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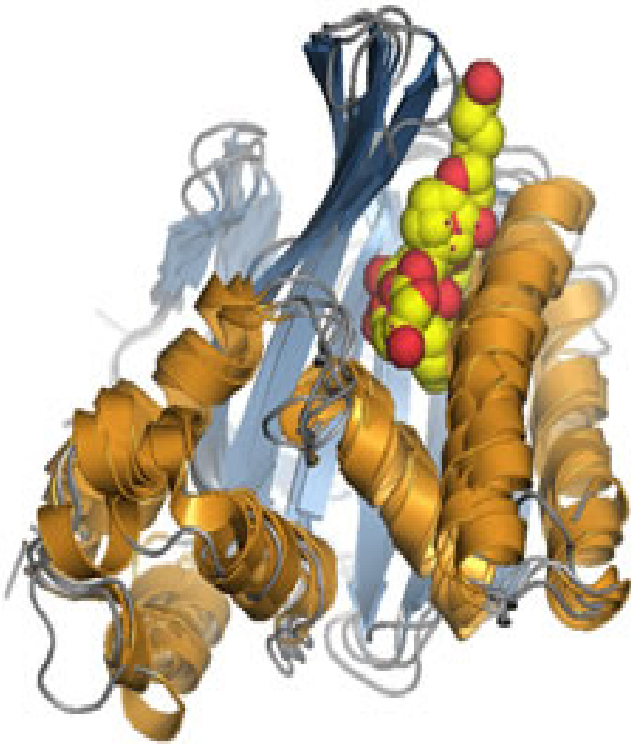

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Antioxidant and cytotoxic activities of three different fractions of *Lonicera quinquelocularis* (Translucent Honeysuckle) plant

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Abstract. *The cytotoxic and antioxidant activities of a plant count for its effectiveness against various lethal and sever diseases such as cancer, Parkinson Alzheimer and Diabetics etc. Free radicals in the form of reactive oxygen species and reactive nitrogen species may be the main cause of these severe health issues. Medicinal plants with antioxidant and cytotoxic properties have been proved to be the most reliable method of treatment of the mentioned diseases. In the present study antioxidant and cytotoxic potential of three different fractions i.e. ethanol, chloroform and ethyl acetate extracted from Lonicera quinquelocularis (Translucent Honeysuckle) plant were tested. The ethanolic and ethyl acetate fraction showed highest scavenging ability than chloroform fraction against DPPH and ABTS free radicals. In cytotoxic assays, chloroform fraction showed highest lethal effects than ethanolic and ethyl acetate fraction. The data obtained from the experimental work revealed that all the three fractions isolated from Lonicera quinquelocularis (Translucent Honeysuckle) possess antioxidant and cytotoxic properties.*

***Key Words*:** Free radicals, anticancer agents, cytotoxic, phytochemicals, antioxidant

Introduction

Antioxidant defense system is a protective system of the body that prevents the body from the harmful effects caused by free radicals. Antioxidant property of a plant represents its effectiveness against diseases originated as a result of free radicals like cancer, cardiovascular diseases and Parkinson disease etc (Sian *et al.*, 2003). Plants which contain phenolic compounds (phenolic acids, flavonoids, quinines, lignin's, tannins),coumarins alkaloids and vitamins are the best sources of natural antioxidant **(**Kahkonen *et al.*,1999**;** Zheng and Wang 2001; Cai *et al.*, 2003). Phenolic compounds are present in plants as secondary metabolites **(**Lin *et al.*,2007**)**. Medicinal plants have played a great role in the treatment of diseases like cancer, aging **(**Finkel

