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RESEARCH ARTICLE

# Antimicrobial Compounds Production by *Pseudomonas fluorescens* and *Bacillus subtilis*

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## ABSTRACT:
In the present study, ten strains of *Pseudomonas fluorescens* and ten strains of *Bacillus subtilis* were isolated from the soil sample. The production of antimicrobial compounds such as HCN, siderophores, salicylic acid and indole acetic acid by the isolates were estimated. Among the isolates AUPF3, AUBS2 and AUBS8 showed maximum production compared to the other isolates. Thus from the above study, it can be concluded that effective isolates can be used as biocontrol agents.

KEYWORDS: *Pseudomonas fluorescens, Bacillus subtilis* HCN, siderophores, salicylic acid ,indole acetic acid

## INTRODUCTION:
The exploitation of biocontrol agent for the management of plant disease have achieved greater significance in recent times due to its readily available nature, antimicrobial activity, easy biodegradation, non-phytotoxicity, besides inducing resistance in host [1]. Among various bacterial and fungal biocontrol agents, *Bacillus*, *Pseudomonas* and *Trichoderma* were most frequently used against various plant diseases. Fluorescent Pseudomonads and *Bacillus* were isolated and screened *in vitro* for their plant growth promoting traits like production of indole acetic acid (IAA), salicylic acid, hydrogen cyanide (HCN) and siderophores[2]. Production of hydrogen cyanide (HCN) is a major factor in the control of soil-borne diseases by *Pseudomonas fluorescens* CHA0[3]

Investigations were conducted to determine the role of salicylic acid (SA) in induced systemic (ISR) resistance against blue mold disease of tobacco elicited by plant growth-promoting rhizobacteria (PGPR). The observations indicate that SA accumulation in tobacco plants may play a role in ISR against tobacco blue mold by PGPR. [4] Siderophores sequesters ferric ions in the environment and the ferric siderophores are taken up in the microbial cells after specific recognition by membrane proteins. The production of siderophores is an important trait of PGPR in their ability to suppress soil-borne pathogens. Competition for ferric iron between the PGPR and the plant deleterious microorganisms is considered the mode of action of these siderophores [5]. Fluorescent Pseudomonads isolated from rhizophere showed biocontrol potential and the isolates displayed the production of siderophore, HCN and IAA.[6] The present study is aimed to investigate the production of antimicrobial compounds from the bacterial isolates.

## MATERIALS AND METHODS:
**Collection and maintenance of bacterial antagonists :**
Ten isolates of *P. fluorescens* and ten isolates of *B. subtilis* were collected from the Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam University for Women, Coimbatore - 43 and pure cultures were maintained on King's B agar and nutrient agar slants respectively.

**Antimcirobial compounds of *P. fluorescens* and *B. subtilis* isolates:**
**Hydrogen cyanide (HCN) production :**
**Qualitative assay :**
*P. fluorescens* and *B. subtilis* strains were grown on Trypticase soy agar (TSA) Filter paper soaked in picric acid solution (2.5 g of picric acid, 12.5 g of $Na_2CO_3$, 1000 ml of distilled water) was placed in the lid of each petridish. Dishes were sealed with parafilm and incubated at 28°C for 48 hr. A change in the colour of the filter paper discs from yellow to light brown, brown or reddish brown was recorded as an indication of weak, moderate or strong in producing HCN by each strain, respectively.[7]

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Quantitative analysis also revealed that among four isolates, AUPF3 produced higher amount of HCN (0.067 OD at 625 nm). This was followed by AUPF4 (0.060 OD at 625 nm), AUBS2 (0.041 OD at 625 nm) and AUBS8 (0.040 OD at 625 nm).

**Salicylic acid production:**

The results of present study revealed that among the four isolates, AUPF3 recorded maximum production of salicylic acid (26.97 µg/ml). This was followed by AUBS2, AUPF4 and AUBS8, which recorded 25.67, 24.5 and 20.44 µg/ml respectively (Table 2).

