function in multiple ways as follows: (i) restricting the expression of free radicals; (ii) reducing the activities of free radicals-producing enzymes; and (iii) enhancing the expression/activities of antioxidant enzymes. The reactive oxygen species (ROS)/ reactive nitrogen species (RNS) must be degraded in order to protect human health by promoting enzymatic and non-enzymatic defense mechanisms[6]. The major enzymatic antioxidant systems in the human body are glutathione peroxidase, superoxide dismutase, peroxidase, and catalase. The non-enzymatic antioxidant system includes vitamin A, vitamin C, vitamin E, and reduced glutathione. According to the World Health Organization (WHO), approximately more than half of the world's population is affected by many diseases and disorders due to inadequate health care services[6,8]. Presently, the synthetic antioxidants available for scavenging free radicals show low solubility, a negative impact on human health, and ineffective antioxidant activity. Scientists have focused on replacing these poor synthetically produced antioxidants with natural antioxidants from plants and food. Hence, there is an emergence of a strategy to use medicinal plants as potential antioxidants for scavenging free radicals produced by the human body in order to reduce tissue injury[9,10].

Medicinal plants have been used for various purposes in traditional points from the beginning of human civilization. Herbal plants play a prominent role in several medicinal systems including Unani, Siddha, and Ayurvedha[11,12]. Nearly, more than 50,000 plants have been recorded for their therapeutic potential across the globe. For the past few decades, medicinal plants have been well known to produce compounds from their various parts like root, stem, leaf, flower, seed, and fruits and it plays a crucial role in pharmacology and drug discovery. Most of the developing countries still depend on traditional medicinal plants for a cure. Not surprisingly, almost all medicinal plants contain therapeutic compounds as an active principle to treat various diseases and disorders. The secondary metabolites or phytochemicals produced by plants have attracted much attention for their therapeutical potential against several diseases and disorders[6].

Couroupita guianensis Aubl. is a popular tree with potential medicinal value among tribal communities. More than 30 species are included in the genus of *Couroupita* and it has been recognized and declared as a State flower by the Puducherry government[13]. It belongs to the Lecythidaceae family with various medicinal and ornamental properties. The tree is commonly referred to as a cannonball tree due to the appearance of fruits as cannonball shapes. This tree is mainly contemplated as a sacred tree known for Lord Shiva. It has large applications in the aspect of ethnomedicine as follows: the younger leaves used to cure toothache; juice from its leaves fights against bacterial and fungal pathogens; fruits also have antimicrobial and wound healing activities; leaf, fruit, and bark are used to treat malaria; and seeds are attributed to cure fertility issues. This property of *C. guianensis* led to the development of many indigenous medicinal products in Ayurveda, Unani, Siddha, Chinese, and others[14-16]. Especially, the leaves of the plant have antioxidant, anthelmintic, immunomodulator, and anti-nociceptive activities[15]. Earlier studies have reported that the phytoconstituents present in the leaves and flowers of *C. guianensis* are as follows: alkaloids, sterols, flavonoids, glycosides, triterpenes, tannins, indirubin, isatin, α-amirin, couroupitine, and β-amirin. Among them, isatin is one of the major alkaloid compounds that possesses several medicinal properties including, antioxidant, antimicrobial, antitumor, and anti-inflammatory. These components in the plant system may act as a driving force for the pharmacological and therapeutic properties of the plant, *C. guianensis*[14,15,17]. The present study was planned to evaluate *C. guianensis* Aubl. for its antioxidant potential using goat liver slices acts as *in vivo* simulated *in vitro* system.

Materials and Methods

Collection of plant materials

The *C. guianensis* Aubl. plant leaf and flower samples were procured from the local area, Coimbatore district (Perur temple), and the plant specimens were identified, certified and the voucher specimen number (2430) was deposited at the Botanical Survey of India, Southern Circle, Coimbatore (Fig. 1).

Fig. 1 — A leaf and flower of *C. guianensis*

Preparation of plant extract

Alkaloid extract and the methanolic extract of flower and leaf samples were taken to investigate the antioxidant status using an *in vivo* simulated *in vitro* model and prepared by following procedures.

***Alkaloid extraction* (*Harborne, 1973*)[18]**

The plant sample was cleaned with tap water followed by distilled water. 5 g of leaf and flower sample of *C. guianensis* was taken and crushed by adding 50 mL of 10% acetic acid in ethanol using a mortar and pestle. The crushed sample was vigorously vortexed and allowed to stand for about 4 h to get the filtrate. The obtained filtrate was evaporated to one-quarter of its original volume by keeping it on a hot plate under 60°C. Alkaloids were precipitated by adding the concentrated ammonium hydroxide drop by drop. The precipitate was centrifuged for 5 min at 2500 rpm to collect the alkaloid residue. The pellet was washed three times with 1% ammonium hydroxide solution and the residue contained the alkaloid, which was dried, weighed, and dissolved in a minimal volume of chloroform or ethanol.

