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Antiviral Activity of Three Plant Species, Rhus coriaria, Chenopodium quinoa, and Ailanthus altissima against Tobacco Mosaic Virus

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ABSTRACT

Research on natural compounds provides new alternatives for effective and sustainable control of plant viral pathogens. Herein, we prepared and investigated the *in vitro* antiviral activity of 60 plant species from 22 families. The hydroethanolic extracts of *Rhus coriaria*, *Chenopodium quinoa* and *Ailanthus altissima* have strong inhibitions on *Tobacco Mosaic Virus* (TMV) infection. Hydroethanolic extract of *C. quinoa* with half-maximal Effective Concentration (EC_{50}) value of 1.64 mg mL^{-1} exhibited the highest inhibitory effect against TMV. The extracts of *R. coriaria* and *A. altissima* with EC_{50} values of 2.82 and 4.42 mg mL^{-1}, being compared with *C. quinoa,* showed an anti-TMV activity at higher concentrations, respectively. The systemic assay indicated that all of the three extracts reduced the symptoms and negative effects of TMV on tobacco plants. The chemical analysis of *C. quinoa* extract demonstrated a rich profile of saponins and anthocyanins, while *A. altissima* and *R. coriaria* extracts were rich in phenolic compounds. These results displayed that *C. quinoa*, *R. coriaria*, and *A. altissima* extracts had significant antiviral activity, and could be used as suitable sources for discovering new antiviral agents.

Keywords: Antiviral agents, Inhibitory effects, Plant viruses, Screening plant extracts.

INTRODUCTION

Plant viruses cause diverse diseases in a wide range of crop plant species and account for a large portion of crop disease epidemics. It is well documented that these pathogens cause significant damages to quantity and/or quality of products in a wide range of crops worldwide. Despite the ambiguity existing about the clear data on the economic impact of plant virus diseases in agriculture, the annual worldwide yield reduction assignable to plant viruses is roughly worth $30 billion (Sastry and Zitter, 2014). Therefore, development of effective disease management strategies against plant viruses remains a major concern for growers. Common control strategies, including resistant hosts and chemical control of vectors, have limited success in controlling plant viruses (Jones and Naidu, 2019; Ritzenthaler, 2005). Researches on bioactive natural products provide new alternatives for developing effective and sustainable control of plant virus diseases. Noteworthy, some natural plant products, in addition to the ability of direct interference with viral replication, can also lead to the induction of immune response against viruses. (Ma *et al.,* 2020; Guo *et al.,* 2020). During the last decades, accumulated data have shown several plant products with potent antiviral activity, which implies the potentiality of plant products for the development of effective and sustainable control measures for plant virus diseases (Zhao *et al.,* 2017).

Tobacco Mosaic Virus (TMV), a member of *Tobamovirus* genus, is one of the most common viral pathogens in plants. Tobamoviruses collectively have a very wide host range and cause significant yield losses in many crops such as solanaceous, brassicas and cucurbits (Jewehan *et al.,*

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2021; Zhang *et al.,* 2016; Alishiri *et al.,* 2013; Choi *et al.,* 2009); Furthermore, various characteristics of TMV, including infection of the host plant by mechanical inoculation and showing local lesion phenomenon in some host plants, have made it particularly amenable as a preferred standard model for screening and evaluating the antiviral activity of numerous compounds (Scholthof, 2004).

Iran has a diverse climate in different geographical areas and provides a unique environment for growing a comprehensive source of plant species. Some of these plant species have been reported to possess antiviral effects, and several of them have been practically used for treating humans and animals infected with several viral diseases (Asadi-Samani *et al.,* 2016; Salehi *et al.,* 2018); Nevertheless, despite the rich biodiversity of unique natural plant species in different Iranian geographical regions, only a few studies have screened and isolated plant-based antiviral compounds (Sanjarian *et al.,* 2021; Biniaz *et al.,* 2021; Gholizadeh, 2019).

Despite the importance of identifying biogenic antiviral compounds with the advantage of high activity and low environmental side-effects, the antiviral effects of plant extracts on plant viruses have been studied in a few cases. In the current study, using ethnomedical approach and literature-based follow-up of the existing studies (Pan *et al*., 2013), we selected 60 plant species, and evaluated hydroethanolic extracts of their antiviral activity on TMV and characterized extracts with the most antiviral effect.

