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Antioxidant and anticancer activity of methanolic extract from the tissue of *Catla catla* collected from Mettur Dam, Tamil Nadu, India

Kalpana Devi K K[1*], Abareethan M[2], Solaiappan A[1]

[1]Government Arts College for Men, Krishnagiri – 635001, Tamil Nadu, India

[2]Research Department of Zoology, Government Arts and Science College (Autonomous), Salem, – 636007, Tamil Nadu, India

*Corresponding Author:
K.K. Kalpana Devi
Head and Assistant Professor
Department of Zoology
Government Arts College for Men
Krishnagiri – 635001

Abstract

The present study was to evaluate the antioxidant activity of methanolic extarct from the tissue of *C. catla*. The methanolic extract showed the total antioxidant activity of 18.21% - 51.90% at 50 - 250µg/ml concentration and the DPPH radical scavenging activity of 17.98% - 49.78% at 50 - 250µg/ml concentration. In addition, the superoxide radical scavenging activity of methanolic extract showed 19.19% - 55.01% at 50 - 250µg/ml concentration and the hydroxyl radical scavenging activity of methanolic extract presented 15.34% - 53.67% at 50 - 250µg/ml concentration. The anticancer activity of methanolic extract recorded 14.79 – 46.35% at 50 – 250µg/ml concentration against A549 cell line. Among the antioxidant and anticancer activities of the methanolic extract may helpful to prevent the oxidative stress and used in pharmacological industries in future.

Keywords: Methanolic extract; *C. catla*; Antioxidant; Anticancer.

INTRODUCTION

Antioxidant plays a significant role in the defense system of organisms against free radical. Free radicals are defined as atoms or molecules to facilitate have one or more unpaired electron and they are harmful byproduct generated during cellular metabolism, which could initiate oxidative damage to the body [1]. It is well known that oxidative damage of biological molecule in human is involved in the degenerative or pathological process such as aging, Chronic Heart Disease (CHD) and cancer. Reactive Oxygen Species (ROS) and free radicals play an important role in many diseases such as cancer, gastric ulcers, Alzheimer, arthritis and ischemic reperfusion [2].

The formation of free radicals such as superoxide anion radical (O2-) and Hydroxyl radical (OH) is an inevitable effect during normal metabolism of aerobic organisms. These radicals are very unbalanced and rapidly respond with other groups or substances in the body, leading to cell or tissue injury [3]. Several synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA), Butylated Hydroxyl Toluene (BHT), α-tocopherol and Ter-Butylhydroquinone (TBHQ) are commercially available and currently used. However, the substance may be inappropriate for chronic human consumption because recent publication has mentioned their possible toxic properties for human health and environment [4]. Hence, the development of alternative antioxidant from natural origins has attached considerable attention and is thought to be desirable development.

Antioxidant found in food and supplements supports human through essential antioxidative production to keep up the inner oxidation level by a variety of processes such as In-situ regeneration of antioxidant molecules (vitamins and enzymes) or instantly neutralization of oxidative compound [5]. In human diet in post- industrial countries has changed in the past few decades towards fast-food preparation and pre-made meals, high in

fat and low in antioxidant compound [6]. The growing overload of pre-oxidant compounds more than antioxidants show to lead to an enlarged threat of physiological disorders for the reason that of probable harm caused by cellular oxidation of very important bio-molecule contains protein, lipid, DNA etc. Hence the present study, to evaluate the antioxidant anticancer activity of methanolic extract from the tissue of *C. catla*.

MATERIALS AND METHODS

Collection of fish

The fish *C. catla* was collected from Mettur dam, Tamil Nadu, India. The material was washed with tap water and distilled water and shade dried. After the materials were cut into small pieces and powdered in a mixer grinder.

