Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Sciences

Elixir Bio Sci. 96 (2016) 41595-41599

ISSN: 2229-712X

# Assessment of Ameliorative Properties of Methanol Extract of Pleurotus ostreatus Cultivated with Extract of Allium cepa on Oxidative Stress Markers of $CCl_4$ Induced Hepatotoxicity in Wistar Rats

Ogbeifun, H. E., Anacletus, F. C. and Ighorodje, C.C.

Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.

**ARTICLE INFO**

**Article history:**

Received: 7 June 2016;

Received in revised form: 8 July 2016;

Accepted: 14 July 2016;

**Keywords**

Pleutotus ostreatus,
Allium cepa,
Catalase,
Superoxide Dismutase and Thiobarbituric Acid Reactive Substances.

**ABSTRACT**

This study was designed to assess the ameliorative properties of methanol extract of *Pleurotus ostreatus* cultivated with extract of *Allium cepa* on oxidative stress markers of $CCl_4$ induced hepatotoxicity in wistar albino rats. Seventy wistar albino rats were used for the study and were grouped into 7 of ten rats in each. Groups 2-7 received a dose of 0.3ml/kg body weight (b.w) of $CCl_4$ in 1:1 olive oil via intraperitoneally twice a week. Those in group 1 received only normal rat chow without $CCl_4$. Groups 3, 4, 5 and 7 orally received a dose of 100mg/kg b.w, 200mg/kg b.w, 300mg/kg b.w and 200mg/kg b.w of the methanol extract respectively. Group 6 and 7 orally received a dose of 5.2mg/kg b.w of livolin and group 7 alone orally received a dose of 50mg/kg b.w of vitamin C. These treatments were administered for 30 days and the following biochemical markers; Thiobarbituric Acid Reactive Substances (TBARS), Catalase (CAT) and Superoxide Dismutase (SOD) were estimated on day 10, 20 and 30 after treatment. Result showed that the values of TBARS significantly increased at $p<0.05$ on day 10, 20 and 30 in group 2 compared to group 1 while the values of CAT and SOD significantly decreased at $p<0.05$ on day 10, 20 and 30 in group 2 compared to group 1. The values of TBARS significantly decreased at $p<0.05$ on day 10, 20 and 30 in groups 3,4,5, 6 and 7 compared to group 2 while the values of CAT and SOD significantly increased at $p<0.05$ on day 10, 20 and 30 in groups 3,4,5,6 and 7 compared to group 2. These findings suggest that methanol fruiting body extract of *Pleurotus ostreatus* cultivated with extract of *Allium cepa* scavenged the free radicals responsible for oxidative stress initiated by $CCl_4$ and subsequently ameliorated and substantially reversed the toxic effect of the tissue specific toxicant.



## Introduction

The large size of the liver is well positioned and properly organized to carry out a major role in protein, fat and carbohydrate metabolism. It is the place where metabolites and waste products like ammonia are converted to harmless substances. It also serves a role of producing plasma proteins including clotting factors and lipoprotein by chemical process and maintains stable blood glucose concentration. It participates in the release of glucose from its storage form (glycogen) decomposing it to glucose when required (glycogenolysis) and producing glucose from non carbohydrate like ammonia (gluconeogenesis) (Ward and Daly, 1999; Kmiec, 2001, Pocock and Gillian, 2006; and Krishnendu, 2012).

Diseases associate with liver have become a major problem all over the world and are connected with high rate of diseases that result to death (Baranisrinivasan *et al;* 2009). In developed countries, the major cause of liver disease is excessive alcohol consumption and viral induced chronic liver diseases while in developing countries hepatitis B and C viruses, parasitic diseases, hepatotoxic drugs, high doses of paracetamol and environmental toxins are the common frequent causes.

Herbal medicine and plant-based preparations are used to alleviate diseases. Over the past thirty years, the use of herbal drugs have increased as it is known that if used properly, herbs can help treat various conditions and have fewer side effects compared to conventional medicine (Kala *et al.,* 2006). Plants can synthesize different chemical compounds and some of these compounds exhibit pharmacological activity, this is the basis of herbal drug. These plants contain bioactive compounds that can affect one or more identified biological process such as improving homeostasis, free radicals scavenging ability, cholesterol lowering capability, anti-inflammatory, antimicrobial, antiviral, anticancer and antiparasitic activity (Manjulika *et al;* 2004).