MATERIALS AND METHODS

Virus and Plant Materials

Tobacco Mosaic Virus (TMV) Shiraz, Iran, was propagated in *Nicotiana tabacum* L. var. Turkish and was purified as described by Gooding and Hebert (1967). The concentration of the virus was measured using a Nanodrop (ND-1000 Spectrophotometer) [$\text{virus concentration} = (A260) / E_{1cm}^{0.1\%,260\ nm}$]. The purified virus was stored at -20°C until use.

The plant materials (used for evaluation of antiviral activity) were collected from the Eram Botanical Garden (Fars Province, Iran), Bamu National Park (Fars Province, Iran), and Dena National Park (Kohgiluyeh and Boyer-Ahmad Province, Iran) in spring and summer 2018, and were identified by the Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Iran.

Preparation of Plant Extracts

The air-dried plant samples were ground to a fine powder using a grinder. Then, about 10 grams of the powdered leaves were extracted using 80% ethanol solvent at room temperature (24 hours). The extracts were then filtered through gauze and were clarified by centrifugation at 12,000×g for 15 minutes. The supernatant was collected and concentrated using a rotary evaporator (Heidolph, Germany) under reduced pressure. Then, the dried plant extracts were weighed, dissolved in a small volume of Dimethyl Sulfoxide (DMSO), and diluted to 20 mg mL^{-1} with distilled water. The DMSO concentration did not exceed 2% (v/v) for any of the solutions (Jing *et al.,* 2012).

Local Lesion Assay

Tobacco (*Nicotiana glutinosa*) plants at the 5-6 leaves stage were used to evaluate the plant extracts' antiviral properties against TMV. Each sample (20 mg mL^{-1}) was mixed with an equal volume of TMV solution (10 µg mL^{-1}) and was immediately inoculated on the half leaves of tobacco, whereas the opposite half leaves were mock (distilled water with 2% DMSO) inoculated, using 500-mesh carborundum as abrasive. The antiviral activity of the plant extracts was estimated according to the inhibition rates of local lesions on the leaves of *N. glutinosa*.

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The local lesions were numbered 3-4 days after inoculation. The MAP30 protein of *Momordica charantia*, a type I ribosome-inactivating protein and an antiviral agent (Moghadam *et al.,* 2016), was used as the positive control. The inhibition rate of viral infection was calculated using the following equation 1 (Verma *et al.,* 1996):

$$\text{Inhibition rate}\ (\%) = (1 - \mathrm{T/C}) \times 100 \quad (1)$$

Where, T is the average mean lesion number of treated half-leaves, and C is the average lesion number of the control halves. Seven replicates of each treatment were randomized on the leaves of test plants. This experiment was performed at least three times.

Leaf-Disc Assay

The extracts that showed more than 50% inhibition of viral infection by the local lesion assay on *N. glutinosa* were further tested with the leaf-disc method. For this method, the growing tobacco (*N. tabacum* L. var. Turkish) leaves were mechanically inoculated with TMV (10 µg mL^{-1}). After 72 h, 12-mm diameter leaf discs that were smooth and thin, and had no major veins, were cut from the leaf surface. The leaf discs were hovered on the solution of each sample (10 mg mL^{-1}) in a petri dish and were then incubated for 48 hours in growth chamber with light intensity of 3,000 lx (25±2°C). The discs that were floated on mock were used as the control. After 48 hours, leaf discs were ground in coating buffer, and using antiserum developed in Plant Virology Research Center, Iran, their viral accumulation were assessed by Enzyme-Linked Immunosorbent Assay (ELISA) (Clark and Adams, 1977) using antiserum developed in Plant Virology Research Center, Iran. Measurements were conducted by absorbance Plate Reader (model ELx808LBS, Biotek, USA), and the mean absorbance value (OD 405 nm) of three replicates for each experimental condition was taken. The inhibition rate was calculated according to Equation (2) (French and Towers, 1992):

$$\text{Inhibition rate}\ (\%) = (1 - \mathrm{C/C_0}) \times 100 \quad (2)$$

Where, C is the virus accumulation in the treated leaf discs, and C_0 is the viral accumulation in the control, using the A405 value of TMV at concentrations of 10, 5, 2.5, 1.25, and 0.625 µg mL^{-1}. TMV accumulation level was estimated from a standard curve generated.