Preparation of methanolic extract

The extract preparation was done followed by the method of Seedevi *et al*. [7]. Briefly 100g of powder samples were extracted in 1L of methanol thrice by soaking for overnight at room temperature. The resulting solution was filtered through Whatman No. 1 sterile filter paper. The extracts from three consecutive soaking were pooled and evaporated under reduced pressure by using rotary evaporator at 40°C. The concentrated extract (methanolic extract) obtained was finally dried under vacuum pressure in desiccators and stored in refrigerated condition at -20°C until used for further study.

Total antioxidant activity

The total antioxidant activity was carried out based on the method described by Seedevi *et al.* [8]. 2 ml of sample at various concentrations (50 - 250µg/ml) was mixed with 1 ml reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min under water bath. After the mixture cooled to room temperature, the absorbance of each solution was measured at 695nm against a blank. The L-ascorbic acid and BHA were used as standards.

Scavenging ability on DPPH radicals

The DPPH free radical scavenging activity of methanolic extract was determined by following the method of Seedevi *et al.* [8]. 0.1mM solution of DPPH was prepared in 100% methanol, and 1 ml of this solution was added to 4 ml of sample in 40% methanol at various concentrations (50 - 250 µg/ml). The mixture was shaken vigorously and incubated for 15 min at 30°C in the dark. The reduction of the DPPH radical was measured by continuous monitoring of the decrease of absorption at 517 nm. The L-ascorbic acid and BHA were used as standards and the DPPH scavenging effect was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = 1 - \frac{\text{A sample} - \text{A blank}}{\text{A control}} \times 100$$

Superoxide radical scavenging assay

The superoxide scavenging ability of methanolic extract was assessed by the method of Nishikimi *et al.* [9]. The reaction mixture, containing sample (50 - 250µ/ml), PMS (30 mM), NADH (338 mM) and NBT (72 mM) in phosphate buffer (0.1 M pH 7.4) was incubated at room temperature for 5 minutes and the absorbance was read at 560 nm against a blank. The capability of scavenging the superoxide radical was calculated using the following equation:

$$\text{Scavenging ability (\%)} = \frac{(\Delta A560 \text{ of control} - \Delta A560 \text{ of sample})}{\Delta A560 \text{ of control}} \times 100.$$

Hydroxyl radical scavenging assay

The reaction mixture containing sample (50 - 250µg/ml) was incubated with deoxyribose (3.75 mM), H_2O_2 (1 mM), $FeCl_3$ (100 mM), EDTA (100 mM) and ascorbic acid (100 mM) in potassium phosphate buffer (20 mM, pH 7.4) for 60 minutes at 37˚C [10]. The reaction was terminated by adding 1ml of TBA (1%, w/v) and 1ml of TCA (2%, w/v) and

then the tubes were heated in a boiling water bath for 15 minutes. The contents were cooled and the absorbance of the mixture was measured at 535 nm against reagent blank. Decreased absorbance of the reaction mixture indicated decreased oxidation of deoxyribose.

Anticancer activity

The anticancer activity of the methanolic extract was examine against lung carcinoma (A549) cell line by using MTT assay as described by Seedevi *et al.* [11]. Vero cells were seeded (3×10^4/well) in 96-well plates in 100µl of growth medium (MEM) containing 10% FCS mixture in each well incubated at 37°C in a 5% CO_2 incubator. After 24 h of monolayer cell cultivation, the medium was removed and replaced by a 100µl of varying concentrations (25 - 250µg/ml) of the sample in MEM medium containing 2% FCS in respective wells. Control cells were maintained in MEM medium containing 2% FCS incubated at 37°C in a 5% CO_2. After 72 h of incubation, 20µl of MTT (5mg/ml) in PBS solution/well were added and incubated at the above said condition for 4 h, and then were observed for the crystal formation. The medium was replaced by 100µl of DMSO solution in each well and the Optical Density (OD) of each well was measured by using an Elisa reader at 620 nm.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) using SPSS Software 16.0 followed by Duncun's multiple range test (DMRT). Results were expressed as mean ± S.D. P values <0.05 were considered as significant.