Preparation of methanol extract

Methanolic extract of leaf and flower of *C. guianensis* was obtained by maceration of the sample (5 g) with 10-20 mL of methanol and it was subjected to centrifugation at 5000 rpm for about 10 min. The resultant mixture was filtered with Whatman no.1 filter paper to collect the extract and the collected filtrate was kept overnight for evaporation. The final residue obtained was weighed and then dissolved in a minimal volume of dimethyl sulphoxide.

***In vitro* model - goat liver slices**

The liver was the organ choice to conduct the study which is the main metabolic organ where major metabolic waste products and xenobiotics are cleared. The goat liver was cut into thin slices and taken in PBS/ HBSS solution. Hydrogen peroxide is the potent free radical and it was used as the oxidant to induce oxidative stress in the liver slices.

The following groups were set for the assay:

Group 1 – Untreated liver slice (Negative control)
Group 2 – Liver slice + Hydrogen peroxide (Positive control)
Group 3 – Liver slice + Alkaloid extract of flower
Group 4 – Liver slice + Alkaloid extract of leaf
Group 5 – Liver slice + Methanol extract of flower
Group 6 – Liver slice + Methanol extract of leaf
Group 7 – Liver slice + Alkaloid extract of flower + Hydrogen peroxide
Group 8 – Liver slice + Alkaloid extract of leaf + Hydrogen peroxide
Group 9 – Liver slice + Methanol extract of flower + Hydrogen peroxide
Group 10 – Liver slice + Methanol extract of leaf+ Hydrogen peroxide

Pre-treatment of liver slices

The goat liver bought from the slaughterhouse is soaked in cold PBS/ HBSS in a beaker. Carefully remove the white fatty layer and cut very thin slices using a sterile scalpel. To each group, 250 mg of liver and 1 mL of PBS/ HBSS was added. Following this, 20 µL of plant extract and 5 µL of 0.1 m hydrogen peroxide were added to corresponding groups.

The above-treated groups were allowed to incubate at 37°C for 60 min in the dark and the tissue liver homogenate was prepared using Teflon homogenizer. The homogenate was subjected to centrifugation at 1500 rpm for 5 min and the supernatant was used for further analysis of enzymatic and non-enzymatic antioxidants.

Enzymatic antioxidant analysis

Estimation of superoxide dismutase* (*SOD*) *activity

The activity of SOD was quantified by the method of Misra and Fridovich, 1972[19]. In this method, 300 µL of reagent mixture (containing 50 mM potassium phosphate buffer (pH 7.4), 45 µM methionine, 5.3 µM riboflavin, 85 µM nitroblue tetrazolium, 20 µM potassium cyanide) and 300 µL of the sample was added in the incubation medium and was made up to 3 mL with water as the final volume. The experimental tubes were kept in an aluminium foil-lined box under 25°C maintained with 15 W fluorescent lamps. With the exposure to light for 10 min, reduced NBT was measured using a spectrophotometer at 600 nm. The maximum reduction of NBT was evaluated in the tube without inoculation of an enzyme. One unit of enzyme activity is defined as the enzyme reaction, which gives 50% inhibition of NBT reduction in one minute under the assay conditions and is expressed as specific activity in units.

Estimation of catalase* (*CAT*) *activity

The activity of catalase was assayed spectrophotometrically at 230-250 nm by the method of Luck (1974)[20]. The experimental method involves

pipetting out 3 mL of H_2O_2 phosphate buffer into the dry test tubes. This was mixed in 10-40 µL of sample with the glass or plastic rod flattened at one end. The time needed for a decrease in the absorbance by 0.05 is noted and the value was taken for further calculations. If 't' was found to be more than 60 seconds, repeat the measurement with an increased concentrated solution of the sample. The concentration of hydrogen peroxide using the extinction coefficient of 0.036 micromole/ milliliters was calculated.

Estimation of peroxidase* (*POD*) *activity

Reddy *et al*.[21], method was used to evaluate the activity of peroxidase. 0.05 M pyrogallol solution (3 mL) was added to the 100 µL of sample in the test tubes. The spectrophotometer was adjusted to '0' at 400 nm. In a test cuvette, 0.5 mL of 1% hydrogen peroxide was added and change in the absorbance was read at 430 nm every 30 seconds up to 3 min.

