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Antiviral activities of *Aporosa lindleyana* Baill

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Abstract

The antiviral activities such as in vitro HBsAg binding, HBV-DNA polymerase inhibition, RT (non-isotopic) inhibition, HSV inhibition were studied for the ethanolic extract of the root of Aporosa lindleyana Baill. It was found to possess potent in vitro HBsAg binding activity. The minimum inhibitory concentration (MIC) was also determined.

Keywords: *Aporosa lindleyana* Baill, HBsAg, HSV inhibition

INTRODUCTION

Aporosa lindleyana Baill. (Fam. Euphorbiacee) is a tree, the root of which is traditionally given for jaundice, fever, headache, seminal loss and insanity [1]. It is distributed in the Western Ghats of India. 50% ethanol extract of the plant is found to be hypoglycemic [2,3]. β-sitosterol and β-sitosterolglucoside are reported on this plant [4]. Hardly a solitary report is available on *Aporosa* genus mentioning the antiviral activity of *Aporosa Villosa* [5]. In this communication, antiviral activities such as *in vitro* Hepatitis B surface Antigen binding, Hepatitis B Virus-Deoxyribo Nucleic Acid polymerase inhibition, Reverse Transcriptase (non-isotopic) inhibition, Herpes Simplex virus inhibition on the root of *A.lindleyana* are presented for the first time.

MATERIALS AND METHODS

Plant material

The root of *Aporosa lindleyana* Baill was collected from Keeriparai area of Kanyakumari District, Tamil Nadu, a part of the Western Ghats of South India in the month of May 2000. The specimens were identified by a taxonomist Dr. V. Chelladurai, Research Officer (Botany), Survey of Medicinal Plants unit, CCRAS, Palayamkottai – 627 002, Tamil Nadu, India and the voucher specimen(No. MSU 001) was submitted at MSU Herbarium, MS University, Tirunelveli, Tamil Nadu, India

Extraction

Dried and ground root of *Aporosa lindleyana* Baill (709g) was successively soxhlet - extracted with light petroleum, chloroform, ethanol and water. Each extract was concentrated in vacuo to dryness yielding: 2.60, 2.80, 8.76 and 8.92%(W/W)respectively. Antiviral screening of the ethanol and aqueous extracts were carried out

Antiviral screening

a) *in vitro HBsAg binding study*

Equal volume of HBsAg positive plasma and the cold and hot ethanol and aqueous extract (5mg/ml) were mixed and incubated at 37ºC for 5 days. The mixture was assayed daily using hepanostika Elisa kits (ELISA KIT HEPANOSTIKA HBsAg Uniform ll HBs Ag kit – Organon Technika, Holland) for 5 days. Control tubes containing plasma with a solvent alone were incubated. The binding effect of the extracts were analysed for ELISA procedure conducted every day [6]. Petroleum ether (40º-60ºC), benzene and chloroform hot extracts were also subjected for this study. Minimum inhibitory concentration (MIC) was determined for the ethanolic extract upto 72 hrs.

b) HBV – DNA polymerase inhibition study

HBV-DNA polymerase inhibition study was performed according to Lofgren *et al*[7] Both the test and control were spotted in Whatmam DE 81 filter paper and DNA was precipitated by adding cold 5% Trichloroacetic acid and 0.1% pyrophosphate solution. The filter paper was washed thrice with the same solution used to precipitate DNA. It was transferred to scintillation cock-tail and radioactivity was measured. The radioactivity was correlated to HBV-DNA polymerase inhibition assay.

c) RT inhibition (non-isotopic)

RT inhibition study was performed as per the procedure adopted by Gopalakrishnan [8]. The reaction mixture containing first strand reaction buffer sodium pyrophosphate, human placental ribonucleus inhibitor, deoxyribonucleotide triphosphate were added and incubated at 42ºC for 40 min acted as a control. In the test, before RT is added to a reaction mixture, it was incubated with the extract and reconstituted in 10% DMSO. The presence or absence of C-DNA band in agarose gel was noted after incubating with reverse transcriptase.

d) HSV inhibition

HSV inhibition assay was carried as per the procedure of Aduma *et al* [9]. The extract was dissolved in Phosphate Buffer Saline (PBS) and centrifuged at 2000 rpm for 10 minutes. The supernatant liquid was filtered in a membrane filter (0.2 micron). The filtrate was used for antiviral studies against Herpes Simplex Virus.

RESULTS AND DISCUSSION

The cold and hot ethanolic and aqueous extracts of the root *A. lindleyana* alone showed positive results in *in vitro* HBsAg binding studies. The minimum inhibitory concentration (MIC) determined for the ethanolic extract was 1.25 mg/ml. No drug in modern medicine is a satisfactory antiviral agent against Hepatitis B. The surface antigen of Hepatiti B is required for the attachment and uncoating of the virus on the target cell. HBs Ag binding ability of a drug would indicate the direct inhibitory impact of the drug on HBV attachment and further complications in viral replication cycle.

Table 1. Antiviral assay of the root of *Aporosa lindleyana* Baill.

Plant extract	Dosage	*In vitro* HBs Ag binding					HBV- DNA Polymerase inhibition	RT inhibition	HSV inhibition
		24hrs	48hrs	72hrs	96hrs	120hrs			
Aqueous extract (Cold)	5 mg/ml	+	+	+	+	+	–	–	–
Ethanol extract (Cold)	5 mg/ml	+	+	+	+	+	–	–	–
Soxhlet extract:									
Petroleum ether (40º – 60ºC)	5 mg/ml	–	–	–	–	–	–	–	–
Benzene	5 mg/ml	–	–	–	–	–	–	–	–
Chloroform	5 mg/ml	–	–	–	–	–	–	–	–
Ethanol	5 mg/ml	+	+	+	+	+	–	–	–
Water	5 mg/ml	+	+	+	+	+	–	–	–
Ethanol extract (hot)	2.5 mg/ml	+	+	+					
	1.25 mg/ml (MIC)	+	+	+					
	0.625 mg/ml	–	–	–					

+ = Positive; – = Negative; MIC = 1.25mg/ml

In the present work, the ethanol and aqueous extracts of *A. lindleyana* were able to uncoat the virus cell efficiently since the result of HBV-DNA polymerase inhibition assay on all graded extracts of *A. lindleyana* were found to be negative, the plant drug is not able to damage viral macromolecular synthesis. The negative results of RT (non-isotopic) inhibition and HSV inhibition indicated that the plant drug was not effective in HIV and HSV infections respectively.

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