RESULTS

Total antioxidant activity

In the total antioxidant activity of methanolic extract showed exhibited moderate to high antioxidant activity in the range of 18.21% - 51.90% at 50 -250 µg/ml concentration (Fig. 1). The maximum of 51.90% inhibition was observed at the concentration of 250µg/ml concentration of methanolic extract. Antioxidant activity of methanolic extract was directly

proportional to the amount of concentration used. The antioxidant activities of BHA and L-ascorbic acid were 80.01% and 86.09% respectively at 250µg/ml concentration respectively.

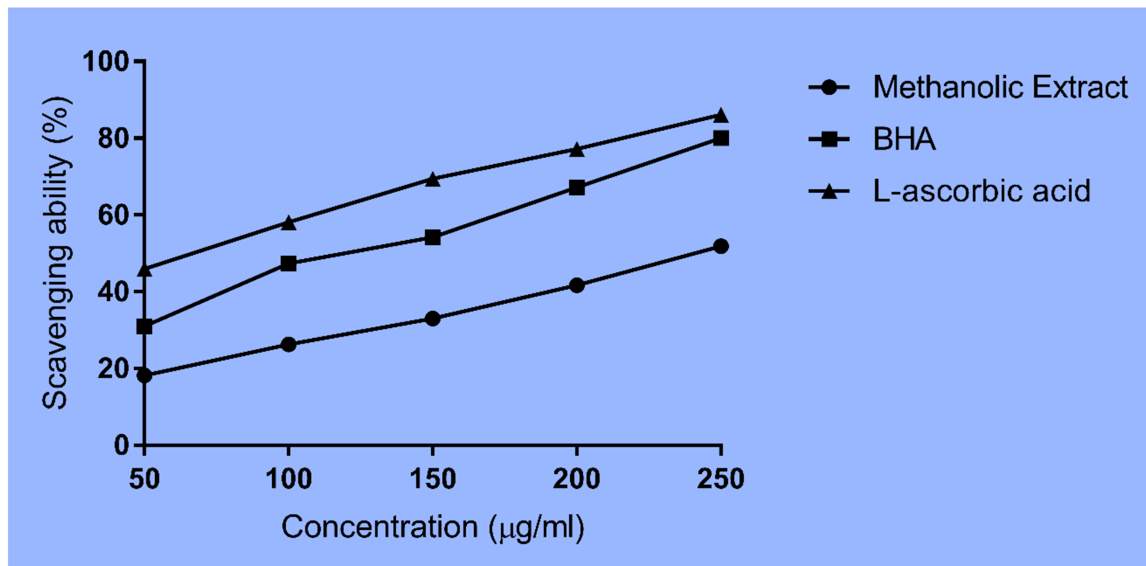

Fig. 1. Total antioxidant activity of methanolic extract from the tissue of *C. catla*

Scavenging ability on DPPH radicals

The scavenging ability of methanolic extract on DPPH radicals showed 17.98% - 49.78% at 50 - 250 µg/ml concentration (Fig. 2). The maximum inhibition of 49.78% was observed at the highest concentration of 250 µg/ml of methanolic extract. However, the BHA and L- ascorbic acid showed moderate to high scavenging abilities of 80.22% and 84.38% at 50-250 µg/ml concentration respectively. In the DPPH radicals activity of methanolic extract showed a moderate scavenging activity, when increase the concentration showed increase the scavenging ability.

