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Research Paper

Inhibitory Effect of Cleistanthus Collinus Plant Extracts and their Fractions on Glutathione S-transferase activity

Agriculture

KEYWORDS : glutathione S-transferase, detoxification enzymes, inhibition, Cleistanthus collinus, Minimum inhibitory concentration (MIC).

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ABSTRACT

Glutathione-S-transferases (GSTs) play a significant role in the detoxification and metabolism of many xenobiotic and endobiotic compounds. GSTs are a potential target for the plant extracts. Naturally occurring enzyme inhibitors play an important role in a drug discovery program. The effect of methanolic extracts of leaf and bark of C. collinus on glutathione S-transferases from P. xylostella was studied by spectrophotometric method. The leaf and bark extracts were used at various concentration such as 1, 2.5, 5, 7.5, 10, 100 µg/ml and 1, 10 mg/ml. Inhibition of crude GST extract was observed at all the concentrations with MIC at 5µg/ml and 2.5 µg/ml for leaf and bark extract respectively.

Introduction

Cleistanthus collinus popularly known as Garadi and Oduvan is a small deciduous tree of the family Euphorbiaceae. *Cleistanthus collinus* is abundantly found in many parts of India, Malaysia and Africa. The leaf, roots and specially the fruits act as violent gastro intestinal irritants. It is also used as cattle and fish poison and also for procuring criminal abortion. The leaf are abortificinet and occasionally used for suicidal purposes (Modi and Coius,1940). Cleistanthin is also used as anticancer (Pradheepkumar et. al,2000).

Host plant possess different amounts and types of secondary metabolites which induce various detoxifying enzymes. Inhibition of these enzymes suggests that the phytochemicals can exert insecticidal role by antioxidant activity (Ayodele et al, 2009). These enzymes and correlated genes can be used in the development of efficient and harmless biotechnological tools for agricultural insect pest control without negative environmental and human health effects (Jackeline et al, 2006).

Materials and methods

Plant materials

The authenticated plant material used for this study was collected from Forest department office at Purkabodi village situated in Bhandara taluka of Bhandara District (Maharashtra states), India.

Rearing of *Plutella xylostella* (DBM)

The larvae and pupae of *P. xylostella* were collected from cabbage and cauliflower field from outskirts of Akola. They were reared in the laboratory on the mustard seedlings upto F4 generations for establishing homologous laboratory population. Mustard seeds (Var. Pusa Bold) were used for raising seedlings. The rearing procedure described by Lu and Sun (1984) was followed to maintain the test culture of *P. xylostella*.

Preparation of plant extract

Fresh leaf and bark of *C. collinus* were dried at room temperature and then ground into fine powder using a grinder. About 20 g of dried powder of leaf and bark were taken in separate 250 ml conical flasks with screw cap, and then 200 ml of methanol was added to the flask. The flasks were kept at room temperature with shaking for 7 days. At the end of the extraction, extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30°C and stored at 4°C until further use. The final crude extract was diluted with methanol to a standard volume and tested separately against the test organisms (Harborne, 1973).

Solvent extraction

Few amount (5 gm) of extract was taken and triturated with MeOH:H_2O (4:1) ratio, filtered and then the residue was separated and it was considered as fraction 1. The filtrate then acidified with 2M H_2SO_4 and was extracted again with $CHCl_3$ for three times. Thus chloroform and aqueous acid layer get separated. Chloroform was evaporated it was considered as fraction 2 while aqueous acidic layer was considered as fraction 3 (Harborne, 1998).

Preparation of enzyme

The third instar larvae (*P. xylostella*)weighing 3.0-4.0 mg approximately were separated and starved for 7-8 hours to remove all digested food particles. Whole larvae were homogenized using mortar and pestle in sodium phosphate buffer (PB) (100 mM, pH 6.5), containing 0.1 mM of EDTA, PTU and PMSF each, insects were chilled in refrigerator before homogenization. The homogenate thus obtained was centrifuged at 10,000 rpm for 15 minutes at 4°C in high speed refrigerated centrifuge, solid debris and cellular material was discarded. The resultant post mitochondrial supernatant obtained was stored at -20°C and used as enzyme source.

