.

2.6. Acute Toxicity Test

The acute toxicity study for ethanolic and aqueous extracts (decocted and macerated) of *J. gossypifolia* leaves were performed using albino mice and rats. The animals were

fasted overnight prior to the experiment and maintained under standard conditions. All the extracts were administered orally in increasing dose and found safe up to dose of 2000 mg/kg for all extracts.

2.7. CCL₄ Induced Hepatotoxicity

Group-I animals served as normal control, treated with vehicle (gum acacia 3% solution). Group-II animals served as toxic control, treated with CCl₄ in a single dose of 1.5 ml/kg, i.p., to produce acute toxicity. Group III served as a standard group, and was administered Liv-52 (Anti-peroxidative and Hepatoprotective) in a dose of 56 mg/kg, p.o. Group-IV, V and -VI animals were treated with daily doses of 100, 300 and 500 mg/kg, p.o., respectively, of ethanolic extract of *J. gossypifolia* for 7 days. The animals of Groups III–VI were given single dose of CCl₄, 1.5 ml/kg, i.p., 6 h after the last treatment. On day 8 the rats were sacrificed by carotid bleeding and liver was rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15M KCl, centrifuged at 800 g for 10 min at 4°C. The supernatant obtained was used for the estimation of catalase, peroxidase, and other enzymes. Further, the homogenate was centrifuged at 1000 rpm for 20 min at 4°C and the supernatant was used for biochemical estimation.

2.8. Biochemical Estimation

2.8.1. Estimation of Glutathione

Glutathione was estimated using Ellman's reagent (5, 5-dithiobis-(2-nitrobenzoic acid) [DTNB]). The sulphhydryl groups present in glutathione forms a colored complex with DTNB, which was measured by colorimeter at 412 nm. The amount of glutathione was determined using its molar extinction coefficient of 13600/m/cm and expressed in terms of $\mu\text{mol/mg}$ of protein [11].

2.8.2. Estimation of SOD

Estimation of SOD was done by auto oxidation of hydroxylamine at pH 10.2, which was accompanied by reduction of NBT, and the nitrite produced in the presence of EDTA was detected colorimetrically. One enzymatic unit of SOD is the amount in the form of proteins present, 10% liver homogenate required to inhibit the reduction of 24 mM NBT by 50% and is expressed as units per milligram of protein.

2.8.3. Estimation of Catalase

Catalase activity was estimated by determining the decomposition of H₂O₂ at 240 nm in an assay mixture containing phosphate buffer. One international unit of catalase utilized is that amount that catalyzes the decomposition of 1 mM H₂O₂/min/mg of protein at 37°C. Catalase activity was calculated using the millimolar

extinction coefficient of 0.07 and expressed in terms of micromole per minute per milligram of protein.

2.8.4. Estimation of Peroxidase

Peroxidase estimation is based on per-iodide formation. Periodide can be spectrophotometrically determined at 353 nm, and this is directly proportional to the peroxidase concentration in the reaction mixture containing approximate amounts of H₂O₂ and enzyme [12]. One unit of peroxidase activity is defined as the change in absorbance per minute and expressed in terms of units per milligram of protein.

2.8.5. Assay of Lipid Peroxidation Using Mouse Brain Homogenates

The brains of young adult male mice were dissected and homogenized with a Polytron in ice-cold Tris- HCl buffer (20

mM, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 12000g for 15 min at 4 °C, and the supernatant was used for in vitro lipid peroxidation assay. A 1 mL aliquot of the supernatant was incubated with the test samples (final extract concentrations in the assays were 1, 5, 10, and 50 µg/mL, respectively) in the presence of 10 µM FeSO₄ and 0.1 mM ascorbic acid at 37 °C for 1 h. The reaction was terminated by the addition of 1.0 mL of trichloroacetic acid (TCA; 28%, w/v) and 1.5 mL of thiobarbituric acid (TBA 1%, w/v), followed by heating at 100 °C for 15 min. After centrifugation to remove precipitated protein, the absorbance of the malondialdehyde (MDA)-TBA complex was measured at 532 nm. (+)-Catechin was used as a positive control. The inhibition ratio (percent) was calculated from the following equation:

$$\% \text{ inhibition} = \left[\frac{(\text{absorbance of control} - \text{absorbance of test sample})}{\text{absorbance of control}} \right] \times 100.$$

2.9. Histopathological Studies

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation, including cell necrosis, fatty change, hyaline regeneration, ballooning degeneration.