et al.,2000**)**, atherosclerosis (Harrison *et al.*,2003**)**, and cardiac arrest (Sharma *et al.*,2007**)**by potent antioxidant components.One of the most common example is the *Zingiber zerumbet*(L) plant which has been traditionally used in many countries for curing cancers**(**Yob*et al.*,2011**)**.
The term **"**Cytotoxicity" is made by the combination of two wards "cyto" which means the living cell and "toxicity" means toxic/dangerous. Cancer which is a severe health problem is malignant neoplasm and involves the propagation of damaging of healthy cells. It is characterized by abnormal and uncontrollable growth. Synthetic drugs are failed in controlling cancer or tumor due to several aspects as the severe toxicity, adverse side effects and being costly. Herbal medicine have been improved a satisfactory way of the treatment of cancer and tumors and many other lethal diseases (Harun-ur-Rashid *et al.,* 2002). Approximately 50% drugs used for the treatment of cancer are isolated from medicinal plants (Newman and cragg, 2007). The anticancer activities of medicinal plants are due to the presence of antioxidants or cytotoxic bioactive molecules such as Vinblastine and vincristine which is present in large quantity in *Catharanthus roseus* (L). Hence, plants are the real rich source of novel anticancer agents throughout the world (Cragg*et al.,* 1996).
The present study aimed to investigate the antioxidant and cytotoxic properties of the three fractions i.e. ethnolic, chloroform and ethyl acetate of *Lonicera quinquelocularis (Translucent Honeysuckle,)* to use as a therapeutic agent for the treatment of cancer and other related diseases caused by free radicals.

Materials and Methods

DPPH radical scavenging activity

DPPH scavenging activity was determined by the method of Gyamfi*et al.,* (1999) with some modification.5mg/5mlmethanolic stock solution for each of the three fraction i.e.ethanolic, chloropharmic and ethyl acetate was prepared from their relative crude extracts of *Lonicera quinquelocularis*. Sub solutions were prepared through dilution of the stoke solutions of each fraction i.e. 50, 100, 150, 200, 250 and500 µg/ml. Ascorbic acid was used as a standard.
Free radical DPPH stock solution was prepared by dissolving 1.5 mg powder in 50 ml methanol. The solution after preparation was covered with aluminum foil to prevent light access and was stored at 20ºC. The absorbance of DPPH solution was measured by means of spectrophotometer at517 nm and was found to be less than 1.
2700 µl of DPPH solution was mixed with 300 µl of each sub solutions with sample and standard solutions as well and stored in incubator in dark for about 30 minutes at 25 ºC. Percentage scavenging of the DPPH radical was calculated using the following equation: DPPH radical scavenging effect (%) = (A1-A2/A1) x100
Where A1 is the absorbance of the control (DPPH solution without test sample) and A2 is the absorbance in the presence of the test sample.

ABTS radical scavenging activity

ABTS assay was run according to the method of Arnao *et al.* 2001 with some modification. ABTS solution was prepared by mixing equal volumes of 7mM ABTS and 35mM $K_2S_2O_8$ (potassium per sulfate) and incubate for 24 hours in dark. Absorbance of the ABTS solution was cheeked at 745nm, it was less than 1. Stoke solutions were made for each of the three fractions of *Lonicera quinquelocularis* plant i.e. Ethanolic, Chloroform and ethyl acetate by dissolving 10mg

of the respective fraction in 10ml of 70% commercial grade methanol. Respective sub solutions of concentration 50, 100, 150, 200, 250µg/ml, for each of the three fractions were prepared. 400µl from each concentration of every fraction was mixed with 600µl ABTS solution. Similar process was repeated for ascorbic acid which was used as a reference. After incubation the Kinetic absorbance was taken at 745 nm by spectrophotometer after 1 and 6minutes for each concentration and mean was taken for each reading. The potential to scavenging the ABTS radical was calculated using the following equation:
ABTS radical scavenging effect (%) = (A1-A2/A1) x100 Where A1 is the absorbance of the control (ABTS solution without test sample) and A2 is the absorbance in the presence of the test sample.