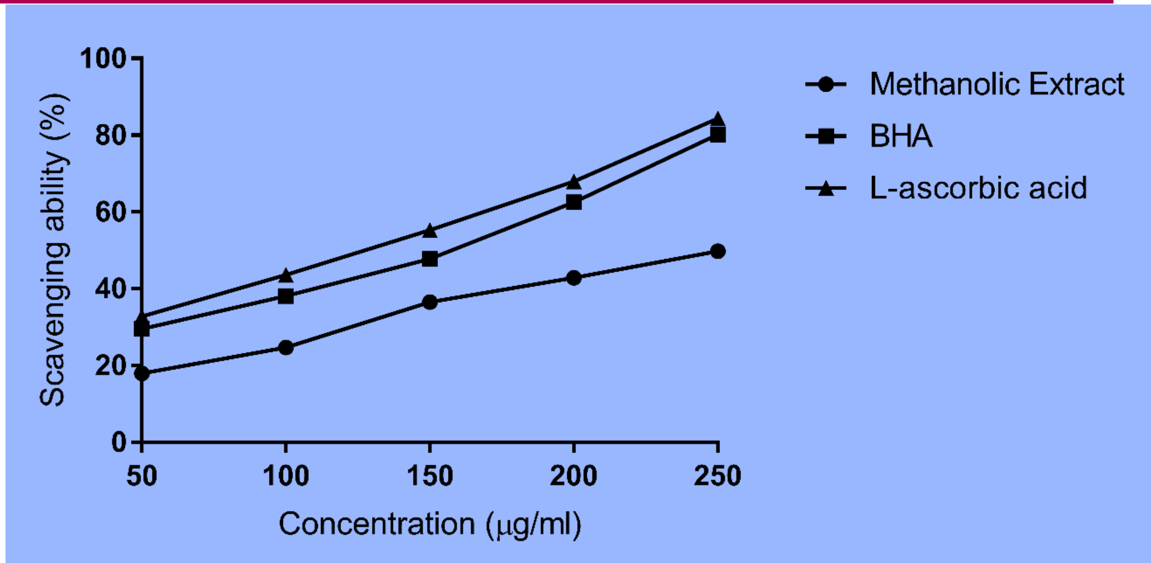

Fig. 2. DPPH scavenging activity of methanolic extract from the tissue of *C. catla*

Superoxide radical scavenging activity

The inhibitory effects of methanolic extract on superoxide radicals were determined, which was found concentration dependent (Fig. 3). The scavenging effect of methanolic extract on superoxide radical was 19.19% - 55.01% at 50 -250µg/ml concentration respectively to significant at all concentrations. The methanolic extract showed maximum superoxide radical scavenging activity of 55.01% at 250µg/ml concentration and minimum of 19.19% 50µg/ml concentration. Whereas, the BHA and L- ascorbic acid showed the inhibition of 80.02% and 81.39% at the maximum concentration of 250µg/ml concentration respectively.