## Materials and Methods

### Experimental Plants

Red *Allium cepa* (Onion) bulbs and *Pleurotus ostreatus* fruiting bodies were bought from Choba Market, Choba, Port Harcourt, Rivers State and identified at Department of Plant Science, University of Port Harcourt, Rivers State Nigeria.

### Experimental Animals

Seventy Wistar rats weighing between 100-200g of three months old breed, purchased from Department of Biochemistry, University of Port Harcourt Animal House were used for this research. The animals were Randomly Selected,

Tele:
E-mail address: francisanacletus@gmail.com

weighed and distributed into seven groups. These animals were put in plastic cages and left under suitable laboratory conditions for two weeks for them to acclimatize to the new environment. The cages were cleaned daily. The animals were fed with commercial growers mash product of Top feeds Ltd., Sapele, Nigeria and water served ad libitum. The animals' body weights were recorded before commencement of treatment.

**Methods**

**Preparation of *Allium cepa* extract by sohxlet method.**

Fresh, healthy Red bulbs of *Allium cepa* were washed, sliced into small pieces and blended in a warring blender. Four hundred grams of the sample was placed in a Sohxlet extractor that was inserted on a filter paper. The extractor was connected to a pre-weighed dried distillation flask and acetone was poured into the distillation flask through the condenser, joined to the Soxhlet extractor. This set up was clamped on a retort stand. Cold water from the jet was permitted to move continuously into the condenser, and the heated solvent refluxed as a result. Onion sample in the solvent that was poured into the distillation flask was extracted in the process of refluxing continuously. When onion extract was observably extracted completely from the sample under test, the condenser and the extractor were disconnected, and the solvent was heated to concentrate the onion extract.

Air oven was used to dry the flask to constant weight and re-weighed to get the crude weight of onion extract (Sheema *et al.*, 2015).

**Method of cultivation of *Pleurotus ostreatus* fruiting body with extract of *Allium cepa* bulb.**

This involves 5 stages:

**(i) Preparation of tissue culture of *Pleurotus ostreatus* using Potato Dextrose Agar (PDA) medium.**

Potato Dextrose Agar (PDA) medium was prepared by pealing 200g Irish potatoes and boiled in water for some minutes. This was filtered and the filterate was made up to 1000ml. Twenty grams of glucose and 20 grams of powdered Agar were added to the filterate and this was stirred properly and shared into two conical flasks of 500ml which were covered with cotton wool and foil paper held with rubber band. The two conical flasks were placed inside the pressure pot and sterilized for 15-20 minutes under pressure. Twenty grams of *Allium cepa* was mixed with the mixture in one of the conical flasks and allowed to cool, and poured out into petri-dishes. Inner tissues of the grown *Pleurotus ostreatus* (that was purchased from Choba market) were removed from the stem of the fruiting bodies and were dropped into the petri-dishes. These were covered with foil paper held with rubber band and kept in dark cupboard for seven days to form mycelium (Vasil and Thorpe, 1998).

**(ii) Grains sterilization**

Two kilograms of guinea corn grains were washed in water to remove the bad ones. The grains were poured into a pressure pot and per boiled in water for some minutes to make it soft. The water was sieved out and the grains were sprayed on the ground for some hours for it to dry properly before they were bottled in spawn bottles.

**(iii) Transfer of the mycelium into the sterilized guinea corn grains in the spawn bottles**

The mycelium that were formed in stage 1 were cut into parts and were added to the grains mixed properly in the spawns bottles and were kept in the inoculation room for two weeks to form spawns.

**(iv) Mushroom substrate preparation**

The substrate used were 93kg of saw dust, 7kg of wheat brown, 400g of calcium carbonate and 60 to 65 % of water. These were mixed properly after which they were bagged and sterilized for 4 hours before the spawns were transferred into them and were kept in the incubation room for one month for ramification to occur.