Cytotoxic assay (Brine shrimp assay)

28 g commercial sea salt (Sigma) was dissolved in one liter of distilled H_2O or 2.8 g of commercial sea salt (Sigma) was dissolved completely in 100 ml distilled water with continuous stirring for 2 hours. For this assay, the stock solution of 10mg/10 ml of methanolic crude extract was prepared in the respective solvent methanol for each of the three fractions. From this stock solution further dilution was made to 10 µg/ml, 20 µg/ml, 50 µg/ml 100 µg/ml 150 µg/ml 200 µg/ml and 250 µg/ml each dilution was made by using sea salt solution, instead of methanol due to its toxicity (M1V1=M2V2). The commercial sea salt solution (media) was put in the two compartment rectangular tray contained with large number of small pores for passage of larvae. Eggs were scattered in dark compartment of tray (covered by aluminum foil) and were placed under the lamp for 24 hours incubation. After 24 hours, the larvae were hatched, migrated to the light end via pores and was collected by pasture pipette. Now 10 shrimps were transferred to each vial by pasture pipette both in the controlled and sample solution vials and raised the volume up to 5 ml in each vial by sea salt solution. In the controlled vials only sea salt solution (media for shrimps) were taken. Then all the vials were incubated at 28 ^{o}C for 24 hours. After 24 hours of incubation, the numbers of alive brine shrimp were counted with the help of 3x magnifying glass.

Results

1 kg powder of the *Lonicera quinquelocularis* was dissolved in ethanol and after filtration and drying, ethanolic extract was obtained. The extract was further fractionated with chloroform,and ethyl acetate. The three different fractions i.e. ethanolic, chloroform and ethyl acetate of the plant*Lonicera quinquelocularis*were then used for antioxidant and cytotoxic activities. The resultsrevealed that all the three fractions of the plant of *Lonicera quinquelocularis* have significant free radicals scavenging abilities. It was noted that all the three fractions have potential to scavenge both DPPH(1, 2-dyphenyl-2-picrylhydrazyl) andABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic) radicals . Various concentrations (50, 100, 150 200, 250 and 500 µg/ml)of each of the three fractions were subjected to DPPH free radicals and was noted that the scavenging ability of all the three fractions increases with an increase in their concentrations(Fig 1-3). Similar phenomenon was noted for ABTS free radicals (Fig 4-6). The significance of the three different fractions of *Lonicera quinquelocularis* for radicals scavenging was concluded by comparison with Ascorbic acid which was used as a standard in both assays.

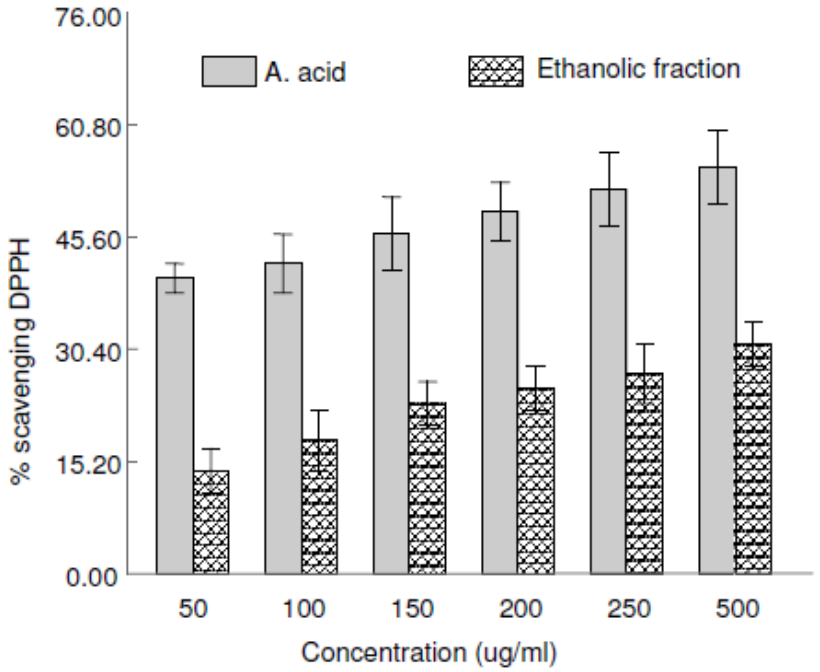

Fig.1. Percentage scavenging of DPPH radicals by various concentrations of ascorbic acid and ethanolic fraction of *Lonicera quinquelocularis***.** The data is the Mean Value ± Standard Deviation of three replicates.