Systemic Assay and Phytotoxicity

Healthy tobacco plants (*N. tabacum* L. var. 'Turkish') were sprayed with 5 mL (10 mg mL^{-1}) of plant extracts, *Ailanthus altissima* (AAE), *Chenopodium quinoa* (CQE) and *Rhus coriaria* (RCE) at the 5-6 leaves stage. After 24 hours, the treated leaves were uniformly inoculated with TMV inoculum (10 µg mL^{-1}) and were kept under greenhouse conditions (25±2°C, 12-hour photoperiods). The experiment involved five treatments: CQE+TMV, AAE+TMV, RCE+TMV, mock (Distilled water with 2% DMSO)+TMV and mock+water (healthy plants). Three plants were used for measuring growth parameters, i.e., height and the fresh weight of aerial parts at 40-days post-inoculation (dpi). The experiment was performed three times.

In a phytotoxicity bioassay, ethanol extracts of the three plant species were used at four concentrations (10, 20, 30, and 40 mg mL^{-1}) in an experimental greenhouse under 12-hr photoperiod, at 25±2°C conditions. One week after treatment, the leaves were visually examined for recording chlorotic and necrotic injury levels (Frąckowiak *et al.,* 2019).

Determination of Half Maximal Effective Concentration (EC_{50})

Dose-dependent inhibition analyses were further carried out to examine the antiviral potential of the selected extracts. The

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hydroethanolic extract of each species was diluted to 20, 15, 10, 5, 4, 2, 1, and 0.5 mg mL^{-1} with distilled water containing 2% DMSO: it was mixed with an equal volume of TMV inoculum (10 µg mL^{-1}) and immediately inoculated on the half leaf of tobacco (*N. glutinosa*). A mixture of DMSO solvent and the inoculum was rubbed on the opposite half leaf as a negative control. The number of developed local lesions was recorded at 3-4 days post-inoculation. The inhibition rate of viral infection was recorded and calculated by equation (1).

LC-ESI-MS Analysis

For the Liquid Chromatography (LC) Electrospray Ionization (ESI) mass Spectrometry (MS) analysis, a Perkin-Elmer API 165 (Norwalk, CT, USA) single quadrupole MS instrument with Turbo-Ion spray interface was applied in the negative and positive ion modes scan spectra (150 to 1000 amu). ESI settings were as follows: temperature 350ºC ~ ion spray 4500V, curtain gas 8 psi, and CEM detector 2300V in negative mode. The MS detector was coupled to Hewlett Packard (Palo Alto, CA, USA) 1100 HPLC system consisting of a high-pressure mixing pump, autosampler, column oven, and DAD. Online UV spectra were determined at 220-500 nm. Firstly, solutions (0.12 µg μL^{-1}) were extracted in formic acid: H_2O: acetonitrile (1:5:94, v/v), and centrifuged at 13,000×g for 3 minutes. Then, five microliters were injected into LC-MS. Linear gradient elution was carried out using H_2O with 0.1% formic acid as eluent A and acetonitrile 0.1% formic acid as B with an elution rate of 500 µL min^{-1}.

Statistical Analysis

Using the paired Student's t-test at P-value< 0.05, the significant difference between the mean values of treatments and controls were analyzed statistically. One-way ANOVA analysis was used for the virus effect assessment on growth parameters data. Group means values were compared using the Tukey method (P< 0.05). Statistical analysis was performed using SAS 9.4 statistical software.

RESULTS

Local Lesion Assay

The concentration of plant extracts used in this research exhibited different inhibition rates at 10 µg mL^{-1} against TMV in the local lesion assay (Table 1). Out of 60 extracts tested, 14 crude extracts had no positive effect on preventing the virus infection; though, the other evaluated hydroethanolic extracts had relative antiviral activity. Among these all, the leaves and flowers of *Ailanthus altissima* (80.1%), seeds of *Chenopodium quinoa* (90.3%), and leaves of *Rhus coriaria* (89.6%) showed higher inhibition rate against TMV infection; whereas MAP30 protein at 100 µg⁄mL resulted in 73.4% inhibition rate.