**(v) Development of the full grown fruiting bodies of *Pleurotus ostreatus***

After ramification had taken place, the bags were cut open and were watered for duration of two weeks before the fruiting bodies started emerging and were harvested after maturation (Vasil and Thorpe, 1998)

**Methanol extraction of *Pleurotus ostreatus* cultivated with *Allium cepa* extract**

Dried *Pleurotus ostreatus* were blended in a warring blender, 300 grams of *Pleurotus ostreatus* sample was macerated in 300ml of methanol for three days in a macerating jar. Then the sample was filtered using a Whatman No.1 filter paper. The filtrate was concentrated with a rotary evaporator at 65$^{o}$ C and was finally dried in a thermostat water bath at 60$^{o}$ C to become an extract (Chaturvedi, 2011).

**Table 1.1. Result of the Effect of Methanol Extract of *Pleurotus ostreatus* on Superoxide Dismutase (SOD) activities (unit/mg).**

| Groups | Treatment | Day10 | Day20 | Day30 |
|---|---|---|---|---|
| 1 | Normal Control (NC). | $2.40 \pm 0.05^{abc}$ | $2.60 \pm 0.07^{ab}$ | $2.58 \pm 0.18^{abc}$ |
| 2 | $CCl_4$ treated only. | $0.29 \pm 0.00^{ab}$ | $0.69 \pm 0.00^{abc}$ | $0.79 \pm 0.00^{ab}$ |
| 3 | $CCl_4$ + 100mg/kg extract. | $0.72 \pm 0.04^{a}$ | $1.78 \pm 0.33^{b}$ | $1.11 \pm 0.00^{a}$ |
| 4 | $CCl_4$ + 200mg/kg extract. | $0.68 \pm 0.13^{a}$ | $2.13 \pm 0.02^{b}$ | $1.47 \pm 0.25$ |
| 5 | $CCl_4$ + 300mg/kg extract. | $0.94 \pm 0.03^{ab}$ | $2.00 \pm 0.00^{b}$ | $1.89 \pm 0.33$ |
| 6 | $CCl_4$ + 5.2mg/kg Livolin. | $0.50 \pm 0.24^{ac}$ | $1.95 \pm 0.00^{bc}$ | $1.32 \pm 0.32^{ac}$ |
| 7 | $CCl_4$ + 200mg/kg extract+5.2mg/kg Livolin+50mg/kg Vitamin C. | $1.07 \pm 0.04^{abc}$ | $2.33 \pm 0.33^{b}$ | $2.33 \pm 0.33^{b}$ |

Values are represented as Mean $\pm$ Standard error of mean; n =3 per group.

Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.

Superscript A ($^{a}$) represents significant difference when group 1 is compared to other groups at $P < 0.05$.

Superscript B ($^{b}$) represents significant difference when group 2 is compared to other groups at $P < 0.05$.

Superscript C ($^{C}$) represents significant difference when group 6 is compared to other groups at $P < 0.05$.

Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

**(vi) Catalase activity determination**

The activity of Catalase was determined as described by Brisswanger, (2004),. Catalase acts to prevent accumulation of $H_2O_2$, by converting it to $O_2$ and $H_2O$. This composition of $H_2O_2$ was monitored spectrophotometrically at 480nm.

**(vii) Determination of Superoxide Dismutase (S.O.D) Activity**

The activity of SOD was determined as described by Fridovich (1997). Adrenaline auto-oxidizes rapidly in aqueous solution to adreno-chrome, whose concentration can be determined at 420nm using spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme SOD inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anions; the degree of inhibition is a reflection of the activity of SOD and is determined at one unit of the enzyme activity.

**(viii) Thiobarbituric Acid Reactive Substances Assay (TBARS)**

TBARS assay is an establishment for quantifying lipid peroxidation by measuring the formation of TBARS according to the method of Tripathi et al., (2001). This assay is based on the reaction of a chromogenic reagent, 2 thiobarbituric acid with malondialdehyde (MDA) at 25$^{o}$C to give a red species absorbing at 535nm.

**Statistical Analysis**

All data were presented as Means ±SD, and were analyzed using the One Way Analysis Of Variance (ANOVA). The results were considered significant when *p* values are less than 0.05 ($p<0.05$) and non-significant when p values are greater than 0.05 ($p>0.05$).

**Discussion**

The effect of methanol fruiting body extract of *Pleurotus ostreatus* cultivated with crude extract of red bulb *Allium cepa on* oxidative stress markers of carbon tetrachloride induced hepatotoxicity in wistar rats were investigated.