Estimation of glutathione - s - transferase* (*GST*) *Activity

The activity of GST was analyzed by the method of Beutler, 1984[22]. 0.1 mL of GSH was added to 0.1 mL of CDNB. The volume was made up to 3 mL using 0.1M phosphate buffer. Added 0.1 mL of sample with the mixture and it was vortexed for a few minutes. The change in absorbance at 340 nm was monitored for every 30 seconds up to 3 min. Blank was conducted without any enzyme source. GST activity in the extract is expressed as a micromole of CDNB - GSH conjugate/ min/ mg protein.

Estimation of glutathione peroxidase* (*GPx*) *activity

Paglia and Valentine method was applied to analyze the activity of glutathione peroxidase[23]. 0.02 mL of phosphate buffer was pipetted out into a tube and 0.2 mL of EDTA, 0.1 mL of sodium azide and 0.2 mL of the enzyme source were added and vortexed vigorously. 0.2 mL of glutathione was added followed by the addition of 0.1 mL of hydrogen peroxide to the reaction mixture. The contents were vortexed and incubated for 10 min at 37°C along with the control tube containing all reagents without the enzyme. The reaction was arrested by adding 0.5 mL of 10% TCA solution after 10 min. Centrifuged the tubes and the supernatant were assayed for the presence of glutathione content by adding 5 mL of phosphate buffer and 0.5 mL of DTNB reagent. The color was recorded at 412 nm. The activity of glutathione peroxidase was expressed as micrograms of glutathione utilized per minute per milligram of protein.

Non-enzymatic antioxidant estimation

Estimation of vitamin A

Vitamin A content was estimated by the method of Bayfield and Coll, 1980[24]. 1 mL of the homogenate was added to 1 mL of saponification mixture containing 2N KOH in 90% alcohol. The tubes were gently swirled for about 20 min at 60°C and cooled at room temperature then added 20 mL of water and vortexed well. Vitamin A was extracted twice with 10 mL portions of petroleum ether (40-60°C). Pooled the extract and washed thoroughly with water and separated the layers by separating funnels. Sodium sulphate (anhydrous) was added to remove moisture, 1 mL of ether extracts was pipetted out to dry at 60°C and the dried tissue was dissolved in 1 mL of chloroform. The standard solution was taken into a series of clean, dry test tubes with a concentration range of 0.7 µg, and the volume in all the tubes was made up to the final volume of 0.1 mL with chloroform. Added 2 mL of TCA reagent to the tubes and vortexed. Recorded the absorbance immediately at 620 nm is a spectrophotometer. The procedure was repeated for sample tubes. Constructed a standard graph and read off concentration in the samples.

***Estimation of ascorbic acid* (*Vitamin C*)**

Ascorbic acid was evaluated by the method of Roe and Keuther (1953)[25]. Ascorbate is converted to dehydroascorbate by the treatment with activated charcoal or bromine. Dehydroascorbic acid reacts with 2, 4 - dinitrophenyl hydrazine (DNPH) to form the osazones, that is known to dissolve in sulphuric acid and produce an orange-colored solution and the absorbance was measured spectrophotometrically at 540 nm. The experimental method involves the plant sample (0.2 mL) being pipetted out into a clean and dry test tube and made up to 2.0 mL with 4% TCA solution. DNPH reagent of volume 0.5 mL and 2 drops of 10% thiourea were added to all the tubes. The tubes were kept for incubation at 37°C for 3 h. The osazones products generated in the tubes were dissolved in 2.5 mL of 85% H_2SO_4 kept in cold condition and it was added drop by drop into the tubes without disturbing the environment temperature. DNPH reagent and thiourea were added after the addition of H_2SO_4 in the control tubes. The developed color was read at 540 nm using a spectrophotometer, after completing the incubation period of 30 min at room temperature. The total content of ascorbic acid was calculated in the sample groups by the standard graph.

***Estimation of α- tocopherol* (*vitamin E*)**

Tocopherol can be estimated using the method described by Rosenberg (1992)[26]. The 3 stoppered centrifuge tubes were labeled as a test, standard, and blank, and added 1.5 mL of sample, 1.5 mL of standard, and 1.5 mL of water respectively. 1.5 mL of ethanol was added to the test and blank tubes whereas to the standard 1.5 mL of water was added. Following that, 1.5 mL of xylene was carefully added to all the labeled tubes and it was vortexed and centrifuged thoroughly. The xylene layer was separated and 1.0 mL was transferred into another dry-stoppered tube, careful precautions were to be undertaken to avoid any other ethanol or protein. 1.0 mL of 2, 2' - dipyridyl reagent was added to all the tubes and stopped and vortexed. For reading the absorbance at 460 nm, 1.5 mL of the mixture was pipetted out into the fresh tubes. 0.33 mL of ferric chloride solutions were added alone to the control tubes. The amount of vitamin E can be calculated using the formula:

Amount of tocopherol =
Reading at 520 nm – Reading at 460 nm
Reading of standard at 520 nm x 0.29 x 15

***Estimation of reduced glutathione* (*GSH*)**

Moron *et al*.[27], method was used to estimate the level of GSH. 0.1 mL of the sample was made up to 1.0 mL with 0.2 M phosphate buffer (pH 8). 2 mL of 0.6 mM DTNB (Dithio 2-Nitrobenzoic acid) solution in 0.2M phosphate buffer to the tubes was added and the yellow color was formed in the tube and the intensity was measured using a spectrophotometer at 412 nm after the reaction mixture was kept at 10 min for incubation at room temperature. A standard graphical curve of GSH was drawn with the concentration ranging from 2- 10 nanomoles of reduced glutathione in 5% TCA.

