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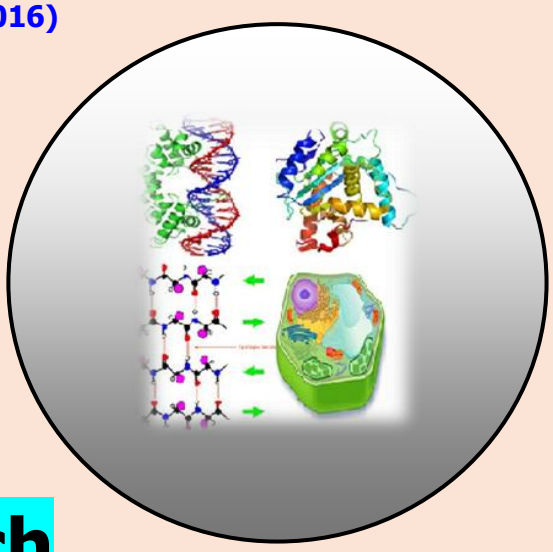

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RESEARCH PAPER

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Croton bonplandianum : Antioxidant, Protease, Catalase and Peroxidase activities

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ABSTRACT

Croton bonplandianum (Euphorbiaceae) is one of the exotic weeds and mostly seen in wastelands, and it is commonly known as 'bantulasi'. Traditionally, this plant is used to treat liver disorders, skin diseases, bronchitis, asthma, jaundice, acute constipation, abdominal dropsy and internal abscesses and for treatment of wounds. Because of its wide availability and pharmacological significance, a study was set out to investigate the in-vitro antioxidant, protease, catalase and peroxidase activities of the latex of C. bonplandianum. The antioxidant activity was analysed using DPPH (1-1, dipenyl-2-picrylhydrazyl) reduction. Protease activity was analysed using the cleavage of milk protein, casein. Catalase activity was studied by the cleavage of hydrogen peroxide and peroxidase activity was determined using hydrogen peroxide and o-phenylenediamine. From the results, all antioxidant, protease, catalase andperoxidase activities were observed in the latex of C. bonplandianum; i.e., 10 µl of latex (undiluted) showed 76 ± 5.4 % of antioxidant activity; 20 µl of latex (undiluted) showed 94.7 ± 7.9 µg/ml/min protease activity;1 ml of latex (diluted 1:500 in distilled water) showed 928 ± 14 U/ml catalase activity; and 25 µl of latex (diluted 1:20 in distilled water) showed 0.576 ± 0.095 OD_{492} peroxidase activity. The plant source has rich amounts of molecules with different bioactivities such as antioxidant, protease, catalase and peroxidase activities. The result of the present study supports the traditional use of the C. bonplandianum with different bioactivities such as wound healing.

Keywords: Croton bonplandianum, wound healing, Antioxidant activity, Protease activity, Catalase activity, Peroxidase activity.

INTRODUCTION

Croton bonplandianum Baill (Euphorbiaceae), commonly known as Bantulsi is a perennial herb, one of the exotic weeds, found in waste lands and road side areas in India, Bangladesh and all other countries of South Asia (Chakrabarty and Balakrishnan, 1992). Flowering and fruiting time of this plant is from September to December (Reddy, 1995). *C. bonplandianum* is a lactiferous, green herb, growing up to 1-2 ft. long. Leaves of the plant are simple, petiolate, alternately arranged, 3-5 cm long, lance shaped with toothed margin. Flowers are small, white, unisexual; contain 5 sepals, 5 petals and numerous long stamens protruding out. Fruits are deciduous with two valved cocci, 5 mm oblong capsule having warty surface. Seeds are small, smooth and albuminous (Sethiand Sharma, 2011). Traditionally, this plant is used to treat liver disorders, skin diseases including ring worm infection, to cure the swelling of body, bronchitis and asthma (Bapuji and Ratnam, 2009; Kuppuswamy et al., 2013). Bark and roots of *C. bonplandianum*are alterative and chologogue (Das et al., 2008).