Onions are common kitchen spices which posses so many health benefits; this vegetable increases the health values of many foods when added to them (Khiari *et al.*, 2009).

The cultivation of *Pleurotus ostreatus with Allium cepa* extract shows a fast growth of the mycelium when compared to the growth of the mycelium without *Allium cepa* extract.

Oxidative stress has been shown to be involved as primary factor in progression of many degenerative ailments like cancer, diabetes type 2, atherosclerosis, cataracts, liver disease, neurodegenerative disorders, etc. (Jayakumar *et al.*,2006) and it occurs when there is an over production of reactive oxygen species.

**Table 1.2. Result of the Effect of Methanol Extract of *Pleurotus ostreatus* on Catalase (CAT) activities (unit/mg).**

| Groups | Treatment | Day 10 | Day 20 | Day30 |
|---|---|---|---|---|
| 1 | Normal Control (NC). | $1.39\pm0.00^{a}$ | $1.31\pm0.01^{ab}$ | $1.47\pm0.03^{ab}$ |
| 2 | $CCl_4$ treated only. | $0.38\pm0.00^{b}$ | $0.20\pm0.00^{abc}$ | $0.23\pm0.00^{ab}$ |
| 3 | $CCl_4$ + 100mg/kg extract. | $1.07\pm0.30$ | $0.98\pm0.06^{b}$ | $0.75\pm0.17$ |
| 4 | $CCl_4$ + 200mg/kg extract. | $1.48\pm0.33$ | $1.16\pm0.15^{b}$ | $0.88\pm0.20$ |
| 5 | $CCl_4$ + 300mg/kg extract. | $1.59\pm0.10^{b}$ | $1.23\pm0.12^{b}$ | $0.99\pm0.34$ |
| 6 | $CCl_4$ + 5.2mg/kg Livolin. | $0.96\pm0.35^{c}$ | $1.05\pm0.01^{bc}$ | $0.75\pm0.07^{c}$ |
| 7 | $CCl_4$ + 200mg/kg extract+5.2mg/kg Livolin+ 50mg/kg Vitamin C. | $1.55\pm0.16^{b}$ | $1.28\pm0.07^{b}$ | $1.19\pm0.16^{b}$ |

Values are represented as Mean ± Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.
Superscript A ($^{a}$) represents significant difference when group 1 is compared to other groups at $P < 0.05$.
Superscript B ($^{b}$) represents significant difference when group 2 is compared to other groups at $P < 0.05$.
Superscript C ($^{C}$) represents significant difference when group 6 is compared to other groups at $P < 0.05$.
Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

**Table 1.3. Result of the Effect of Methanol Extract of *Pleurotus ostreatus* on TBARS Levels (µmol/mg).**

| Groups | Treatment | Day10 | Day 20 | Day 30 |
|---|---|---|---|---|
| 1 | Normal Control (NC). | $1.12\pm0.00^{ab}$ | $1.20\pm0.00^{a}$ | $1.18\pm0.00^{abc}$ |
| 2 | $CCl_4$ treated only. | $1.31\pm0.01^{abc}$ | $1.30\pm0.01^{b}$ | $1.36\pm0.10^{abc}$ |
| 3 | $CCl_4$ + 100mg/kg extract. | $1.12\pm0.01^{b}$ | $1.09\pm0.04$ | $1.06\pm0.07^{bc}$ |
| 4 | $CCl_4$ + 200mg/kg extract. | $1.00\pm0.00^{b}$ | $0.68\pm0.33^{b}$ | $0.99\pm0.01^{ab}$ |
| 5 | $CCl_4$ + 300mg/kg extract. | $0.99\pm0.02^{b}$ | $0.07\pm0.03^{abc}$ | $0.89\pm0.03^{ab}$ |
| 6 | $CCl_4$ + 5.2mg/kg Livolin. | $1.11\pm0.00^{bc}$ | $1.01\pm0.00^{c}$ | $0.87\pm0.29^{abc}$ |
| 7 | $CCl_4$ + 200mg/kg extract + 5.2mg/kg Livolin + 50mg/kg Vitamin C. | $0.98\pm0.07^{abc}$ | $0.09\pm0.00^{abc}$ | $0.83\pm0.01^{ab}$ |

Values are represented as Mean ± Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.
Superscript A ($^{a}$) represents significant difference when group 1 is compared to other groups at $P < 0.05$.
Superscript B ($^{b}$) represents significant difference when group 2 is compared to other groups at $P < 0.05$.
Superscript C ($^{C}$) represents significant difference when group 6 is compared to other groups at $P < 0.05$.
Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

These reactive species if not deactivated, their chemical reaction can cause injury to macromolecules in cells like proteins, carbohydrates, lipids, and nucleic acids. High level of ROS are dangerous and can cause oxidation of bimolecular substances that can result to cell damage and eventual death which results to various ailments and disorders (Halliwell and Gutteride, 2000).