Statistical analysis

The values are given as mean±SD for the triplicates performed. One-way analysis of variance (ANOVA) was done to compare the statistical differences in the obtained results using the SigmaStat statistical package (version 3.1). If the P values are 0.05 or less, the results will be taken as statistically significant.

Results

Enzymatic antioxidants

Enzymatic antioxidants have plenty of protective roles against the deleterious effects caused by free radicals and researchers and scientists have been working to elucidate the antioxidant profile of several medicinal plants. The enzymatic antioxidants analyzed in the studies were SOD, CAT, POD, GST, and GPx.

SOD activity

Superoxide dismutase provides protection against the generated free radicals and the damage caused by the oxidants using defense mechanisms. Figure 2(a) depicts SOD activity in hydrogen peroxide treated liver slices (goat) with and without the addition of alkaloid and methanolic extracts of leaves and flowers of *C. guianensis* Aubl. While administrating the alkaloid and methanolic extract of *C. guianensis* Aubl., a remarkable increase in the activity of SOD was observed. Significant elevation of SOD was also noted in the extracts with the exposure of standard oxidant, hydrogen peroxide. The treatment with hydrogen peroxide alone showed a significant ($p<0.05$) decrease in superoxide dismutase activity. The alkaloid and methanolic extract of *C. guianensis* Aubl. caused a significant ($p<0.05$) elevation in the SOD activity in the treated groups. The SOD activity in treated groups of alkaloid fraction and methanolic extract of leaf was found to be 10.904 unit/g tissue and 9.163 unit/g tissue, respectively. While the flower extracts were shown drastic SOD activity in alkaloid fraction (11.330 unit/g tissue) than the methanolic extract (9.760 unit/g tissue). It was statistically significant when compared with the untreated group and plant group.

CAT activity

Catalase catalyzes the decomposition of H_2O_2 to molecular oxygen and water, thereby, protecting cells from the toxic effects of H_2O_2. The effect of alkaloid and methanolic extracts of *C. guianensis.* Aubl leaves and flowers on the catalase activity with the exposure of standard oxidant using the liver slices is depicted in Figure 2(b). The toxicological properties of H_2O_2 were ignored by the subsequent treatment with the alkaloid and methanolic fractions of *C. guianensis.* Aubl. The CAT activity observed in the control groups was found to be 450.6 units/g tissue (without treatment) and 245.9 units/g tissue (with treatment). The CAT activity in the hydrogen peroxide exposed goat liver slices was gradually decreased ($p<0.05$) in the extracts and along with the hydrogen peroxide treated groups. The methanolic extract was found to show the CAT activity in the hydrogen peroxide treated group

is elevated as 563 units/g tissue for the flower sample and 524 units/g tissue for the leaf sample ($p<0.05$). Besides, alkaloid fractions were shown to elevate the SOD activity in hydrogen peroxide treated flower extract was found to be 720 units/g tissue and 676 units/g tissue for the leaf sample ($p<0.005$). The leaf and flower samples showed a significant elevation ($p<0.05$) of CAT activity in the treatment groups. The activity of CAT was found to reduce in the standard oxidant treated liver slices. The leaf and flower samples by themselves significantly increased the CAT activity compared to the control.

POD activity

The peroxidase activities in the hydrogen peroxide exposed goat liver slices with and without the addition of alkaloid and methanolic extracts of leaves and flowers of *C. guianensis* Aubl. is presented in Figure 2(c). The peroxidase activity has been found to decrease exponentially ($p<0.05$) with the hydrogen