2.10. Statistical Analysis

All analyses were run in triplicates. Data were analyzed by an analysis of variance (ANOVA). Statistical analysis was performed by one-way ANOVA followed by post hoc Dennett's test.

3. Result

3.1. Phytochemicals Investigation

It was found that ethanolic extract contained carbohydrates, amino acids, steroids, flavonoid, alkaloids, glycosides and tannins.

3.2. Acute Toxicity Studies

Ethanolic and aqueous extracts did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

3.3. In-Vivo Antioxidant Activity

Phytochemical screening of the plant shows the presence of flavanoids in ethanolic extract. Therefore, the present study was undertaken to assess the *in vivo* antioxidant effects of ethanolic extract of *J. gossypifolia* on CCl₄ induced hepatotoxicity in rats. The result showed that the activities of glutathione, SOD, catalase and peroxidase in group treated

with CCl₄ declined significantly than that of normal group. Co-administration of ethanolic extract of *J. gossypifolia* at a dose of 100, 250 and 500 mg/kg for 7 days markedly prevented these CCl₄ induced alteration and maintained enzymes level near to normal values. Standard (Liv 52) treated group also significantly increased the level of glutathione, SOD, Catalase and peroxidase in CCl₄ induced hepatotoxic rats. Lipid peroxidation (LPO) levels of liver homogenate were summarized in Table 1.

Lipid peroxidation decreased towards the normal value in ethanolic extract treated carbon tetrachloride intoxicated rats. The antioxidant activity or the inhibition of the generation of free radical is important in the protection against CCl₄-induced liver lesion. The level of lipid peroxide is a measure of membrane damage alterations in its structure function. The level of MDA, which is one of the end products of lipid peroxidation in liver tissue, was found to be high in CCl₄ control group (shown in Table 1) implying enhanced lipid peroxidation leading to tissue damage failure of antioxidant defense mechanisms against free radicals.

3.4. Histopathological Observations

Histology of the liver sections of control animals (Group I) showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus and visible central veins. The liver sections of CCl₄-intoxicated rats showed massive fatty changes, necrosis, ballooning degeneration and broad infiltration of the lymphocytes and the loss of cellular boundaries. The histological architecture of liver sections of the rats treated with ethanolic extracts showed more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the control and Liv 52 treated groups (Fig. 1).