Carbon Tetrachloride has been established to cause hepatotoxicity in both man and experimental subjects (Adewale *et al.,* 2013) and has since been used in suitable modern form for the screening of hepatoprotective activities of different sources of natural products. Administration of $CCl_4$ to experimental rats induced chronic liver injury that results to fibrosis, scar production and damage of normal tissue architecture (Chaudhary *et al.,* 2010).

Medicinal plants owe their therapeutic features to the presence of various phytochemicals in their leaves, stems, barks, roots, and fruits (Sofowora, 2008). Fruiting body extract of *Pleurotus ostreatus* has been recognized to be possible source of antioxidants and have the ability to highly stop lipid peroxidation (Chaudhary *et al.,* 2010). The occurrence of compounds like phenolic compounds, and flavonoid are responsible for the fruiting bodies of *Pleurotus ostreatus* protection due to their antioxidant features (Liu, 2004; Gupta *et al.,* 2011) that scavenge reactive oxygen species.

The result of Table 1.1 and 1.2 on day 10 shows a significant decrease at $P<0.05$ in the activities of SOD and CAT in group 2 when compared to group 1. Groups treated with the dosage of methanol extract of *Pleurotus ostreatus* showed a significant increase at $P<0.05$ in the activities of SOD and CAT when compared to group 2. This explanation was also applicable to the activities of SOD and CAT on day 20 and 30.

This improvement to near normal level was an indication of stabilization of plasma membrane as well as repair of hepatic parenchyma.

The result of Table 1.3 on day 10 shows a significant increase at $P<0.05$ in the levels of TBARS in group 2 when compared to group 1. Groups treated with the dosage of methanol extract of *Pleurotus ostreatus* showed a significant decrease at $P<0.05$ in the level TBARS when compared to group 2. This explanation is also applicable to TBARS on day 20 and 30.

**Conclusion**

From this research, it has been established that $CCl_4$ induced a significant damage to the liver tissues causing high level of lipid peroxidation, but methanol fruiting body extract of *Pleurotus ostreatus* cultivated with red bulb *Allium cepa* effectively ameliorated this effect and as such can be used in the treatment of Liver diseases.

**References**


Adewale, A. and Abiodun, O. (2013): Antioxidant and Hepatoprotective Effect of Hibiscus Polyphenol Rich Extract (HPEEE) Against Carbon Tetrachloride ($CCl_4$) - Induced Damage in Rats.*Medicine and Medical Research British Journal,* **3** (4):1574-1586.

Baranisrinivasan, P., Elumalai E. K., Sivakumar C., Theresa S. V. and David E. (2009) Hepatoprotective effect of *Enicostemma littorale blume* and *Eclipta alba* during ethanol-induced oxidative damage in albino rats. *Pharmacology International Journal*; **5** (4):268-272.

Bora, P.S., Srivastava, L.M. and Bhatt, S.D. (1995).Metabolic and histopathological effects of propanol and insulin. *Experimental Biology.Indian Journal,***23**:22-26.

Chaturvedi, P. (2011). Effects of Methanol extract of bark of the Bauhinia purpura on alcohol- induced liver toxicity in albino rats. *Biology and Chemical Science International Journal,* **5** (3): 944-952.

Chaudhary, G.D., Kamboj, P., Bingh, I. and Kalia.(2010). Herbs as liver savers; *Natural Products and Resources Indian Journal,***1** (4):397-408.

Dianzani, M.U., Biocca, M.E., Canuto, R.A. and Muzia, G. (1991).Caffeine in rats induced lipid peroxidation in fatty liver.*International Journal of Tissue Reaction,***113** (1)79-85.