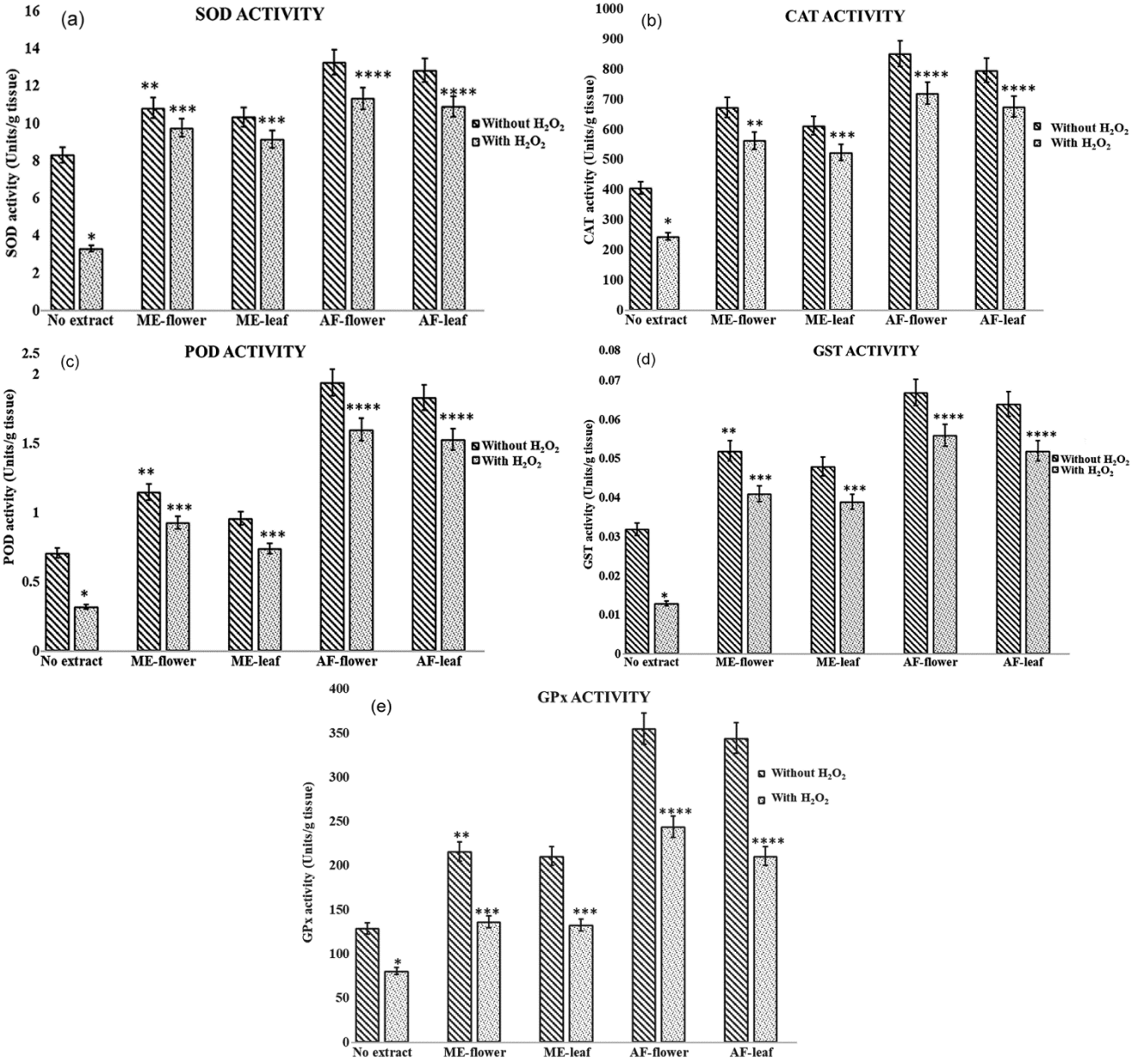

Fig. 2 — Enzymatic Antioxidants activity of leaf and flower sample of *C. guianensis* on H_2O_2 treated liver samples - A) SOD; B) CAT; C) POD; D) GST; E) GPx. Triplicates of each experiment were performed. Statistically significant $p<0.05$

ME- flower: Methanolic Extract –flower, ME- leaf: Methanolic Extract – leaf, AF- flower: Alkaloid Fractions- flower, AF-leaf: Alkaloid Fractions- leaf

peroxide treatment. Exposure to the alkaloid fraction of the flower of *C. guianensis* Aubl. caused remarkable changes (1.60 units/g tissue) in the activity of peroxidase when compared with the control (0.32 units/g tissue) in the presence of free radicals. The POD activity in the methanolic extract of leaf and flower samples was found to be 0.74 units/g tissue and 0.93 units/g tissue, respectively ($p<0.05$), statistically significant compared to the untreated group, free radical treated group and plant group) in hydrogen peroxide treated groups. The flower and leaf extracts of *C. guianensis* were found to be very effective in reversing reduced peroxidase activity induced by hydrogen peroxideto a higher extent.