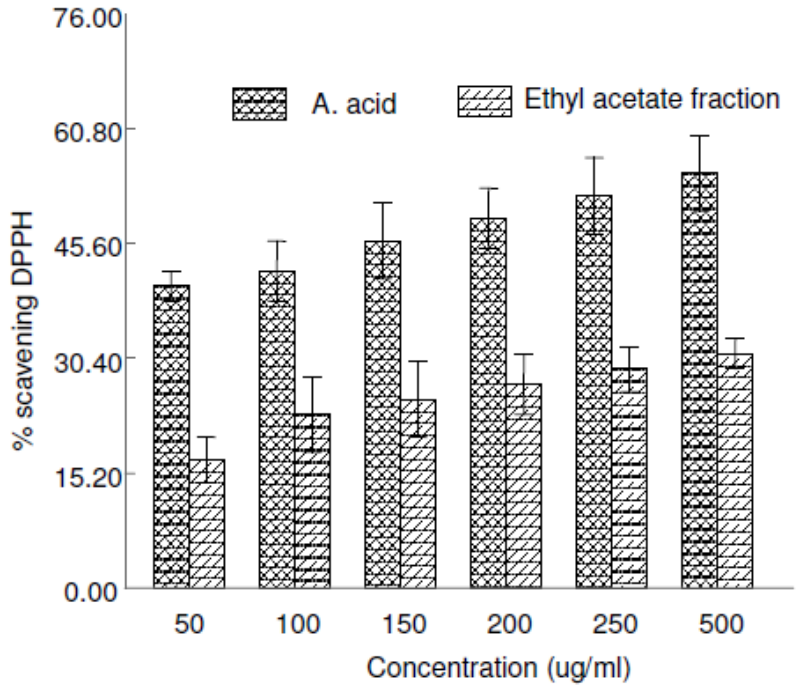

Fig.2.Percentage scavenging of DPPH radicals by various concentrations of ascorbic acid and ethyl acetate fraction of *Lonicera quinquelocularis*. The data is the Mean Value ± Standard Deviation of three replicates

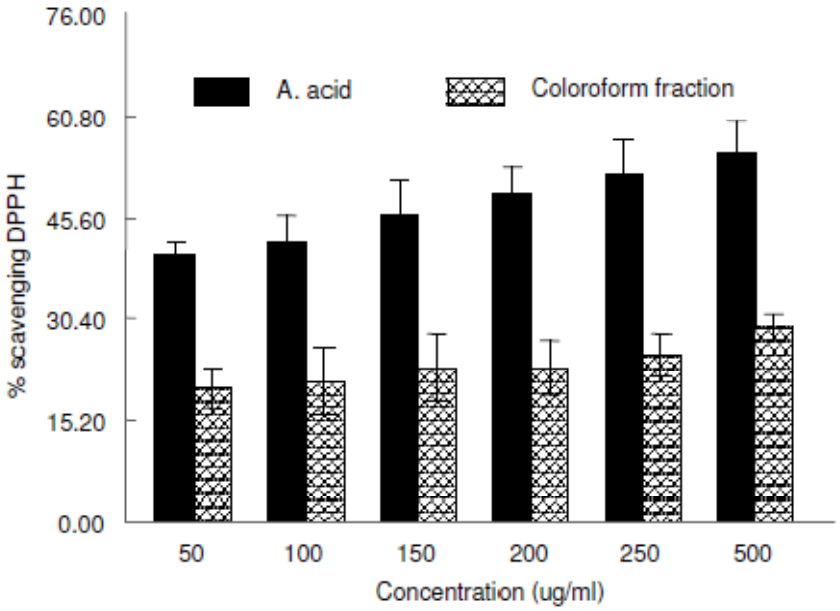

Fig.3. Percentage scavenging of DPPH radicals by various concentrations of ascorbic acid and chloroform fraction of *Lonicera quinquelocularis*. The data is the Mean Value ± Standard Deviation of three replicates. Error bars represent standard deviation.