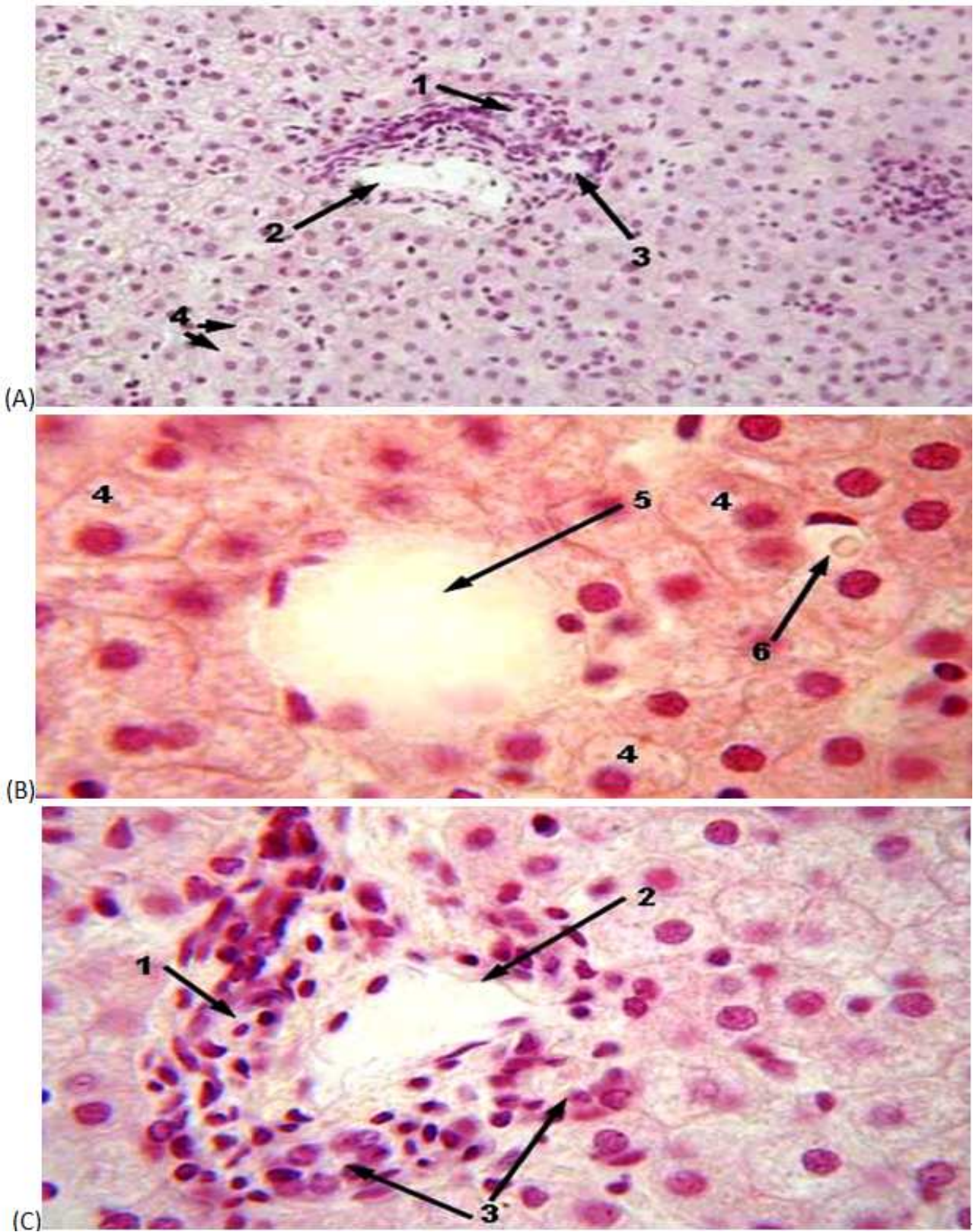


Fig. 1. (A) Liver sections of normal control rats showing: normal hepatic cells with well-preserved cytoplasm; well brought out central vein; prominent nucleus and nucleolus. (B) Liver section of CCl₄ treated rats showing: massive fatty changes, necrosis, ballooning degeneration, and broad infiltration of the lymphocytes and the loss of cellular boundaries. (C) Liver section of rats treated with CCl₄ and extracts showing: well brought out central vein, hepatic cell with well-preserved cytoplasm, prominent nucleus and nucleolus (H, hepatocytes; N, nucleus; CV, central vein; CP, cytoplasm; B, ballooning; NC, necrosis).

Table 1. Effect of ethanolic extract of *Jatropha gossypifolia* on biochemical parameters.

Treatment	Glutathione SOD Catalase Peroxidase ($\mu\text{mol}/\text{mg}$ of protein)	SOD (u/mg of protein)	Catalase (uM/min/mg of protein)	Peroxidase (u/mg of protein)	Lipid Peroxidation [MDA content (in nano mole/milligram)]
Normal control(Vehicle treated)	18.5 \pm 0.094 **	32 \pm 0.02**	11 \pm 0.03**	84 \pm 0.09**	20.78 \pm 0.757
Hepatotoxic Control (CCl ₄ Treated)	8.4 \pm 0.7	19 \pm 0.01	1.1 \pm 0.01	13 \pm 0.02	99.31 \pm 5.59**
Standard Liv 52	16.3 \pm 0.07**	31 \pm 0.01**	8.5 \pm 0.1**	67 \pm 0.15**	39.40 \pm 4.0398*
EEJG 100 mg/kg	9.9 \pm 0.06	23 \pm 0.02	1.6 \pm 0.2	15 \pm 0.06	15.39 \pm 0.479*
EEJG 250 mg/kg	12.9 \pm 0.1*	25 \pm 0.01	1.8 \pm 0.4	17 \pm 0.03	18.34 \pm 0.348
EEJG 500 mg/kg	15 \pm 0.9**	27 \pm 0.05*	5.1 \pm 0.4**	63 \pm 0.06**	75.28 \pm 7.35*

EEJG: Ethanolic Extract of *Jatropha gossypifolia*

Values are in Mean \pm SEM.

Where* $p < 0.05$ and ** $p < 0.01$

4. Discussion

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be involved in a high number of diseases [13]. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

In the present study, the ethanolic extract was selected as they contain alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds. This may have active constituents for producing the free radical scavenging effect.