GST activity

Glutathione transferases belong to a multi-group of enzymes that participate in the metabolization of various substrates including exogenous and endogenous. Figure 2(d) represents the activity of GST in the standard oxidant exposed liver slices with and without the presence of alkaloid and methanolic extracts of both leaves and flowers of *C. guianensis* Aubl. The untreated group was shown reduced glutathione transferase activity in 0.013 units/g tissue, in the presence of H_2O_2. The GST activity in the alkaloid fraction of leaf and flower samples showed 0.052 units/g tissue and 0.056 units/g tissue, respectively in hydrogen peroxide treated groups. The values were found to be statistically significant compared to all the groups treated ($p<0.05$). Methanolic extract of leaf and flower expressed the GST activity in hydrogen peroxide treated groups, 0.039 units/g tissue and 0.041 units/g tissue, respectively. The values were statistically significant compared to all the tested groups ($p<0.05$).

GPx activity

The Glutathione peroxidase activities in the H_2O_2 exposed liver slices of goat were observed with and without the presence of alkaloid and methanolic extracts of leaves and flowers of *C. guianensis* Aubl. is shown in Figure 2(e). The treatment of the goat liver slices with *C. guianensis* Aubl. leaf and flower extracts in the presence of standard free radicals showed a significant difference ($p<0.05$) in the activity of GPx. The Gpx activity in the untreated control group was found to be 129.08 units/g tissue (without H_2O_2) and 80.81 units/g tissue (with H_2O_2). Whereas the methanolic extract was found to show the GPx activity in hydrogen peroxide treated groups, 132.80 units/g tissue (leaf) and 136.40 units/g tissue (flower). The alkaloid fractions of leaf and flower showed pronounced glutathione peroxidase activity, 210.78 units/g tissue, and 244.20 units/g tissue, respectively in the hydrogen peroxide treated groups ($p<0.05$, significant compared to all the tested groups). The alkaloid flower extracts were noticed to reduce the toxic level of hydrogen peroxide in liver slices of goats than the methanolic extracts

Non-enzymatic antioxidants

The antioxidant system of a cell comprises mainly enzymatic and non-enzymatic antioxidants. In the study, vitamins A, C, and E and reduced glutathione levels were quantified in the liver slices treated with hydrogen peroxide in the presence and absence of the sample extracts of *C. guianensis* Aubl. The results obtained are discussed below.

Vitamin A level

Vitamin A level in the treatment groups treated with the standard oxidant, hydrogen peroxide was estimated with and without the addition of extracts of *C. guianensis* Aubl. is represented in Figure 3(a). The exposure to hydrogen peroxide exhibited remarkable changes ($p<0.05$) in vitamin A levels when compared with the untreated control. The *C. guianensis* Aubl. extract treatment was found to be effective in mitigating the toxicity caused by H_2O_2. The alkaloid fraction of both leaf and flower showed a remarkable vitamin A level in the treated hydrogen peroxide group was found to be 130.1 µg/g tissue and 136.4 µg/g tissue, respectively. Compared to that, the vitamin A level in the methanolic extract of the H_2O_2 group was noticed as 108.4 µg/g tissue for the leaf sample and 112.3 µg/g tissue for the flower sample ($p<0.05$, statistically significant compared to the test groups). The exposure of the liver lices to *C. guianensis* Aubl. alkaloid fraction of the flower was found to show a significant difference ($p<0.05$) from the leaf and flower extracts in the vitamin A levels.

Vitamin C level

Figure 3(b) depicts the effects of the *C. guianensis* Aubl. samples on the vitamin C level with the presence of hydrogen peroxide treatment using *in vitro*. The treatment with the liver slices to the hydrogen peroxide and the *C. guianensis* Aubl. sample extracts exhibited a significant elevation in the vitamin C level when compared with the untreated control and hydrogen peroxide alone treated

experimental groups. Gradually the levels of vitamin C were increased by the alkaloid flower extract (0.532 mg/g tissue) followed by alkaloid leaf (0.526 mg/g tissue) and methanolic extract of flower (0.489 mg/g tissue) and leaf (0.473 mg/g tissue), but hydrogen peroxide treated groups showed a noticeable ($p<0.05$, statistically significant compared to all the tested groups) depletion in the levels of vitamin C. The values were found to be 0.5 mg/g tissue (alkaloid flower), 0.49 mg/g tissue (alkaloid leaf), 0.432 mg/g tissue (methanolic flower) and 0.418 mg/g tissue (methanolic leaf).

Vitamin E level

The remarkable changes in the levels of vitamin E observed in different groups are represented in Figure 3(c). Increased elevation of vitamin E levels showed a noticeable difference ($p<0.05$) in the plant extract-treated groups. Vitamin E levels were found to increase in the alkaloid fraction of flower-treated groups (23.22 µg/g tissue) in hydrogen peroxide treated liver slices. 23.22 µg/g tissue vitamin E level was expressed by an alkaloid fraction of the flower sample in the presence of standard oxidant ($p<0.05$, statistically significant compared to all the tested groups). Methanolic extract of leaf and flower was expressed as 15.04 µg/g tissue and 17.32 µg/g tissue, respectively in the oxidant-treated groups ($p<0.05$). A decreased level of vitamin E was noticed in the group treated with the standard oxidant, hydrogen peroxide when compared to the untreated control group.