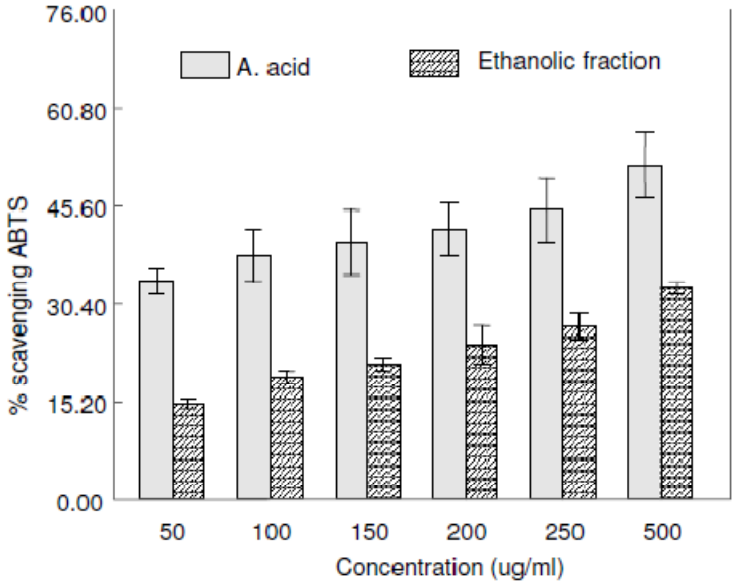

Fig.4. Percentage scavenging of ABTS radicals by various concentrations of ascorbic acid and ethanolic fraction of *Lonicera quinquelocularis***.** The data is the Mean Value ± Standard Deviation of three replicates. Error bars represent standard deviation.

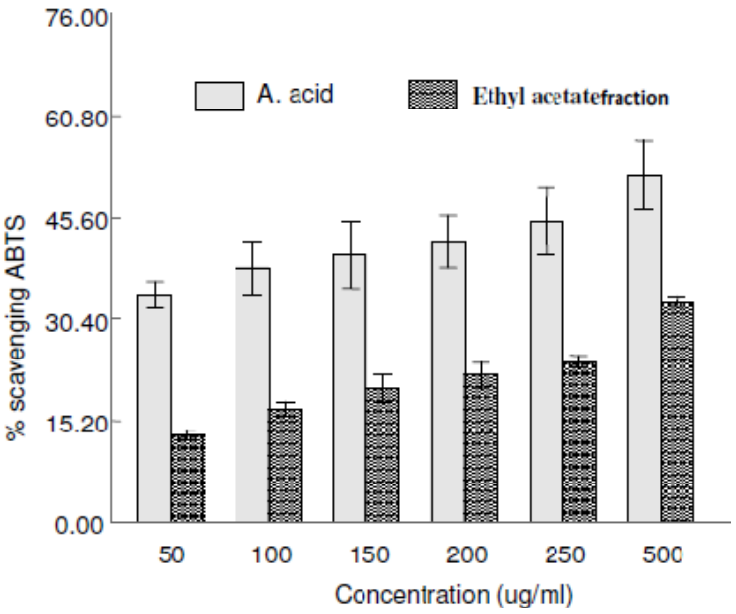

Fig.5.Percentage scavenging of ABTS radicals by various concentrations of ascorbic acid and ethyl acetate fraction of *Lonicera quinquelocularis***.** The data is the Mean Value ± Standard Deviation of three replicates