Glutathione-S-transferase (GST) quantification

GST quantification was carried out by method described by Kao et al. (1989). Fifty microlitre of 50 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 150 µl of reduced glutathione (GSH) were added in 2.79 ml PB (100 mM pH 6.0, 0.1 mM PTU). Ten microlitre of enzyme stock of the DBM was added in above mixture. Reaction was carried out in triplicate set. The contents were gently shaken and incubated for 2 to 3 minutes at 20°C and then transferred to cuvette of the UV spectrophotometer. Three milliliter of the reaction mixture without enzyme was placed in the cuvette present in reference slot. Absorbance was read for 6 to 7 minute at 340 nm by employing time scan menu of the spectrophotometer. The percent inhibition of the enzyme activity was calculated by using following formula. The experiment was analysed statistically to confirm the significance of the findings.

$$I = \frac{C - T}{C} X100$$

Where,

I = Percent Inhibition C = Control T = Treatment

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Result and conclusion

The leaf and bark extracts were used at various concentration such as 1, 2.5, 5, 7.5, 10, 100 µg/ml and 1, 10 mg/ml. Inhibition of crude GST extract was observed at all the concentrations with minimum inhibitory concentration (MIC) at 5 µg/ml and 2.5 µg/ml for leaf and bark extract respectively (4.18 % and 4.07 %) and maximum inhibition with increasing the concentration so that a concentration dependent activity was observed. Our preliminary investigation showed that both leaf and bark extract used in this study bring about the inhibition of crude GST extract from the *P. xylostella.* This suggests that the extract binds to the active site of the enzyme and therefore prevent detoxification role of the enzyme. After solvent extraction three fractions were obtained fraction 1 of residues, fraction 2 of chloroform and fraction 3 of aqueous acid fraction. The maximum yield was obtained in case of leaf (Fr 3) i.e. 3298 mg while minimum yield was obtained in leaf (Fr 1) i.e. 426 mg. Since the residue i.e. Fraction 1 is a neutral extract and contains mostly carbohydrates and mucilages fraction 1 was not used for further screening of inhibition activity. The effect of fractions of methanolic extracts of leaf and bark of *C. collinus* on glutathione S-transferases from *P. xylostella* was studied at 10 % concentration by spectrophotometric method. The prominent activity was observed in chloroform (Fraction 2) leaf and bark extract. In case of chloroform leaf fraction 27.33% inhibition was observed and in chloroform bark fraction 30.83% was observed. The bark extracts activity was marked as compared to leaf extracts activity. Both leaf and bark extract were found to have GST inhibition activity. The solvent Chloroform fraction of both leaf and bark extracts was found to have potential GST inhibitory activity. The bark extract was found to be more potent extract

Table 1 : Detoxifying enzyme (GST) activity inhibition

Concentration	Inhibition %	
	Leaf extract	Bark extract
10 mg/ml	36.10	48.36
1 mg/ml	21.15	40.61
100 µg/ml	15.50	33.26
10 µg/ml	12.37	27.95
7.5 µg/ml	9.10	16.08
5 µg/ml	4.18	8.63
2.5 µg/ml	0.0	4.07
1 µg/ml	0.0	0.00
F test	Significant	Significant
S.E.(m)±	0.025	0.018
CD	0.111	0.089

Table 2: Solvent extraction of crude methanol extract

S. N.	Extract	Weight of extract			
		Initial weight of Dried Methanol (gm)	Fraction 1 (Residues) Mg	Fraction 2 (Chloroform) Mg	Fraction 3 (Aqueous acid) Mg
1	Leaf	5	426	652	3298
2	Bark	5	784	1560	2616

Table 3: Detoxifying enzyme activity inhibition of solvent fractions at 10% concentration

Fraction	Inhibition %	
	Leaf extract	Bark extract
Chloroform fraction	27.33	30.83
Aq. Acid extract	14.06	20.27

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