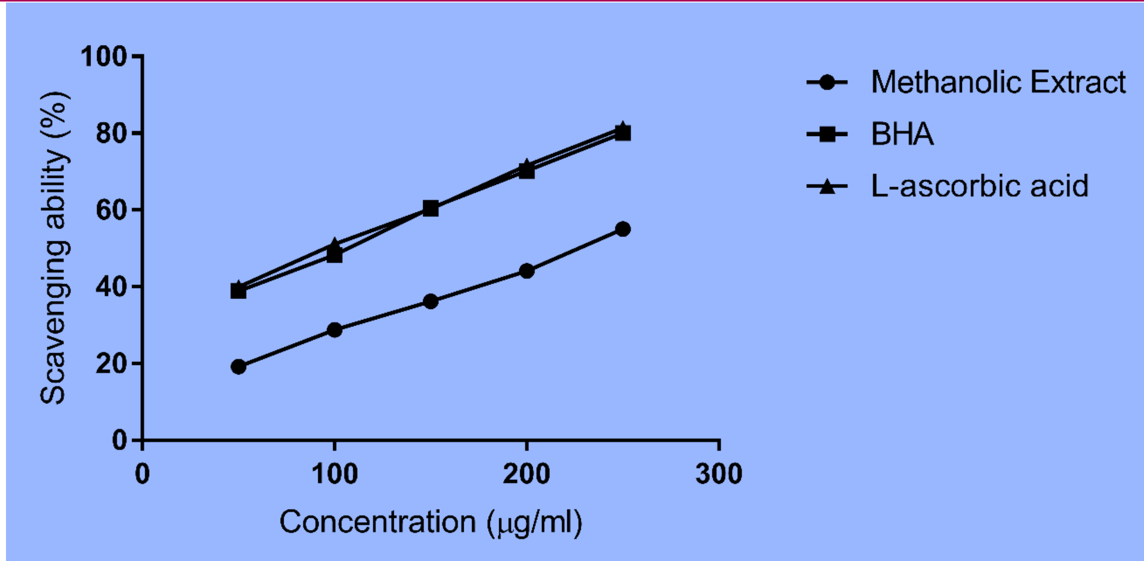

Fig. 3. Superoxide radical scavenging activity of methanolic extract from the tissue of *C. catla*

Hydroxyl radical scavenging activity

The effect of methanolic extract on oxidative damage induced by hydroxyl radical at different concentrations (50 – 250µg/ml) was found between 15.34% and 53.67% respectively. The hydroxyl radical scavenging activity of the methanolic extract showed a maximum of 53.67% inhibition at the highest concentration of 250µg/ml and minimum of 15.34% 250µg/ml concentration (Fig. 4). The hydroxyl radical scavenging activities of BHA and L- ascorbic acid were 73.98% and 77.04% at 250µg/ml concentration.

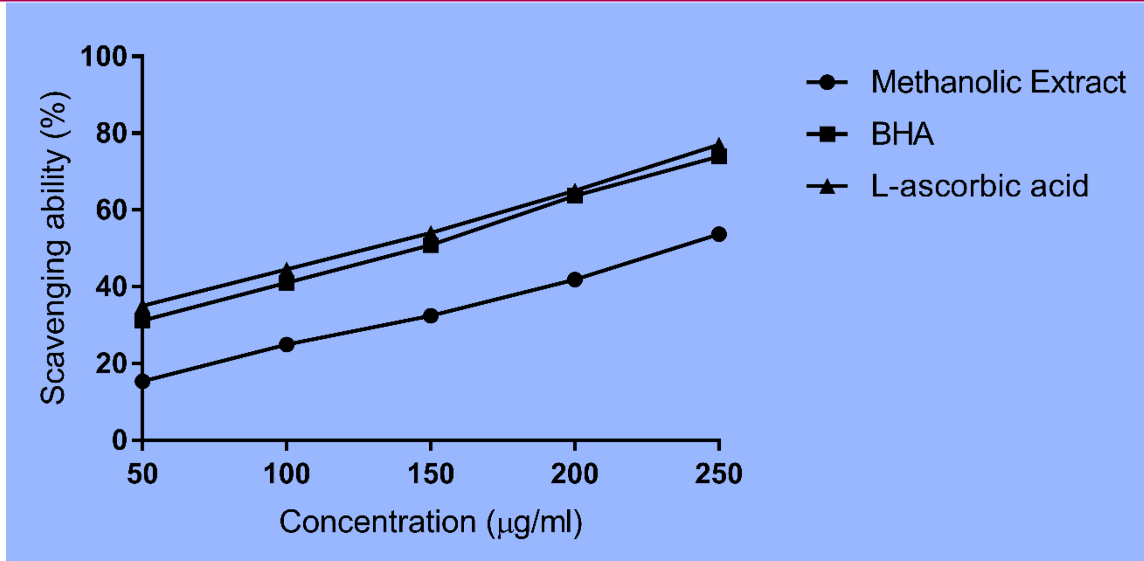

Fig. 4. Hydroxyl radical scavenging activity of methanolic extract from the tissue of *C. catla*

Anticancer activity

In the present study, the anticancer activity of methanolic extract from the tissue of recorded 14.79 – 46.35% at 50 – 250µg/ml concentration against A549 cell line (Fig. 5). The highest cancer inhibition was recoreded 46.35% at 250µg/ml concentration. The anticancer activity of methanolic extract showed dose depend activity; whereas in increasing concentration of methanolic, the activity level also increased.