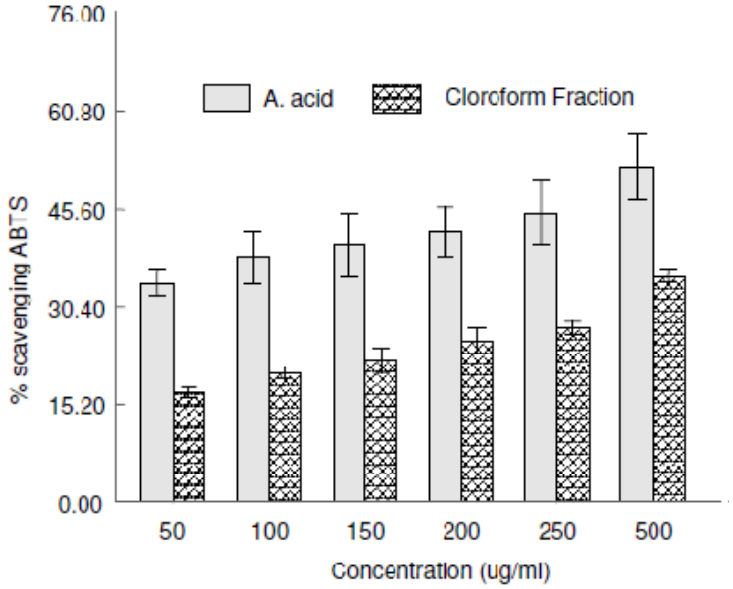

Fig.6. Percentage scavenging of ABTS radicals by various concentrations of ascorbic acid and chloroform fraction of *Lonicera quinquelocularis***.** The data is the Mean Value ± Standard Deviation of three replicates.

Cytotoxicity of a substance accounts for its anticancer and antitumor properties. The three different fractions of *Lonicera quinquelocularis* were tested for cytotoxic properties through brine shrimp assays. The results obtained showed that all the above mentioned three fractions have

lethal effects on the brine shrimp, means that all these three fractions have cytotoxic properties. It was noted from the results that with an increase in the concentrations of each of the three fractions, there was also increased in lethal effects. (Figure7-9)

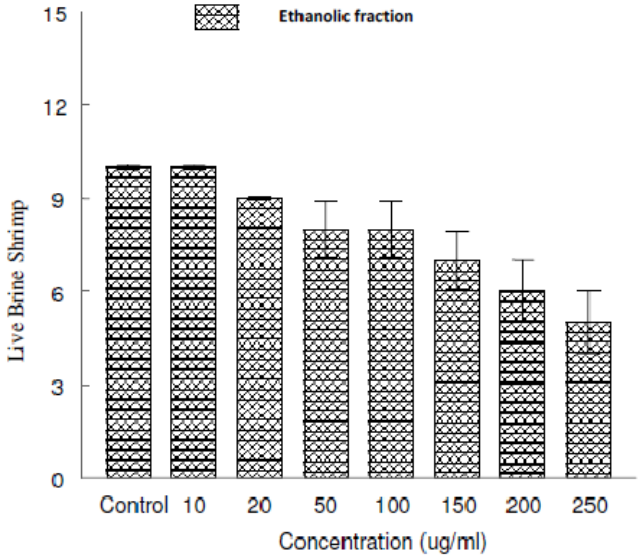

Fig 7.survival rate of brine shrimp at various concentrations of ethanolic fraction of *Lonicera quinquelocularis***.** The data is the Mean Value of three different experiments ± Standard Deviation.

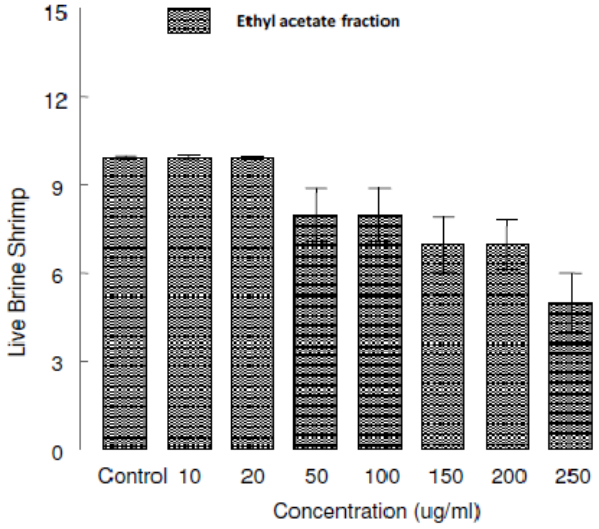

Fig.8. No of living brine shrimps at various concentrations of ethyl acetate fraction of *Lonicera quinquelocularis*. The data is the Mean Value of three different experiments ± Standard Deviation.

