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Original Article

EFFECT OF *COSTUS PICTUS* D. DON. ON PEPSIN ENZYME

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ABSTRACT

Objective: Pepsin has a close resemblance with HIV-protease in proteolytic activity as both of them belong to same aspartate enzyme family. Hence in the present study, pepsin was used as a substitute for HIV-protease. Pepsin assay could be used for preliminary screening of natural products for probable HIV-protease inhibitory activity. Therefore the objective of present study was to prepare 4 extracts namely, Fresh-Aqueous, Fresh-Hydroalcoholic (HA), Dry-Aqueous and Dry-Hydroalcoholic (HA) from fresh as well as air dried leaves of *Costus pictus* D. Don. and to evaluate their effect on pepsin enzyme.

Methods: Pepsin assay was carried out using hemoglobin as a substrate. Further, the antioxidant activity of extracts was monitored using DPPH assay, whereas, phenolic content was estimated using Folin-Ciocalteu reagent.

Results: All the extracts showed potent inhibitory effect against pepsin enzyme, wherein Fresh-HA revealed the highest inhibitory activity (93.49%) followed by Dry-HA (88.11%). The highest antioxidant potential was exhibited by Dry-HA extract (IC_{50}= 1.03mg/ml).

Conclusion: The present study suggests that *C. pictus* might be useful as a HIV protease inhibitor. The inhibitory activity could be attributed to phenolic content of the extracts.

Keywords: Anti-HIV, HIV-Protease (PR), Pepsin assay, *Costus pictus,* Antioxidant, Phenolics.

INTRODUCTION

Aspartic proteases include pepsin, cathepcin D, renin, chymosin and the proteases isolated from numerous fungi [1]. HIV protease belongs to class of aspartic proteases and has similar structural features and mechanism to aspartic protease enzymes [2]. HIV protease plays a vital role in viral replication cycle [3]. Blockage of HIV protease leads to formation of immature non-infectious virions [4]. Hence it has become an important target in HIV drug development. Several natural products from traditional medicine have been shown to possess HIV-protease inhibitory activity [5,6].

Costus pictus D. Don. was included in the present *in vitro* study. It is one of the folk medicines used for the treatment of diabetes mellitus [7]. However, it has been found to possess antibacterial [8,9], anthelmintic [10] and anticancer [11,12] activities. Hence in the present study, aqueous and hydroalcoholic (HA) extracts of *Costus pictus* D. Don. were evaluated for their effect on pepsin enzyme, which was used as a substitute for HIV-protease. Pepsin assay is useful for preliminary screening of natural products for probable HIV-protease inhibitory activity.

MATERIALS AND METHODS

Collection of the material

Costus pictus D. Don was collected from Goa by Dr. Ramkrishna Y. Ambaye. It was identified by Dr. Rajendra D. Shinde, Associate Professor, Department of Botony, St. Xaviers College, Mumbai. Fresh specimen was deposited at the Blatter Herbarium of St. Xaviers College that matched with the Blatter Herbarium specimen AM-1.

Preparation of extracts

Finely chopped fresh leaves of *C. pictus* were used to prepare aqueous as well as hydroalcoholic (HA) extracts. Aqueous extract was obtained by hot decoction method using distilled water, whereas, hydroalcoholic extract was prepared by cold maceration using 20% ethanol. Similarly, the leaves were shade dried, powdered and then subjected to aqueous and hydroalcoholic extraction as stated above. All the extracts were made free from solvents using boiling water bath and percentage yield of individual extract was calculated. Thus, there were 4 extracts, namely, Fresh-Aqueous, Fresh-HA, Dry-Aqueous and Dry-HA. Extracts were kept at 4^0C until further use.

Assessment of pepsin enzyme inhibitory activity

Pepsin enzyme inhibitory activity was carried out using hemoglobin as a substrate. Preparation of hemoglobin and the pepsin assay was carried out as stated earlier [13].

DPPH radical-scavenging assay

The free radical scavenging activity of *C. pictus* was measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay [14]. For this, 1 ml of DPPH solution (0.1mM) in methanol was added to different concentrations of extracts. After incubating for 30 minutes in dark, the absorbance was measured at 517nm using Multimode Reader (Synergy HT, BioTek). Ascorbic acid at various concentrations (3-7 µg/ml) was included as a standard. A negative control without extracts was run in parallel. The percent DPPH-scavenging activity was calculated as, DPPH scavenged (%)= $A_{\text{Negative control}} - A_{\text{Test}}/A_{\text{Negative control}}$ X 100, where, A is absorbance. The antioxidant activity of *C. pictus* is also expressed as IC50 value.

Phenolic content estimation

The total phenolic content of *C. pictus* was determined using Folin-Ciocalteu reagent according to the method of Rege et al. [14]. Gallic acid at various concentrations (4-20 µg/ml) was included as a standard. All the determinations were done in triplicate. Mean values of triplicate determinations were used to plot the graph. Total phenolic content was calculated from the equation (y= 0.045x, R2= 0.997) obtained from the Gallic acid standard curve. The total phenolic content was expressed as Gallic acid equivalent (GAE) in mg/g of dry sample.