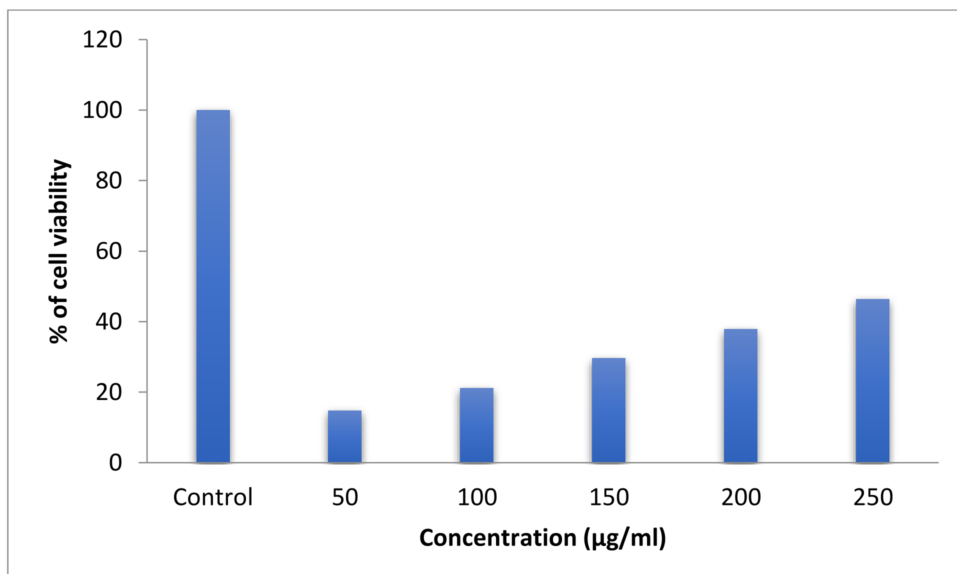

Fig. 5. Anticancer activity of methanolic extract from the tissue of *C. catla*

DISCUSSION

Biological antioxidants are necessary components of the tissues and cells of living organisms, in which free-radical auto-oxidation processes that occur in the presence of molecular oxygen are maintained at a steady-state level of normal physiological concentrations of antioxidants [12]. The antioxidant activity of extracts depends on the type and polarity of extraction solvent, the isolation procedures, purity and identity of antioxidant active components from the raw materials. The use of synergistic mixtures of antioxidants allows a reduction in the concentration of each substrate and also increases the anti-oxidative effectiveness as compared with the activity of each separate compound [13]. Many findings showed that different solvent extract extracted from marine organisms present significant antioxidant activity in *in-vitro* conditions [14, 15].

In the present study, the total antioxidant activity of methanolic extract from the tissue of *C. catla* showed maximum of 51.90% inhibition was observed at the concentration of 250µg/ml concentration. In the present study, the total antioxidant activity of methanolic extract from the tissue of *C. catla* was maximum in lower concentration when compared to the previous study of the ethanolic extract from *Teucrium barbeyanum* showed total antioxidant activity of 0.57% at the maximum concentration of 0.004µg/ml and polysaccharide from *Gardenia jasminoides* was found as 97.10% at 4 mg /ml concentration [16]. The three polysaccharide fractions (RBP1, RBP2 and RBP3) from Rice bran recorded the total antioxidant activity of 90.1, 18.2 and 2.5% at the concentration of 2mg/ml [17]. In the present study, the results indicated that the methanolic extract is potent total antioxidant capability it may be the presence of hydroxyl functional groups.