Leaf-Disc Assay

Based on the results of the local-lesion assay, 8 crude extracts were assessed using the leaf-disc assay. Except for *Ferula aucheri* (-3.55%) and *Rydingia persica* (Burm.f.) Scheen and V. A. Albert (-.51%), all the other crude extracts had inhibition effects against TMV replication with the inhibition rate ranging from 21.63% to 42.93%, repectively. The extract of *Ziziphus spina-christi* leaves had the lowest inhibition rate (21.63%), while *Ailanthus altissima* (AAE, 36.44%), *Chenopodium quinoa* (CQE, 42.02%), and *Rhus coriaria* (RCE, 42.93%) extracts had the highest inhibition rate (Table 2).

Systemic Assay

Our results showed that three extracts could reduce the negative impact (including,

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Table 1. The inhibition rate of plant extracts against *Tobacco Mosaic Virus* (TMV) infection on *Nicotiana glutinosa* by local lesion assay.[a]

Family	Plant name	Location GPS	Part of plant used	Inhibition rate (%)
Amaranthaceae	*Amaranthus blitoides* S.Watson	293650 N, 524215 E	AP	37.4
Anacardiaceae	*Rhus coriaria* L.	293813 N, 523127 E	L	89.6
Apiaceae	*Echinophora platyloba* DC.	293604 N, 524505 E	AP	8.5
Apiaceae	*Foeniculum vulgare* Mill.	293813 N, 523127 E	S	42.4
Apiaceae	*Ferulago angulata*	305214 N, 513021 E	AP	NI
Apiaceae	*Ferula aucheri*	310810 N, 505516 E	L	72.2
Apiaceae	*Prangos ferulacea* (L.) Lindl.	304133 N, 513827 E	AP	19.0
Apiaceae	*Trachyspermum ammi* (L.) Sprague	293813 N, 523127 E	S	NI
Apocynaceae	*Vinca major* L.	293813 N, 523127 E	L	7.9
Araceae	*Arum rupicola* Boiss.	304138 N, 513828 E	L	42.2
Asteraceae	*Lactuca serriola* L.	294342 N, 523520 E	L	NI
Asteraceae	*Calendula officinalis*	293813 N, 523127 E	AP	32.9
Asteraceae	*Cichorium intybus*	293813 N, 523127 E	L	28.0
Asteraceae	*Achillea millefolium* L	293813 N, 523127 E	AP	NI
Asteraceae	*Artemisia absinthium* L.	293813 N, 523127 E	AP	31.1
Asteraceae	*Tanacetum polycephalum* Sch.Bip.	304140 N, 513827 E	AP	NI
Asteraceae	*Achillea santolinoides* Lag.	304138 N, 513827 E	AP	32.5
Asteraceae	*Artemisia sieberi* Besser	293637 N, 525321 E	AP	37.4
Asteraceae	*Dahlia pinnata* Cav.	293813 N, 523127 E	AP	24.0
Boraginaceae	*Onosma rostellatum* Lehm.	293637 N, 525321 E	AP	NI
Boraginaceae	*Heliotropium europaeum* L.	294134 N, 525331 E	AP	21.5
Chenopodiaceae	*Chenopodium quinoa* Willd.	294346 N, 523515 E	S	90. 3
Chenopodiaceae	*Atriplex leucoclada* Boiss.	293650 N, 524215 E	AP	48.5
Chenopodiaceae	*Salsola imbricata* Forssk.	293650 N, 524215 E	WP	44.1
Euphorbiaceae	*Euphorbia erubescens* Boiss.	304139 N, 513828 E	AP	42.0
Hypericaceae	*Hypericum perforatum* L.	294354 N, 523535 E	AP	NI
Lamiaceae	Thymus vulgaris L.	294353 N, 523536 E	AP	19.8
Lamiaceae	Thymus daenensis Celak	293813 N, 523127 E	AP	26.1
Lamiaceae	*Stachys aucheri* Benth.	304141 N, 513829 E	AP	49.4
Lamiaceae	*Satureja bachtiarica Bunge*	293637 N, 525321 E	AP	26.8
Lamiaceae	*Ajuga austroiranica* Rech.f.	293637 N, 525321 E	WP	23.6
Lamiaceae	*Hyssopus officinalis* L.	294353 N, 523536 E	AP	36.3
Lamiaceae	*Nepeta persica*	293813 N, 523127 E	AP	9.3
Lamiaceae	*Melissa officinalis*	294353 N, 523536 E	L	40.0
Lamiaceae	*Zataria multiflora*	294353 N, 523536 E	AP	30.2
Lamiaceae	*Lamium album* L.	304140 N, 513829 E	L	NI
Lamiaceae	*Salvia officinalis*	293813 N, 523127 E	AP	51.6
Lamiaceae	*Stachys benthamiana* Boiss.	293637 N, 525321 E	AP	59.6
Lamiaceae	*Marrubium vulgare* L.	293925 N, 525312 E	AP	29.7
Lamiaceae	*Rydingia persica* (Burm.f.) Scheen & V.A.Albert	293637 N, 525321 E	R	56.7
Lamiaceae	*Micromelia persicae*	293637 N, 525321 E	AP	NI
Lamiaceae	*Hyoscyamus bornmuelleri* Khat.	293637 N, 525321 E	AP	37.1
Lamiaceae	*Lophanthus depauperatus* (Benth.) Levin	293925 N, 525312 E	AP	NI
Lamiaceae	*Ballota aucheri* Boiss.	293925 N, 525312 E	AP	42.6