Table 2: Salicylic acid production by antagonistic bacterial isolates

| S. No. | Antagonistic bacterial isolates | Salicylic acid production* (µg/ml) |
|---|---|---|
| 1. | AUPF3 | 26.97[a] |
| 2. | AUPF4 | 24.5[c] |
| 3. | AUBS2 | 25.67[b] |
| 4. | AUBS8 | 20.44[d] |

* Mean of three replications

In a column, means followed by a common letter(s) are not significantly different (P=0.05) by DMRT.

**Siderophore production:**

Analysis of nature of siderophore produced by antagonistic bacterial isolates revealed that all the isolates were known to produce hydroxymate type of siderophore. Quantitative analysis reported that AUPF3 and AUPF4 produced maximum amount of siderophore, which recorded 43.66 and 42.74 µg/ml respectively. This was followed by AUBS2 (19.47µg/ml) and AUBS8 (16.33 µg/ml) (Table 3).

Table 3: Siderophore production by antagonistic bacterial isolates

| S. No. | Antagonistic bacterial isolates | Siderophore production* (µg/ml) |
|---|---|---|
| 1. | AUPF3 | 43.66[a] |
| 2. | AUPF4 | 42.74[a] |
| 3. | AUBS2 | 19.47[b] |
| 4. | AUBS8 | 16.33[c] |

* Mean of three replications

In a column, means followed by a common letter(s) are not significantly different (P=0.05) by DMRT.

**Indole acetic acid (IAA) production:**

In the present study, four isolates of antagonistic bacteria were evaluated for their efficacy in producing IAA. Among the four isolates, AUPF3 produced maximum amount of IAA (2.34 µg/ml). The isolates AUPF4, AUBS2 and AUBS8 recorded 1.97, 1.78 and 1.6 µg/ml respectively (Table 4).

Table 4: Indole acetic acid production by antagonistic bacterial isolates

| S. No. | Antagonistic bacterial isolates | Indole acetic acid production* (µg/ ml) |
|---|---|---|
| 1. | AUPF3 | 2.34[a] |
| 2. | AUPF4 | 1.97[b] |
| 3. | AUBS2 | 1.78[bc] |
| 4. | AUBS8 | 1.6[c] |

* Mean of three replications

In a column, means followed by a common letter(s) are not significantly different (P=0.05) by DMRT.

## DISCUSSION:

*Pseudomonas* and *Bacillus* species produces various secondary metabolites. Moreover, HCN and siderophores have been found to be inhibitory against different phytopathogens [15]. It has been documented that HCN and cell wall degrading enzymes could be involved in antagonistic activity toward phytopathogens and contributes to the biocontrol of plant diseases [16]. Study revealed that among the four isolates, AUPF3 and AUPF4 recorded moderate HCN production whereas isolates AUBS2 and AUBS8 recorded weak production of HCN.

Several authors have reported that hydrogen cyanide played an important role in disease suppression by *Pseudomonas* species.[17-20]

**Salicylic acid production:**

A number of elicitors may be produced by the PGPR strains upon inoculation, including salicylic acid, siderophore, lipopolysaccharides and 2,3-butanediol and other volatile substances. [21] The results of present study showed that among the four isolates, AUPF3 recorded maximum production of salicylic acid followed by AUBS2, AUPF4 and AUBS8.

Twelve isolates of *P. fluorescens* were tested for their ability to produce salicylic acid. Among the 12 isolates, PfUA 7 was resulted maximum amount of salicylic acid (159.7 µg/ml). This was followed by PfUA 6 (95.60 µg/ml) and PfUA 11 (73.80 µg/ml). The other isolates showed the production of salicylic acid ranging from 11.08 to 64.72 µg/ml.[22]

A study on four different *B. subtilis* isolates for salicylic acid production showed that the *B. subtilis* isolate BS13 produced maximum amount of salicylic acid accounting for 0.47 µg/ml. However other *B. subtilis* isolates (BS8, BS9 and BS11) produced less quantity compared to BS13.[23]