Leaves of this plant are medicinally used for the treatment of cut and wounds, venereal sores and cholera (Asolkar et al., 1992). The seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses (Reddy, 1995). Fresh juice of the plant is used in headache (Saggoo et al., 2010). The pharmacological activities of *C. bonplandiamun* has been investigated and reported by various researchers. This plant has showed cardio and hepatotoxic effect (Ahmad et al., 2008), antibacterial and antifungal activity (Saggoo et al., 2010; Asthma et al., 1989), antioxidant, anti-tubercular, cytotoxic (Qaisar et al., 2013; Kuppuswamy et al., 2013) and also possess antitumor activity (Islam et al., 2010). Croton is rich in secondary metabolites including alkaloids and terpenoids (Rizk, 1987).

Because of its wide availability and pharmacological significance, a study was set out to investigate the in-vitro antioxidant, protease, catalase and peroxidase activities of the latex of *C. bonplandianum*.

MATERIAL AND METHODS

Sample collection

The plant (Fig.1) located within the university campus was identified by the Department of Botany, Visva-Bharati, Santiniketan. Latex is collected from *Croton bonplandianum* freshly before the analysis. The twig of the plant was thoroughly cleaned with wet cotton and then tissue paper, a small scratch was made with a blade and the latex was collected into a micro tube. With this fresh latex sample all the experiments were conducted at Palli Siksha Bhavana, Institute of Agriculture, Visva-Bharati, Sriniketan.

Figure 1. ***Croton bonplandianum.***

In-vitro antioxidant activity

The scavenging ability of the natural antioxidants of the plant extracts towards the stable free radical DPPH (1-1, dipenyl-2-picrylhydrazyl) was measured by the method of DPPH assay (Mensor et al., 2001). 10µl of latex was taken into a clean test tube and to this 1 ml of phosphate buffer (pH=7.0) is added. To this tube 1 ml of DPPH (0.4mM in ethanol) was added. The mixture was allowed to react at room temperature for 10 minutes. In the control tube no plant source was added, 1010 µl of buffer and 1 ml of DPPH was taken. The discoloration of the purple color was measured at 518 nm in a UV-Vis Spectrophotometer (Systronics-2201). The % of antioxidant activity was calculated.

Determination of protease activity

Proteases hydrolysethe protein (ex. Casein) into peptide fragments and amino acids. The free aromatic amino acids yield blue color under alkaline medium with Folin-Ciocalteau reagent (FC reagent), which was measured at 700 nm in an UV-Vis spectrophotometer (Systronics-2201). The proteolytic activity is calculated and expressed as µg of tyrosine liberated in 1min, at 37^0C, at pH 7, per 1ml of enzyme.

(T) Test: Reaction was carried out in micro Eppendorf tubes. 20 µl of the latex sample (undiluted) was taken into each Eppendorf tube and then 730 µl of casein (1% dissolved in the phosphate buffer, pH=7) was added to the tube. The mixture was incubated at room temperature for 15 minutes and at the end of this incubation, 750 µl of trichloro acetic acid (10% TCA in distilled water) was added to stop the reaction. The tubes were shaken thoroughly and centrifuged at 5000xg for 15 minutes. After centrifugation, 0.5 ml of the supernatant was taken and transferred into a clean and dry glass test tube, to this, 5 ml of 0.5M NaOH solution was added and mixed, then finally 0.5 ml of FC reagent was added and mixed thoroughly. The intensity of the blue color developed was measured in a UV-Vis Spectrophotometer (Systronics-2201) at 700 nm.

(C)Control: The control reaction was carried out similarly except that the TCA was added to the latex prior to the addition of the substrate casein.

(S)Standard: 0.5 ml of Standard Tyrosine solution (100 µg/ml) was taken and 5 ml of NaOH and 0.5 ml of FC reagent was added.

(B)Blank: Instead of sample aliquot 0.5 ml of buffer was taken and 5 ml of NaOH and 0.5 ml of FC reagent was added. The proteolytic activity of trypsin is calculated. The enzyme activity is expressed as µg of tyrosine liberated/min/ml of enzyme.

Peroxidase activity

Peroxidase activity was determined by using o-phenelynediamine as chromogen and H_2O_2 as substrate. 25 µl of latex (diluted by 1:20 with distilled water) was taken in clean glass test tube and to this 2 ml of the substrate mixture (containing 2 mg of o-phenelynediamine and 4 µl of H_2O_2 in distilled water) was added and the reaction was continued for 15 minutes. The intensity of the brownish yellow color developed was measured in a UV-Vis Spectrophotometer (systronics-2201) at 492 nm. The blank was setup similarly by taking 25 µl of distilled water instead of diluted plant latex.