The scavenging ability of methanolic extract on DPPH radicals showed 17.98% - 49.78% at 50 - 250 µg/ml concentration. In the present study, the DPPH radical scavenging

activity of methanolic extract showed a moderate scavenging activity and also lower when compared the ethanolic extract from the leaf of *Ocimum basillicum* and *Alpinia calcarata* were 96.18 and 94.63% 100mg/ml at concentration[18]. Mak *et al*. [19] statement the DPPH free radical scavenging activity of ethanol and water extract from the flower hibiscus (*Hibiscus rosa-sinensis*) and Cassia (*Senna bicapsularis*) were 3.08±0.1 & 97.35± 0.6 and 99.51±0.2 & 96.51± 0.3 at the concentration of 2349.06 and 2883.23µmoles/100g. The DPPH free radical scavenging activity of methanolic extract from the orchid *Sarcanthus pauciflorus* found to be 87% at 400µg/ml concentration [20]. The previous reports on various flower extracts, which have been shown to exhibited high level of antioxidant activities [21, 22].

Superoxide radical scavenging activity of the methanolic extract recorded 19.19% - 55.01% at 50 -250µg/ml concentration respectively. The superoxide radical activity of methanolic extract from *C. catla* was higher when compared to the methanolic extract from the leaf *Justicia wynaadensis* illustrated 74.5% at 1mg/ml concentration [23]. Hefnawy *et al*. [24] depicted the superoxide anion-scavenging activity of rice bran polysaccharide fractions (RBP1) was 82.3% at 1mg/ml concentration. Guo *et al*. [25] claimed the superoxide radical scavenging effect of ultrasonic-assisted polysaccharide from *Cyclina sinensis* showed 50.44 ± 3.04% - 89.98 ± 0.79% at the concentration of 2.5 to 30mg/ml concentration. When the results are compared, the methanolic extract had stronger scavenging activity for superoxide radical ability. The ability to appearance hydrogen bond declines stridently and the hydroxyl and amino groups are activated, so this is supportive to the reaction with superoxide anion [26].

Hydroxyl radical is a most reactive free radical, can cause the ageing of human body and some diseases, the hydroxyl radical can induce significant damage to adjacent biomolecules. Hence removing hydroxyl radicals is important for antioxidant defense in

living cell systems. In the present study, the methanolic extract showed the hydroxyl radical scavenging activity of 15.34% and 53.67% at 50 – 250µg/ml respectively. The hydroxyl scavenging activity of the ethanolic extract of *G. asiatica* proved 72.01% at 120ug/ml concentration [27]. Likewise Harsha *et al*. [28] reported the hydroxyl scavenging activity of crude protein *Leucas linifolia* (CPLL) was 78 % at 150ug/ml concentration. In addition Sivaperumal *et al*. [29] noticed the hydroxyl radical scavenging effect of purified fraction of haemolymph protein from *O. macrocera* was 91.68 % at the concentration of 100µg/ml. Maharani *et al*. [30] stated the hemolymph protein from *Doclea cravis* proved the hydroxyl radical scavenging activity of 86.11 ± 3.4% and 74 ± 53.8 at 50 and 100mg/ml concentration.

In the present study, the anticancer activity of methanolic extract from the tissue of recorded 14.79 – 46.35% at 50 – 250µg/ml concentration against A549 cell line. The anti-cancer activity of methanolic and aqueous extracts of *Euphorbia tirucalli* showed 53% of inhibition at 50µg/ml concentration against human pancreatic cancer cell line (MiaPaCa2) [31]. The anticancer activity of methanolic extract may depend greatly on their chemical composition, and degree of branching which may be the reason for the difference in the anti-proliferative activity.

CONCLUSION

In conclusion, the present study, the methanolic extract from the tissue of *C. catla* showed good *in vitro* antioxidant activity, especially total antioxidant activity, DPPH radical scavenging activity, superoxide radical scavenging activity and hydroxyl radical activity, and anticancer activity. Hence, the antioxidant and anticancer activities of the methanolic extract may helpful to prevent the oxidative stress and used in pharmacological industries in future.

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