**Siderophore production:**

Under conditions of low iron availability, most aerobic and facultative anaerobic microorganisms including fluorescent Pseudomonad spp. produce low molecular weight, $Fe^{3+}$ specific chelators, siderophores. Competition for ferric ion between these microorganism and plant pathogens is considered to be the mode of action of these siderophore [24]. Analysis of nature of siderophore produced by antagonistic bacterial isolates revealed that all the isolates were known to produce hydroxymate type of siderophore. Isolates AUPF3 and AUPF4 produced maximum amount of siderophore followed by AUBS2 and AUBS8.

Nine *Pseudomonas fluorescens* isolates were screened for their production of siderophore. The result indicated that all the fluorescent isolates produced siderophores and the concentration ranges between 13.6 to 196.3 µg/ml with the highest production by strain Psd 2 and lowest by strain Psd 9.[25]

**Indole acetic acid (IAA) production:**

The ability to produce indole-3-acetic acid (IAA) is widespread among soil and plant-associated microorganisms [26]. The antagonistic bacteria were evaluated for their efficacy in producing IAA. Among the four isolates, AUPF3 produced maximum amount of IAA compared to the other isolates.

A report on the the ability of *Pseudomonas* isolates obtained from rice rhizosphere to produce IAA, was almost threefold higher than *Pseudomonas* isolates obtained from maize rhizosphere and they resulted in the higher ability to control fungal pathogen than isolates producing low amount of IAA.[27].

In the present study, among the various isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* AUPF3, AUPF4, AUBS2 and AUBS8 showed maximum production of antimicrobial compounds such as HCN, IAA, salicylic acid and siderophores .The antagonistic bacterial isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* can be used as a suitable candidate for the management various plant diseases caused by the fungal pathogens.

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# *In vitro* prevention of oxidative damage by *Curcuma amada* in goat liver slices exposed to oxidative stress

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## ABSTRACT

Liver, the largest organ in vertebrate body, has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Even common dietary antioxidants can provide such protection from liver damage caused by oxidative mechanisms of toxic chemicals. A phytotherapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants. Search for pure phytochemicals as drugs is time consuming and expensive. In the present study one such medicinal plant *Curcuma amada* has been tested for their antioxidant activity in precision cut goat liver slices exposed to oxidative stress by hydrogen peroxide treatment. The activity of enzymic antioxidants and the levels of non enzymic antioxidants which decreased initially by $H_2O_2$ treatment was found to be increased on treatment with methanolic extract of both the leaves and rhizomes of *Curcuma amada.*

**Key words:** *Curcuma amada*, liver, antioxidants, phytochemicals, hydrogen peroxide, oxidative damage

## INTRODUCTION

In the human and animal body, Reactive Oxygen Species (ROS) can be neutralized by antioxidant defence systems including antioxidant enzymes and antioxidant compounds [1]. Oxidative stress occurs when the production of reactive oxygen species overrides the antioxidant capacity in the target cell, resulting in the damage of macromolecules such as lipids, nucleic acids and proteins, causing alterations in the target cell function and leading to cell death [2]. Oxidative stress significantly impacts multiple cellular pathways that can lead to the initiation and progression of varied disorders throughout the body [3].

Antioxidant supplements or foods rich in medicinal plants are used to help the human body in reducing oxidative damage by free radicals and active oxygen. Currently, research interest has been focussed on the role of antioxidants as well as antioxidant enzymes, in the treatment and prevention of many diseases [4]. Antioxidants may guard against ROS toxicities by the prevention of ROS construction, by disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack [5].