Catalase activity

Catalase molecule catalyzes the decomposition of H_2O_2, which was monitored by the decrease in the absorbance at 240 nm over a period of 3 min using an UV-Vis spectrophotometer (Systronics-2201). 1 ml of latex (diluted 1:500 in distilled water) and 1 ml of distilled water is taken in a quartz cuvette and 1 ml of H_2O_2 is added. Absorbance readings of the initial and three consecutive 1-minute intervals were takenat 240 nm andthe catalase activity was calculated (Aebi, 1984).

RESULT AND DISCUSSION

From the results (Table 1), it was observed that latex of *Croton bonplandianum*has exhibited very strong antioxidant activity (76 ± 5.4 %). It was observed that the violet color of the DPPH solution is disappeared immediately (within a second) after the addition of the *C. bonplandianum* plant latex to the DPPH solution. This indicates that the latex of *C. bonplandianum* was a rich source of molecules with very strong antioxidant activity. Similar results were observed in other plant sources, it was reported that the *Artocarpus heterophyllus* and *Manilkara zapota* seeds extract show the significant antioxidant potential in-vitro compared with standard in terms of DPPH scavenging activity (Shanmugapriya et al., 2011). It was reported that the methanolic and dichloromethane extracts of *C. bonplandianum* possess appreciable amounts of natural antioxidant and cytotoxic potential compounds (Qaisar et al., 2013). However, in this study we have found very strong antioxidant activity in the latex of the same plant source.

Since protease activity was not reported so far in *C. bonplandianum*, we have studied and found the protease activity in the latex of this plant source. From the results (Table.1), it was observed that *C. bonplandianum* latex has exhibited the protease activity (94.7±7.9µg/ml/min). Similar results were observed in other plant sources, it was reported that the floral extract of *Jasminum grandi florum* possess the protease activity. Proteases occur naturally in living organisms. Growth and development in all organisms occur as a result of an overall balance between protein synthesis and proteolysis (Vidyalakshmi and Selvi, 2013). Proteases are effective in removing damaged and infected tissues from wounds and thus play an important role in the wound healing process. Proteases from various sources such as plant, microbes, maggots and animals were found to be useful in wound debridement (Walsh, 2003). Since the microorganisms, i.e., parasites, fungal forms, and bacteria are made up of protein, hence, proteases play an important role in degrading these microorganisms and keeping the host safe by preventing the survival and multiplication of these microbes. *C. bonplandianum* plant was reported to possess antifungal and antibacterial activity (Saggoo et al., 2010; Asthma et al., 1989). The presence of protease molecules in *C. bonplandianum* found in this present study may prove the antifungal and antibacterial activity of this plant source.

Catalase is an important enzyme of cell defence mechanisms against oxidative stress in living organisms. This antioxidant enzyme widely distributed in a variety of life forms, including microorganisms, plants and animals (Kirkman and Gaetani, 2007). In plants, multiple isoforms of the enzyme are usually present, and they are expressed in different tissues and developmental stages. In green leaves a majority of catalase activity is found in peroxisomes (Foyer and Noctor, 2000; Scandalios et al., 1997). Catalase activity has been reported in several plants, such as spinach, maize, cotton, sunflower, tobacco, van apple, and parsley (Garcia et al., 2000; Mullen and Gifford, 1993; Yoruk et al., 2005; Ozturk et al., 2007). However, in *C. bonplandianum*, catalase activity was neither studied nor reported so far. In the present study, we analyzed and found the catalase activity in the latex of this plant source. From the results (Table.1) it was observed that *C. bonplandianum* has exhibited very strong catalase activity (i.e.928 ± 14 U/ml). Several harmful chemicals with the oxidizing capacity may be generated within the individual due to metabolism of the individual or microbes and also may be ingested through various sources such as diet. These chemicals may be detrimental to the health of the individual.