Diplock, A.T., Charleux, J.L., Crozier-Willi, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., S tahi, W. and Vina-Ribes, J. (1998).Functional food science and defense against reactive oxidative species.*Brazilian Journal of Nutrition,***8**:77-1112.

Doherty, R.E. (2000). The production and use of carbontetrachloride, tetrachloroethylene, trichloroethylene and 1,1,1- trichloroethane in the United States; Part 1- Historical background; Carbontetrachloride and tetrachloroethylene. *Environmental Forensics,***1** (1): 69-81.

Doumas, B. T., Watson, W.A. and Biggs, H.G. (1991).Clinical Chim.Acta.31: 87.

Fredovich, I. (1997). Superoxide anion radical ($O_2^-$), superoxide dismutases and related matters.*The Biological Chemistry Journal,***272** (30):18515-18517.

Gupta, S.K. and Sindhvi, I.J. (2011). Herbal and Hepatoprotective drugs acting on peptic ulcer and liver disease: *A review International Journal of pharmacology and technology,* **3** (1): 824-853.

Halliwell, B. and Gutteridge J.M.C. (2009). Endogenous and diet-derived antioxidant defences in Free Radicals in Biology and Medicine. *4th Edition. Oxford University Press: Clarendon;* 79-81.

Jayakumar, T.,Geraldine, P. andRamesh, E. (2006). Antioxidant activity of the oyster mushroom (*Pleurotus ostreatus) on* $CCl_4$ –induced liver damage in rats. *Food and chemical toxicology,***44**:1989-1996.

Jendrassik, L and Grof, P. (1938).Vere infachte photometrische Method zur Beshmung des Bilirubins.*Biochemistry,* **297:81**-89.

Kala C. P, Dhyani P.P and Sajwan B.S, (2006). The Medicinal plants developing sector in Northern India: Challenges and Opportunities. *Ethnobiology and Ethno Medicine Journal*; **2** (32)1-15.

Khiari, Z., Makris, D. and Kefalas, P. (2009). An investigation on the recovery of antioxidant phenolics from onion solid waste based solvent system. *Food Bioprocess Technology,***2**: 337-343.

Kind P.R.N. and King E.J. (1954).Estimation of plasma phosphatase by determination of hydrolyzed phenol with antipyrine. *Journal of Clinical Pathology*; **7**: 322–6.

Kmiec, Z. (2001). Liver cells Cooperation in disease and health. *Advanced Anatomy Embryology cell biology,***161**:1-151.

Krishnendu, A., Soumya, C., Gunjan, B., Aniruddha, C and Goutam, K. S. (2012). Hepatoprotective effect of a Wild Edible Mushroom on carbon tetrachloride in mice .*International Journal Pharmacological Science.***4** (3)**:** 285-288.

Liu, R.H. (2004). Potential synergy of phytochemicals in cancer prevention mechanism of action. *Nutrition Journal,* **134**: 34795-34855.

Manjulika, Y., Sanjukta, C., Sharad, K. and Geeta, W. (2014). Preliminary phytochemical screening of six medicinal plants

used in traditional medicine. . *Journal of pharmacy and pharmaceutical sciences*, **6**: 539-542.

Pocock and Gillian (2006). Human physiology (3rd edition).*Oxford University Press.*P.404.

Reitman, S. and Frankel A.S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *America Journal of Clinical Pathology;* **28**: 56–63.

Sheema, B., Leena, S., Anupma, M., Pooja, B. and Sunita, D. (2015). Bioactive compounds and pharmacological potential of *Rosa indica* L. and *Psidium guajava* L. methanol 184.

Tietz, N.W. (1995). Clinical guide to laboratory tests.3rd edition.*Saunder Company Philadelphia,*Pp. 518-519.

Tripathi, Y.B., Upadhyay, A.K. and Chaturvedi, P. (2001). Antioxidant properties of smilax China. *Indian Journal of Experimental Biology*,**39**:1176-1179.

Vasil, I.K. and Thorpe, T.A. (1998). Plant cell and tissue culture. Dordrecht: *Kluwer Academy*.

Ward, F. M. and Daly, M. J. (1999). Hepatic Disease. In: *Clinical Pharmacy and Therapeutics*, Walker, R and Edwards, C (Eds.), 195- 212, Churchill Livingstone, New York.