Precision-cut tissue slices represent an organ mini-model that closely resembles the organ from which it is prepared, with all cell types present in their original tissue-matrix configuration [6]. Organ slices, an *in vitro* model representing the multicellular, structural and functional features of *in vivo* tissue, is a promising model for elucidating mechanisms of drug-induced organ injury and for characterizing species susceptibilities. The liver is the major organ used in organ slice studies [7]. In this model, cells are refrained in an environment with normal cell–cell and cell–matrix contacts, and remain to express high levels of metabolic enzymes [8]. This tissue shows a high rate of free radical generation with high metabolic and detoxifying capacity [9]. The advantage of precision-cut liver slices is based on the juxtaposition of cellular assays and tissue morphology [10]. Hence in the present study precision cut goat liver slices subjected to oxidative stress by $H_2O_2$ was used for evaluating the antioxidant potential of the methanolic extract of leaves and rhizomes of the medicinal plant *Curcuma amada.*

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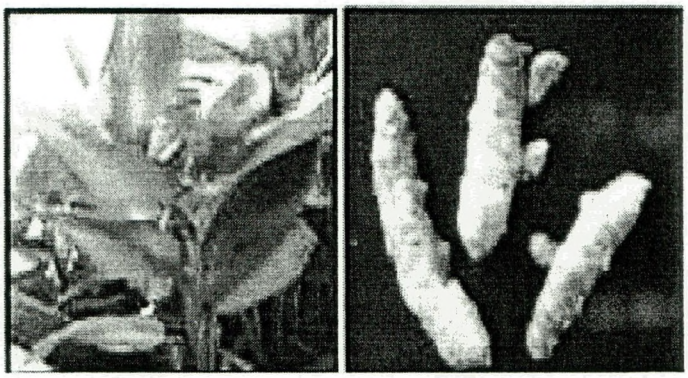
## MATERIALS AND METHODS

### Plant material

*Curcuma amada* rhizomes were procured from Arya Vaidya Pharmacy, Centre for Indian medicinal plant heritage, Kanjikode, Kerala and were grown as pot culture in our university herbal garden. Both leaves and rhizomes were collected fresh for the study. Previous studies conducted by us showed that the methanolic extract of the leaves and rhizomes were rich in antioxidants [11].



Leaves Rhizomes

**Figure 1: *Curcuma amada* leaves and rhizomes**

### Extract preparation

The leaves and rhizomes collected fresh were rinsed with tap water blotted dry using a filter paper and used for extract preparation. The components present in the leaves and rhizomes were extracted using methanol. The methanolic extract prepared after evaporation of methanol was dissolved in

DMSO (Dimethyl sulfoxide) [20mg plant extract in 50µl DMSO]. For each antioxidant assay 2.5mg of rhizome extract and 1.5mg of leaf extract dissolved in 5µl DMSO were used.

**Preparation of goat liver slices**

The goat liver was collected fresh from a slaughter house, plunged into cold sterile PBS and maintained at 4°C till use. Thin slices of 1mm thickness were treated with the oxidising agent $H_2O_2$ (200µM) and/or rhizome and leaf extract and incubated at 37°C with mild shaking for one hour. Appropriate controls were also set up. The homogenate was used for the assay.

**Treatment groups**

1.Liver slices
2.Liver slices + $H_2O_2$
3.Liver slices+ methanolic extract of *C.amada* leaves (CAL)
4.Liver slices+ CAL+ $H_2O_2$
5.Liver slices+ methanolic extract of *C.amada* rhizomes (CAR)
6.Liver slices+ CAR+ $H_2O_2$

**Determination of the activity of enzymic antioxidants**

**Assay of Superoxide dismutase (SOD)**

The incubation medium contained a final volume of 3ml, 50mM potassium phosphate buffer, 45µM Methionine, 5.3µM Riboflavin, 84µM NBT and 20mM potassium cyanide and enzyme source. The tubes were placed in an aluminium foil-lined box maintained at 25°C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the amount of SOD giving 50% inhibition of the reduction of NBT [12].