Free radicals are produced under certain environmental condition and during normal cellular function in the body. These molecules are missing an electron, giving them an electric charge. To neutralize this charge, free radicals try to withdraw an electron from, or donate an electron to, a neighboring molecule. Other antioxidants works against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage [14]. For example, certain enzymes in the body, such as superoxide dimutase, work with other chemical to transfer free radical into harmless molecules. Vitamin C an antioxidant that may prevent cataracts and cancers of the stomach throat, mouth, and pancreas [15]. It may also prevent the oxidation of LDL cholesterol, lowering the risk of heart disease. This investigation revealed that the *Jatropha gossypifolia* contains pharmacologically active substance (s) such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the antioxidant activity.

5. Conclusion

As ethanolic extract of *J. gossypifolia* in the dose of 500 mg/kg, p.o., has improved the glutathione, SOD, catalase, and peroxidase levels significantly, which were comparable

with Liv 52. On the basis of the study we conclude that ethanolic extract of *J. gossypifolia* possesses *in-vivo* antioxidant activity and can be employed in protecting tissue from oxidative stress.

References

- [1] Geronikaki, A., Litina, D. H., Chatziopoulos C, Soloupis, G. (2003). Synthesis and Biological Evaluation of New 4, 5-Disubstituted-Thiazolyl Amides, Derivatives of 4-hydroxy-piperidine or of 4-N-Methyl piperazine. *Molecules* 8: 472-479.
- [2] Wasana, P., Anchalee, P., Nipon, C., Siriporn, C. (2008). Ethnobotany & ethnopharmacology of *Tabernaemontana Divaricata*, *Indian J Med Res* 127: 317- 335.
- [3] Patil, S., Jolly, C. I., Narayanan, S. (2003). Free Radical Scavenging Activity of *Acaci catechu* and *Rotula aquitica* implication in Cancer Therapy, *Indian Drugs* 40: (6), 328-332.
- [4] Sharma, P. C., Yelane, M. P., Dennis, T. J. (2002). Data base on Medical Plants used in Ayurveda. New Delhi: Central council for research in Ayurveda and Siddha 1: 45-47.
- [5] Chhaged, M. R., Khedekar, P. B., Mundhey, A. S. (2007). Synthesis and free radical scavenging activity of some 1, 3, 4-thiazole derivative. *Indian journal of heterocyclic chemistry* 16: 259-262.
- [6] Khandelwal, K. R. (2000). Practical Pharmacognosy techniques and experiments. (2nd ed). Nirali Prakashan, Pune, 34-38.
- [7] Kokate, C. K. (1999). Practical Pharmacognosy. (4th ed). vallabh Prakashan, New Delhi, 230-34.
- [8] Siddique, N. A., Mujeeb, M., Najmi, A. K., Akram, M., (2010). Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of *Aegle marmelos*. *African Journal of Plant Science* 4: 001-005.
- [9] Kuriakose, G. C., Kurup, M. G., (2011). Antioxidant and antihepatotoxic effect of *Spirulina laxissima* against carbon tetrachloride induced hepatotoxicity in rats. *Food Function* 2: 190-196.
- [10] Bumrela, S. B., Naik, S. R., (2011). Identification of β -carotene and β -sitosterol in methanolic extract of *Dipteracanthus patulus* (Jacq) nees and their role in antimicrobial and antioxidant activity. *International Journal of Phytomedicine* 3: 204-215.

- [11] Bagul, M. S., Niranjana, S. K., Rajani, M. (2005). Evaluation of free radical scavenging properties of two classical polyherbal formulations, Indian Journal of Experimental Biology 43:732-36.
- [12] Marzouk, A. M., (2009). Hepatoprotective triterpenes from hairy root cultures of *Ocimum basilicum* L. Zeitschrift für Naturforschung Teil C: Biochemie Biophysik Biologie Virologie 64: 201–209.
- [13] Usha, S. S., Vilasrao, J. K., Rumi, G., (2008). Hepatoprotective activity of Livo Bond, a polyherbal formulation against carbon tetrachloride induced hepatotoxicity in rats. International Journal of Pharmaceutics 4: 472–476.
- [14] D’Mello, P. M., Jadhav, M. A., Jolly, C. I. (2000). Free Radical Scavenging Activity of *Syzygium Cumini* and *Ficus bengalensis* Plants Used in Ayurveda for Diabetes Mellitus. Indian Drugs 37: (11), 518-520.
- [15] Atawodi, S. E., Onaolapo, G. S., (2010). Comparative invitro antioxidant potential of different parts of *Ipomoea asarifolia*, Roemer and Schultes, *Guiera senegalensis*, J. F. Gmeland *Anisopus manii* N. E. Brown Brazilian Journal of Pharmaceutical Sciences 46: 245–250.