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Screening of antiviral property against tobamoviruses in latex of E*uphorbia tirucalli* L.

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ABSTRACT

In the present study Dichloromethane-methanol and Pet ether extracts of *Euphorbia tirucalli* latex were prepared and evaluated for antiviral activity against tobamo viruses *viz*. Tobacco Mosaic Virus (TMV) and Tomato Mosaic Virus (ToMV). Three concentrations of the respective extracts (50,100,150ppm) were taken for antiviral studies. Among the three concentrations, both Dichloromethane-methanol and Pet ether extracts showed maximum protection at 150ppm. Dichloromethane-methanol extract showed maximum protection (81%) than Pet ether extract against Tobacco Mosaic Virus (TMV) at 150ppm, whereas Pet ether extract showed maximum protection (80%) against Tomato Mosaic Virus (ToMV) at 150ppm when compared to that of Dichloromethane-methanol extract.

KEYWORDS

Latex;
Antiviral;
Tobacco mosaic virus;
Tomato mosaic virus.

INTRODUCTION

Euphorbia tirucalli (Euphorbiaceae) is a succulent plant commonly distributed to tropical areas and rainforests in the Amazon, Madagascar and South Africa. The plant is commonly called as Vajradruma (Sanskrit), Indian tree spurge or Milk bush (English) and Bontakalli (Kannada). The latex is used as an application for warts, rheumatism, neuralgia and tooth ache[1]. Latex is also used as antimicrobial, antiparasitic in treatments of coughs, cancer and other maladies as folk remedy[2]. The bark of this plant is used to treat fractures[1].

Viral pathogens have become major constraints to the production of tomato and pepper. Tobacco mosaic (ToMV) and Tomato mosaic tobamovirus (TMV) are the important viral pathogens. Various mechanisms are involved in resistance phenomena against plant viruses including localization, local acquired resistance, systemic acquired resistance, green islands and chemically induced resistance[3]. Effective viricides are lacking for the control of viral diseases of plants. However, safer botanical extracts are gaining importance in modern days for crop protection against pests and diseases including viral infections. Plant extracts have also been found effective against wide range of pathogens[4]. Higher plants possess endogenous virus inhibitors, of which proteina-

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TABLE 1 : Qualitative analysis of phytochemicals of latex extracts

Extracts	Alkaloid	Flavanoid	Steroid	Saponin	Cardiac-glycoside	Phenols	Tannins	Anthraquinone
Dichloro Methane-methanol	+	+	+	-	+	+	+	+
Pet ether	+	+	+	-	-	+	+	-

ceous antiviral substances are of particular interest[5]. Plant seed oils were mainly used to control plant pathogens *viz.*, fungal, bacteria and viral pathogens. Very little information is available on the effect of plant extracts on plant viruses. The latex of *Euphorbia tirucalli* is reported to contain antiviral property against some animal viruses *viz.* Herpes simplex, Hepatitis B, SARS etc. But such an activity has not been investigated involving plant viruses. Hence the present investigation is undertaken in order to evaluate the antiviral property in the extracts of latex of *Euphorbia tirucalli.*

Figure 1: Lesions on a tobacco leaf

MATERIALS AND METHODS

Plant material

The fresh latex of *E.tirucalli* was collected from the region of Chitradurga, Karnataka and authenticated by a Taxonomist at Department of Botany, Sahyadri Science College, Shimoga.

Preparation of extract

The latex material was dried in shade and powdered mechanically, for the preparation of dichloromethane-methanol (1:1) and pet-ether extracts, the dried powdered latex was subjected to cold extraction. The solvent was removed completely over the water bath and finally dessicator dried.

Phytochemical screening

Standard methods[7,8] were used for preliminary phytochemical screening of the extracts to know the nature of phytoconstituents present (TABLE 1).

Preparation of standard virus inoculum

The virus inoculum was prepared by using the pre-maintained virus infected leaf. 0.1 g of leaf material from Tomato (TMV) and Tobacco (ToMV) were homogenized with 0.1 M phosphate buffer in a pre-chilled pestle and mortar. After homogenization, the extract was filtered through the muslin cloth and the filtrate was used as a source of inoculum.

Plant used

Nicotiana glutinosa, which reacts necrotically to the inoculation with the Tobacco mosaic tabovirus (ToMV) and Tomato mosaic tabovirus (TMV).

Efficacy of extracts on *Nicotiana glutinosa*

The extracts were screened for their efficacy against ToMV and TMV on *Nicotiana glutinosa.* Three concentrations of the Dichloromethane-methanol and Pet ether extracts of *Euphorbia tirucalli* latex were prepared (50,100 and 150ppm) .The extracts were sprayed using a hand sprayer (Misty, Varun Industries) on to the labeled primary leaves of two months old *N.glutinosa* plant. The control plants were sprayed with distilled water. After 24 hours of treatment, the leaves were inoculated with TMV and ToMV. Observations on development of local lesions were recorded after 48 hours of inoculation. Each treatment contains five plants and the experiment was repeated three times.

The percentage of inhibition of local lesion formation by each local lesion formation by each treatment over the control was calculated based on the number of local lesion produced using the formula:

$$I=(C-T)/C\times 100$$

Where, I=Percent inhibition of lesion formation over control, C=Number of local lesions in control, T=Number of local lesions in plants treated.

TABLE 2 : Antiviral screening of *E.tirucalli* latex extracts

Extract used	PPM used	No. Of local lesions formed/100Cm^2		% Protection	
		TMV	ToMV	TMV	ToMV
Dichloromethane -Methanol	50	49	56	68	55
	100	37	47	76	62
	150	29	34	81	73
Pet ether	50	65	54	59	57
	100	39	39	75	69
	150	34	24	78	80
Control		157	126	-	-

RESULTS AND DISCUSSION

Treatment with Dichloromethane-methanol and Pet ether extracts of *Euphorbia tirucalli* latex showed reduced number of local lesions after inoculation with tobamo viruses at all three concentrations of 50,100 and 150ppm. Among the extracts, dichloromethane-methanol extract showed maximum protection (81%) than Pet ether extract and control against Tobacco Mosaic Virus (TMV) at 150ppm, whereas pet ether extract showed maximum protection (80%) against Tomato Mosaic Virus (ToMV) at 150ppm when compared to that of dichloromethane-methanol extract and control. (TABLE 2.).

The present investigation clearly showed the effectiveness of the respective extracts in promoting growth of the plant and induction of resistance against tobamoviruses. The results suggested that the antiviral concentration was reduced in treated plants when compared to untreated ones. Antiviral resistance by plant extracts could be due to one of the following mechanisms i.e. de novo synthesis of antiviral substances, production of virus inhibiting agents and production of mobile inducing signal that bind to host plant surface, which produces virus inhibiting agents[6]. The Flavanoids, tannins, terpenoids have been reported to show antiviral properties[12]. Further investigation is warranted in order to identify the specific compound which is responsible for the antiviral activity.

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