**Assay of Catalase**

3ml of $H_2O_2$ in phosphate buffer (0.067M, pH 7.0) was taken in a quartz cuvette and the baseline was adjusted at 240nm. 20µl of homogenate was added rapidly and mixed thoroughly. The time interval required for decrease in absorbance by 0.05 units was recorded at 240nm. The concentration of $H_2O_2$ was calculated using the extinction coefficient 0.036 per µM/cm. One unit is the amount of enzyme activity required to decrease the absorbance at 240nm by 0.05 units [13].

**Assay of Peroxidase**

3ml of pyrogallol solution (0.05M in 0.1M phosphate buffer) and 0.2ml of the homogenate were pipetted out into cuvette. Adjusted the spectrum to read zero at 430nm. 0.5ml of $H_2O_2$ was added in the test cuvette. The change in absorbance was recorded every 30 seconds upto 3 minutes. One unit of peroxidase activity is defined as the change in absorbance/ min at 430nm [14].

**Assay of Glutathione reductase**

The assay system contained 1ml of potassium phosphate buffer (0.12M, pH7.2), 0.1ml EDTA (15mM), 0.1ml Sodium azide (10mM), 0.1ml of oxidized glutathione (6.3mM) and 0.1ml of liver homogenate. The final volume was made up to 2ml using water. Then 0.1ml 0f 6.3mM NADPH was added. The absorbance at 340nm was recorded at an interval of 15 seconds for 2-3 minutes. The enzyme activity was expressed as mM NADPH oxidized/min/g liver [15].

**Assay of Glutathione S-transferase**

The substrates for GST (1mM GSH and 1mM CDNB, 0.1ml each) were taken in a test cuvette along with 0.1M phosphate buffer (pH 6.5) to make a volume of 2.9ml. The reaction was started by the addition of 0.1ml of the enzyme source to this mixture. The readings were recorded against distilled water blank for a minimum of 3 minutes. The complete assay mixture without the enzyme source served as the control. The enzyme activity was determined by recording the changes in absorbance at 340nm. One unit of GST activity is defined as the nM of CDNB conjugated/minute [16].

**Determination of the level of non-enzymic antioxidants**

**Estimation of Ascorbic acid**

Aliquots (0.2 to 1.0ml) of the working ascorbate solution (1mg/ml 4%TCA) were made upto 2.0ml with 4% TCA. 0.5ml DNPH reagent (2% DNPH in 9N $H_2SO_4$) was added to each tube, followed by two drops of 10% thiourea solution and incubated at 37°C for 3 hours. The osazone crystals formed were dissolved by the addition of 85% $H_2SO_4$ (2.5ml) in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of $H_2SO_4$. After incubation for 30min at room temperature the absorbance was read at 540nm [17].

**Estimation of Tocopherol**

The liver homogenate (1.5ml), 1.5ml standard (10ng/l alcohol) and water (1.5ml) were pipetted out into three centrifuge tubes namely test, standard and blank respectively. To all the tubes, xylene (1.5ml) was added, stoppered, mixed well and centrifuged. The xylene layer (1.0ml) was taken and transferred to another set of stoppered tubes. 1.0ml of 2,2' dipyridyl (1.2g/l n-propanol) was added to each and mixed. The reaction mixture (1.5ml) was taken in a spectrophotometric cuvette and the extinction of test and standard were read against the blank at 460nm. 0.33ml Ferric chloride solution (1.2g/l ethanol) was added and after exactly 15 minutes, the absorbance of the red colour was read against blank at 520nm. The amount of tocopherol in the sample was calculated using the formula,

Tocopherol (µg) = [(Reading of standard at 520nm- Reading at 450nm)/ (Reading at 520nm)]X 0.29 x 0.15

The results are expressed as µg tocopherol/g sample [18].