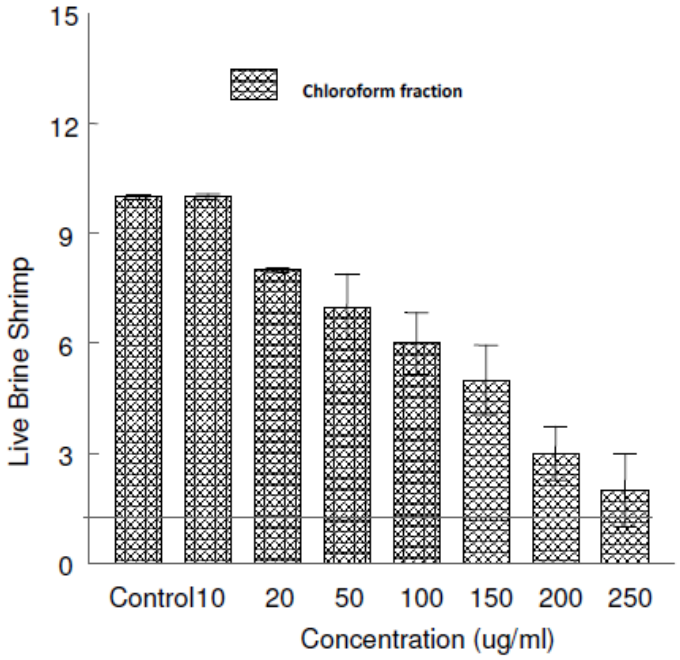

Fig.9. No of living brine shrimps at various concentrations of chloroform fraction of *Lonicera quinquelocularis*. The data is the Mean Value of three different experiments ± Standard Deviation.

Discussion

Free radicals such as ROS and NOS are highly unstable substances and beyond a specific level they cause oxidative stress which is associated with life threatening diseases including cancers, cardiovascular diseases, inflammation neurodegeneration (Wiseman and Halliwell, 1996), a number of free radicals generated during normal metabolism of the body.Due to high reactivity and instability free radicals, attack on bio-molecules including DNA, lipids, proteins and carbohydrates, causing injury and severe diseases (Ivanova*et al.,* 2000).Antioxidant prevents oxidative damages to various body tissues by scavenging free radicals. Medicinal plants are the richest sources of natural anti-oxidant. Man had utilized the benefits of plants and took its advantages by using them for curing various diseases i.e diabetics, alzehmirs, Parkinson, ischemia etc caused due to free radicals. Medicinal plantshave been improved in alleviating illness and promoting human health. In the present study, *Lonicera quinquelocularis* plant showed free radical scavenging ability. The three different fractions i.e. ethanolic, chloroform and ethyl acetate fractions of *Lonicera quinquelocularis* were found very active against DPPH (Figure 1-3) and ABTS free radicals (Figure 4-6). In comparison, ethanolic and ethyl acetate fraction showed highest free radical scavenging property than chloroform fraction, Fig (Figure 1-6).Our results show similarity with the finding of Hagerman *et al.* (1998) and Falleh *et al.* (2008).

Many medicinal plants and their purified ingredients have revealed as useful therapeutic agents. It is estimated that more than 200 types of different cells are affected by cancer. Both in the developed and developing countries of the world, cancer which is one of the most life

threatening diseases and serious public health problem has given much massive damage to the society (Hanahan et al., 2000; Gennari et al., 2007).In the treatment of cancer, there are many difficulties but the drug resistance, toxicity, and low specificity are the most serious one. The synthetic drugs are failed in controlling of cancer or tumors due to the severe toxicity and adverse side effects. The conventional treatments are not fruitful against cancer therapy. Therefore, use of herbal medicine is highly recommended for cancer therapy. The ethanolic, ethyl acetate and chloroform fractions of *Lonicera quinquelocularis* plant were subjected to Brine shrimp assay. From the results it was concluded that all fration had cytotoxic effects (Figure 7-9). It may due to the various compounds present in each fraction that caused cytotoxicity. Zaidi*et al.* (2006) reported that methanolic fraction of *Arceuthobium oxycedri* exhibited higher cytotoxicity for brine shrimps at high dose which are in friendship to our results where's it showed dose dependent lethality (Figure 7-9).

Conclusion

In conclusion, the results of present study revealed that various fractions of *Lonicera quinquelocularis* plant possess bioactive constituents that exhibit antioxidant and anticancer properties that can be used for the treatment of free radical related diseases and also in formulation of anticancer drugs. Further, investigations are recommended for isolation and purification of these biological active constituents.

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