Statistical analysis

All the determinations were done in triplicate. Means and standard deviations were calculated using a Microsoft Excel program. Statistical analysis was done using one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Prism 5 software and $P<0.05$ was regarded as significant.

RESULTS AND DISCUSSION

The development of safe, effective and low-cost anti-HIV agents is among the top global priorities of drug development, since the long-term complications of this disease are multifactorial and can be related to the virus itself or to adverse effects of antiretroviral therapy [15]. Pepsin has a close resemblance with HIV-protease in proteolytic activity as both of them belong to same aspartate enzyme family [16]. Hence, in the present study, pepsin was used as a substitute for HIV-protease. Similar studies have been carried out by Singh et al. [17] and Govindappa et al. [18]. In general, all the extracts revealed potent inhibitory activity against pepsin enzyme, with Fresh-HA showed the highest inhibitory activity followed by Dry-HA (Table 1), suggesting that *C. pictus* might be useful as a HIV protease inhibitor. As various previous studies suggested structural and functional similarity between pepsin and HIV protease [13], *C. pictus* extracts that showed inhibitory activity of pepsin enzyme should also inhibit activity of HIV protease.

Oxidative stress plays a critical role in HIV expression and AIDS development [19]. Antioxidants offer a promising, natural and inexpensive remedy to alter the course of HIV infection to AIDS [20]. Hence in present study, antioxidant effects of *C. pictus* extracts were evaluated by testing their ability to bleach (purple to yellow color) the stable DPPH radical which is a widely used rapid and simple method. Among the four extracts, Dry-HA exhibited strong antioxidant effect (Table 2). A study by Jayasri et al. [21], also reported antioxidant activity of *C. pictus* in an *in vivo* study.

Table 1: Effect of extracts on pepsin enzyme

Extract	% Inhibition (Mean ± SD)
Fresh- Aqueous	77.47±9.1
Fresh- HA	93.49±2.8*
Dry- Aqueous	56.12±6.2**
Dry- HA	88.11±8.6
Pepstatin A (Standard)	78.56±0.8

*P<0.05 and **P<0.01 (as compared with Standard)

Table 2: Effect of *C. pictus* on DPPH

Extract	Conc. (mg/ml)	% DPPH Scavenged (Mean ± SD)	IC_{50} (mg/ml)
Fresh-Aqueous	0.5	18.24 ± 2.7	
	1	30.33 ± 3.5	2.82
	2	44.38 ± 1.9	
	3	55.55 ± 3.6	
	4	62.20 ± 1.2	
Fresh- HA	0.5	21.51 ± 1.7	
	1	30.62 ± 1.3	2.43
	2	49.31 ± 0.3	
	2.5	54.65 ± 0.5	
	4	66.97 ± 1.7	
Dry- Aqueous	0.2	12.68 ± 3.3	
	0.4	24.69 ± 2.7	1.28
	0.8	38.16 ± 3.2	
	1.6	60.79 ± 6.7	
	2	70.46 ± 1.7	
Dry- HA	0.1	5.81 ± 5.9	
	0.2	10.49 ± 2.2	1.03
	0.4	21.36 ± 2.8	
	0.8	42.49 ± 3.1	
	1.2	56.79 ± 1.9	
Ascorbic Acid (Standard)	-	-	5.61 µg/ml

Pepsin enzyme inhibitory and antioxidant activities of *C. pictus* can be attributed to its phenolic content (Table 3). Phenolic components have shown antioxidant effect by various mechanisms [22]. Hence total phenolic content of *C. pictus* extracts was determined by Folin-Ciocalteu method.

Table 3: Phenolic content estimation of extracts

Extract	Gallic acid equivalent (mg/gm)*
Fresh- Aqueous	43.9
Fresh- HA	60.1
Dry- Aqueous	93.2
Dry- HA	78.8

*Mean of triplicate determinations

Group of phenolics includes tannins, simple phenols and phenolic acids, quinones, flavonoids, flavones and flavonols, coumarins to name a few [23]. These phenolics have shown HIV protease inhibitory activity in the past [24, 25]. Besides phenols, preliminary phytochemical investigation of *C. pictus* revealed presence of steroids, alkaloids, glycosides, quinones, coumarins and flavonoids. Further, the phytochemical profile was found to be stable in spite of varied geographical locations and environmental variables [26]. These phytoconstituents have shown anti-HIV activity by different mechanisms, such as, inhibition of 3 viral enzymes and/or interfering with HIV gp120/CD4 interaction [27,28]. *Lageratroemia speciosa* has been used traditionally for treating diabetes and obesity. It has found to inhibit HIV protease and reverse transcriptase enzymes. The phytochemicals present were thought to be responsible for this anti-HIV activity [29]. Hence *C. pictus* should be further explored for novel anti-HIV compounds with possible mode(s) of action. Furthermore, *C. pictus* also revealed good antioxidant potential which would help in decreasing damage caused by oxidative stress in AIDS.

CONCLUSION

The present study indicates that *C. pictus* might be of value as putative HIV-protease inhibitor.

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