**Estimation of Vitamin-A**

Liver homogenate (1.0ml) was mixed with 1.0ml of saponification mixture (2N KOH in 90% alcohol) and refluxed for 20 minutes at 60°C in the dark. All the steps subsequent to saponification were carried out in the dark. Vitamin A was extracted twice with 10ml of petroleum ether (40-60°C). The extracts were pooled, washed thoroughly with water and the layers were separated using a separating funnel. When the petroleum ether fraction was clear, a pinch of sodium sulphate (anhydrous) was added to remove the excess moisture. The volume of the extract was noted and a fraction (1.0ml) was evaporated to dryness at 60°C. The residue was dissolved in 1.0ml of chloroform. Aliquots of the standard (vitamin A palmitate) were pipetted out into a series of clean, dry test tubes in the concentration ranging from 0 to 7.5µg. The volume in all the tubes was made up to 1.0 ml with chloroform. TCA reagent (2.0ml) was added rapidly, mixed well and the absorbance of blue colour was read immediately at 620nm in a spectrophotometer. The vitamin A level was expressed as µg/g tissue [19].

**Estimation of Reduced glutathione**

0.1ml of sample was made upto 1ml with 0.2M Sodium phosphate buffer (pH 8). 2.0ml of freshly prepared DTNB solution (0.6M in phosphate buffer) was added and the intensity of the yellow color formed was read at 412nm in a spectrometer after 10 minutes. A standard curve of GSH was prepared between the concentration range of 2 to 10nM. The values were expressed as nM GSH/g liver [20].

**Statistical analysis**

The parameters of the experiment are expressed as Mean ±S.D. Statistical evaluation of the data was done using one way ANOVA with the level of significance at $P<0.001$ in sigma stat package version 3.1.

## RESULTS AND DISCUSSION

The activities of all the enzymic antioxidants analyzed decreased significantly on exposure to $H_2O_2$. The activities of enzymic antioxidants in the liver slices upon exposure to the leaf and rhizome extracts significantly

**Table1. Effect of *C.amada* leaf and rhizome extracts on the activities of enzymic antioxidants in goat liver slices exposed to $H_2O_2$ *in vitro***

| Parameters | Liver slices | Liver slices+ $H_2O_2$ | Liver slices+ CAL | Liver slices+ CAL+ $H_2O_2$ | Liver slices+ CAR | Liver slices+ CAR+ $H_2O_2$ |
|---|---|---|---|---|---|---|
| SOD[1] | 11.49±1.2 | 8.45±0.56[a] | 13.91±0.34[ad] | 11.7±0.19[bc] | 16.22±0.46[a] | 13.52±0.09[abc] |
| CAT[2] | 360±28.28 | 138.9±4.08[a] | 329.7±17.84 | 296.2±18.23[ab] | 324.55±21.86 | 319.39±17.85[b] |
| POD[3] | 32.28±0.2 | 23.18±0.72[a] | 47.57±0.11[a] | 47.64±2.38[ab] | 49.02±0.44[a] | 46.15±2.38[ab] |
| GR[4] | 7.25±0.16 | 4.29±0.86[a] | 9.98±0.12[ad] | 9.56±0.08[ab] | 8.96±0.32[ad] | 8.52±0.05[ab] |
| GST[5] | 0.44±0.03 | 0.26±0.002[a] | 0.47±0.03 | 0.38±0.007[bc] | 0.43±0.03 | 0.41±0.08[b] |

***Values are expressed as Mean± SD of triplictes, values are expressed in Units/g liver tissue***
***1- 1 Unit is defined as the amount of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 min***
***2- 1 Unit is defined as the amount of enzyme required to decrease the absorbance by 0.05 units at 240nm***
***3- 1 Unit is defined as the Change in absorbance at 430nm per minute.***
***4-1 Unit is defined as the milli moles of NADPH oxidized / min.***
***5- 1 Unit is defined as the nano moles of CDNB conjugated / min.***
***a- Statistically significant (P<0.05) compared to untreated control***
***b- Statistically significant (P<0.05) compared to H2O2 alone treated group***
***c- Statistically significant (P<0.05) compared to the respective extract treated group***
***d- Statistically significant (P<0.05) compared to the rhizome extract treated group***