GSH level

The levels of reduced glutathione with the exposure of hydrogen peroxide on the goat liver slices and the effect of the *C. guianensis* Aubl. samples extracts were analyzed and the results are represented in Figure 3(d). Oxidant-exposed liver slices exhibited

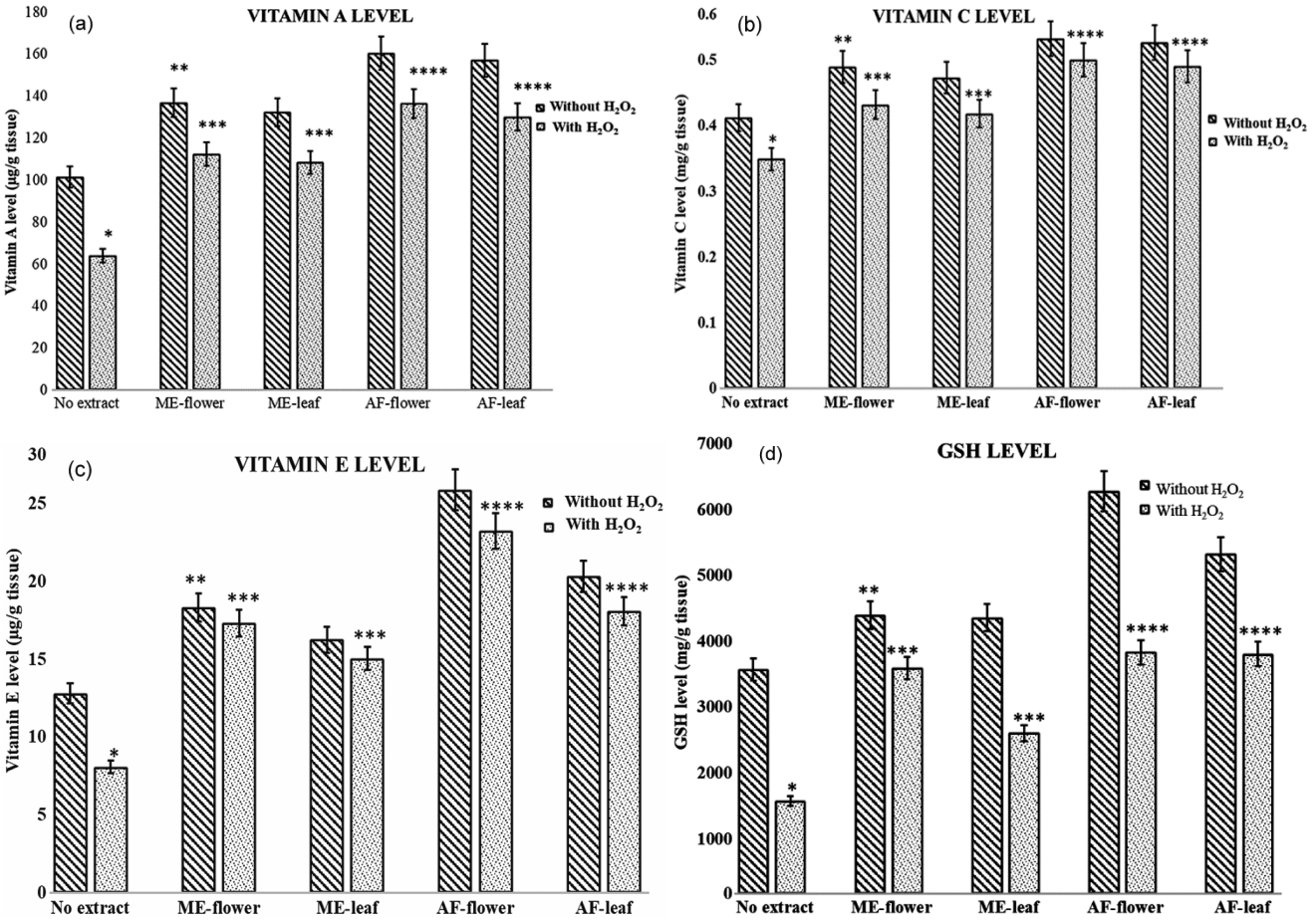

Fig. 3 — Non-enzymatic Antioxidants activity of leaf and flower sample of *C. guianensis* on H_2O_2 treated liver samples – A) Vitamin A; B) Vitamin C; C) Vitamin E; D) GSH. Triplicates of each experiment were performed. Statistically significant $p<0.05$