So, these harmful chemicals should be neutralized or modified to harmless or comparatively less harmful compounds. Catalase plays very important role in performing this job, whereby confirming the safety of the individual. Catalase scavenges H_2O_2 generated during β-oxidation of the fatty acids, electron transport in mitochondria and photorespiratory oxidation (Havir and Mchale, 1987; Kunce and Trelease, 1986). Catalase is the most efficient enzyme as an antioxidative enzyme which lowers hydrogen peroxide or superoxide to accumulate to toxic levels in plant growth (Bowler et al., 1992). Catalase also neutralize the toxins by oxidizing them using hydrogen peroxide, such as phenols, formic acid, formaldehyde and alcohols (Scandalios et al., 1997; Chaudiere and Ferrari-Lliou, 1999; Karra-Chaabouni et al., 2003).

Peroxidases are among the most ubiquitous enzymes in plant species. These enzymes occur widely in animals, plants and microorganisms, where their repertoire of activities include catalytic hydroxylation and oxidative reactions. These are heme-containing proteins that use H_2O_2 to oxidize a large variety of hydrogen donors such as phenolic substances, amines, ascorbic acid, indole and certain inorganic ions (Diao et al., 2011; Dunford, 2010; Murphy et al., 2012; Vernwal et al., 2006). In the plants, peroxidases play many roles but mainly serve to catalyze the reductive destruction of hydrogen peroxide, which otherwise could lead to lipid peroxidation (Nwanguma and Eze, 1995). In most cereals lipid peroxidation causes reduction in quality and shelf life of the products (Hilderbrand, 1992). They participate in the lignification process (Wakamatsu and Takahama, 1993) and in the mechanism of defence in physically damaged or infected tissues in the plants (Biles and Martin, 1993).In animal tissues and microorganisms, peroxidases play a role of protection against toxic peroxides (Welinder, 1992). Different isoenzymes of peroxidases are known to occur in a variety of plant types and tissues. Barley, wheat, buckwheat, soybean etc. have been shown to exhibit high levels of peroxidase activities (Nnamchi et al., 2013). From the results of the present study (Table 1) the latex of *C. bonplandianum* has also exhibited the peroxidase activity (i.e. 25 µl of latex, diluted 1:20 in distilled water, gave 0.576 ± 0.095OD_{492}). The presence of catalase and peroxidases along with protease might be responsible for various pharmacological activities of *C. bonplandianum*.

The effect of *C. bonplandianum* leaves on experimental wounds studied and observed that the plant showed a definite, positive effect on wound healing (Divya et al., 2011). It was reported that the efficacy of this plant in wound healing may be due to its chemical constituent rutin and antioxidant enzymes, thereby justifying the traditional claim (Divya et al., 2011).However, it was neither studied nor reported, which kind of molecules are responsible for this definite activity. In another study, it was reported that the protease activity found in the floral extract may be responsible for wound healing property of the flowers of *Jasminum grandi florum* (Vidyalakshmi and Selvi, 2013). These researchers have studied and reported the presence of the protease activity, which is shown responsible for the wound healing property of *J. grandi florum*. However, in our study we have found protease catalase and peroxidase enzyme activities in the latex of *C. bonplandianum* along with antioxidant activity. The result of this present study indicates that the latex is rich source of bioactive molecules and plays important role in the immunity/defense system of the plant. The molecules identified in the present study,i.e., antioxidant, protease, catalase and peroxidases may play important role in the wound healing process and also various pharmacological activities attributed to *C. bonplandianum*.

Antioxidant activity :	76 ±5.4 %
Protease activity :	94.7±7.9µg/ml/min
Catalase activity :	928±14 U/ml
Peroxidase activity :	0.576±0.095 OD_{492}

Table 1. Different bioactivities observed in the latex of *C. bonplandianum.*

CONCLUSION

The latex of the plant source has rich amounts of molecules with different bioactivities such as antioxidant, protease, catalase and peroxidase activities, and these molecules are vital in the immunity/defense system of the plant. The identified protease, catalase and peroxidase enzyme molecules along with antioxidant molecules play an important role in the wound healing process and may also responsible for various pharmacological activities attributed to *C. bonplandianum*. The result of the present study supports the traditional use of the *C. bonplandianum* with different bioactivities such as wound healing.

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