**Table2. Effect of *C.amada* leaf and rhizome extracts on the levels of non-enzymic antioxidants in goat liver slices exposed to $H_2O_2$ *in vitro***

| Parameters | Liver slices | Liver slices+ $H_2O_2$ | Liver slices+ CAL | Liver slices+ CAL+ $H_2O_2$ | Liver slices+ CAR |
|---|---|---|---|---|---|
| Vitamin-C (mg/g liver) | 0.54±0.02 | 0.23±0.002[a] | 0.86±0.009[a] | 0.75±0.011[ab] | 0.71±0.02[ad] |
| Vitamin-E (µg/g liver) | 3.05±0.13 | 1.07±0.19[a] | 3.19±0.33 | 2.64±0.2[bc] | 3.15±0.13 |
| Vitamin-A (µg/g liver) | 72.01±0.33 | 54.53±0.44[a] | 78.4±0.66[ad] | 71.62±0.44[bc] | 81.71±0.76[a] |
| Reduced glutathione (mM/g liver) | 5.92±0.05 | 3.95±0.05[a] | 5.75±0.04[a] | 5.52±0.02[abc] | 6.35±0.04[ad] |

*a-Statistically significant (P<0.05) compared to untreated control*
*b-Statistically significant (P<0.05) compared to H2O2 alone treated group*
*c-Statistically significant (P<0.05) compared to the respective extract treated group*
*d-Statistically significant (P<0.05) compared to the rhizome extract treated group*

increased compared to untreated control. The presence of the extracts along with the oxidant, recorded an increase in the activities of enzymic antioxidants when compared to the oxidant treated group. The same trend was recorded in the level of non-enzymic antioxidants also in which the exposure of liver slices to leaf and rhizome extract significantly increased the non-enzymic antioxidants levels which were initially diminished by the oxidant treatment.

Our results correlated with the study which reported that the 43kD protein fraction from the leaves of *Cajanus indicus* significantly improved SOD, catalase and glutathione S-transferase activities in chloroform administrated mice liver *in vivo* [21]. The above findings also correlated with another study which showed increased level of enzymic antioxidants in liver of arthritis rats treated with *Withania somnifera* root powder extract [22]. *Trianthema decanda* root extract administration to Wistar rats also improved the antioxidant status by increasing the activities of liver CAT, SOD, GPx and GR in rats subjected to CCl4 treatment[23]. The level of GST was found to be increased in the heart of myocardial injured rats after administration with *Ficus hispida* extract [24].

Increase in the level of vitamin C in the liver of Wistar rats was observed after administration of ethanolic extract of *Piper longum* in alloxan induced diabetic rats [25]. The results obtained correlated with the report that the administration of saponin containing compounds from *Aesculuc hippocastanum, Medicago sativa* and *Spinacia oleracea* reversed the plasma carotene and retinol level to normal in rats exposed to X-radiation [26]. *Piperculina turpethum* extract administration also increased the level of enzymic antioxidants (CAT, SOD and GPx) and non-enzymic antioxidants vitamin E and C in the liver of Sprague–Dawley rats [27]. The level of glutathione was found to increase in rat liver after treatment with *Helicteres isora* extract in rats subjected to oxidative stress by CCl4[28].

The administration of *Solanum nigrum* leaf extracts to the Swiss albino mice also showed an increase in the activities of enzymic and levels of non-enzymic antioxidants in the liver of mice subjected to oxidative stress [29]. Administration of methanolic extract of *Nyctanthes artortris-tis* leaves also improved the enzymic antioxidant status of goat liver slices exposed to oxidative stress [30] and *Alternanthera sessilis* leaf extract treatment also increased the antioxidant status of rat liver which was decreased by oxidant treatment [31].

## CONCLUSION

Thus, the results of the study using precision-cut goat liver slices revealed that the methanolic extract of leaves and rhizomes of *Curcuma amada* possess antioxidant principles that can fight against free radical mediated disorders.

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