ME- flower: Methanolic Extract –flower, ME- leaf: Methanolic Extract – leaf, AF- flower: Alkaloid Fractions- flower, AF-leaf: Alkaloid Fractions- leaf

remarkable ($p<0.05$) changes in the reduced glutathione level in the treatment groups with the plant extracts and along with the standard oxidant. The depleting effect of standard oxidant exposure was reverted by the co-treatment with the leaf and flower extracts of *C. guianensis* Aubl. The untreated group showed a reduced GSH level in the H_2O_2 group (1408.4 mg/g tissue). The alkaloid fraction of flower treatment caused a noticeable ($p<0.05$, statistically significant compared to all the tested groups) elevation in the GSH levels (3666 mg/g tissue) when compared to the alkaloid extraction of leaf (3643.6 mg/g tissue) and the methanolic fractions of flower (3428.4 mg/g tissue) and leaf (2440.8 mg/g tissue) in the hydrogen peroxide treated groups.

Discussion

Free radical production is touted as one of the major biochemical changes that occur in the host system. These molecules may interfere with other stable molecules and cause various disturbances to the host. The reactive oxygen species can directly or indirectly affect the major molecules in the host and lead to various cellular dysfunctions[28-29]. Plants have been contemplated as a rich source for the production of bioactive compounds with therapeutic potential. *C. guianensis* is a large tree and is known to exhibit numerous activities for the well-being of humans. The flowers and leaf parts of this plant have enormous functions in treating cold, intestinal gas formation, and stomach aches. The phytoconstituents namely, isatin isolated from the leaf and flower extracts have shown effective anticancer and antioxidant activity. Flowers are yellow, red, and pink with stunning scenic colors rich in secondary metabolites exerting various pharmacological and therapeutic properties. Antioxidants from plant sources have been involved in the neutralization of oxidants and used to reduce the progression of many diseases and disorders. Enzymatic and non-enzymatic antioxidants effectively participate in the neutralization of the action of various free radicals generated in the body[8,16,30].

In the present study, we evaluated the antioxidant profile of *C. guianensis*. Aubl. using the goat liver slices as an *in vivo* simulated *in vitro* system. The liver slices were exposed to the standard oxidant, hydrogen peroxide. The alkaloid fraction and methanolic extract of both leaf and flower of *C. guianensis* Aubl. was prepared to identify their efficacy in combating oxidative stress-associated disorders by means of evaluating their antioxidant properties. Based on the observations, the alkaloid and methanolic extract of the flower sample was found to have an effective antioxidant activity compared to the leaf extract. Leaf and flower samples have the ability to elevate the level of catalase in the oxidant treated sample. Decreased level of all the antioxidant levels has been noticed in the untreated H_2O_2 group. The non-antioxidant levels have been effectively elevated in the alkaloid extraction of flowers. The effect of the alkaloid extract is more pronounced than the methanolic extract, as it is a concentrated fraction of the phytochemical present in methanolic extract. Flower samples are found to be more effective in scavenging free radicals than the leaf extracts. Earlier studies of our laboratory have supported the bio-therapeutic potential of leaf and flower extracts of *C. guianensis* Aubl.[31]. The antioxidant and antimicrobial properties of *C. guianensis* were conducted by Augusco *et al*.[15]. It has revealed the higher antioxidant activity in ethanolic extract (2.98±0.96 – DPPH; 4.93±0.90 – $ABTS^{\cdot+}$). Besides, the antimicrobial activity against the oral pathogens was also significantly enhanced[15]. The flowers of *C. guianensis* have significantly exhibited antioxidant activity due to the presence of phenolic compounds in the petals[30]. Methanolic extract of *C. guianensis* has more pronounced antioxidant activity due to contain high phenolic contents[32]. The antioxidative potential of propolis collected from the local area of Chandigarh was evaluated using the animal model. The liver, spleen, and kidney were isolated and the antioxidative potential was evaluated using the marker enzymes[33].

Conclusion

Among the flower and leaf samples analyzed, the flower extracts were found to possess significant enzymatic and non-enzymatic antioxidant activity. The results have proven the overall performance of *C. guianensis* Aubl. was found to be effective against oxidative stress. Based on the outcome, it could be concluded that *C. guianensis* Aubl. has good antioxidant potential and is able to combat oxidative damaging systems, using an alternative model (goat liver slices). The *in vivo* simulated *in vitro* model system was found to mitigate the sacrifice of animals by mimicking the *in vivo* condition for research purposes.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

KD: Conceptual design, and manuscript editing; MM: Experimental studies, data acquisition, and statistical analysis; RR: Literature search, manuscript preparation, and review; KC: Manuscript editing and review.

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