[a] NI: No Inhibition; L: Leaves; R: Roots; S: Seed; WP: Whole Plant; B: Bulb; AP: Aerial Part, F: Flower. Inhibition rate (%) was calculated as outlined in the text. [b] The MAP30 protein was used as the positive control.

Table 1 continued…

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Continued of Table 1. The inhibition rate of plant extracts against *Tobacco Mosaic Virus* (TMV) infection on *Nicotiana glutinosa* by local lesion assay.[a]

Family	Plant name	Location GPS	Part of plant used	Inhibition rate (%)
Lamiaceae	*Hyssopus officinalis* L.	294353 N, 523536 E	AP	33.0
Liliaceae	*Fritillaria imperialis* L.	304138 N, 513830 E	B	16.5
Malvaceae	*Malva sylvestris* L.	304138 N, 513827 E	AP	NI
Malvaceae	*Malva neglecta* Wallr.	294342 N, 523520 E	AP	43.6
Malvaceae	*Alcea kurdica* Alef.	303633 N, 513642 E	F	38.3
Meliaceae	*Melia azedarach* L.	293734 N, 523141 E	L	19.3
Nitrariaceae	*Peganum harmala* L.	293637 N, 525321 E	AP	28.0
Plantaginaceae	*Veronica orientalis* Mill.	304138 N, 513827 E	AP	41.9
Plantaginaceae	*Plantago major* L.	293813 N, 523127 E	S	NI
Polygonaceae	*Rumex crispus* L.	293814 N, 523127 E	L	12.4
Pteridaceae	*Adiantum capillus-veneris*	293813 N, 523127 E	WP	31.1
Urticaceae	*Parietaria judaica* L.	293925 N, 525312 E	AP	NI
Rhamnaceae	*Ziziphus spina-christi* (L.) Desf.	293813 N, 523127 E	L	54.9
Rosaceae	*Crataegus ambigua* C.A.Mey. ex A.K.Becke	293813 N, 523127 E	L	NI
Simaroubaceae	*Ailanthus altissima* (Mill.) Swingle	293816 N, 523127 E	L, F	80.1
Zygophyllaceae	*Tribulus terrestris*	294353 N, 523536 E	AP	41.3
	MAP30[b]			73.4

[a] NI: No Inhibition; L: Leaves; R: Roots; S: Seed; WP: Whole Plant; B: Bulb; AP: Aerial Part, F: Flower. Inhibition rate (%) was calculated as outlined in the text. [b] The MAP30 protein was used as the positive control.

Table 2. The antiviral activity of 8 crude extracts on the viral accumulation in the leaf-disc assay.

Name	OD_{405}[a]	Viral concentration (µg mL^{-1})	Inhibition rate (%)
Ailanthus altissima	0.706 [a]	3.52	36.44
Chenopodium quinoa	0.614 [a]	3.21	42.02
Ferula aucheri	1.305 [b]	5.53	-3.55
Rhus coriaria	0.599 [c]	3.16	42.93
Rydingia persica	1.281 [b]	5.45	-0.51
Salvia officinalis	0.804 [d]	3.85	30.49
Stachys benthamiana	0.906 [d]	4.19	24.30
Ziziphus spina-christi	0.950 [d]	4.34	21.63
Control	1.307 [b]	5.54	

[a] Data are expressed as the mean of three biological replicates. Different letters indicate statistically different values ($P < 0.05$, Tucky test).

symptoms and reduction in plant growth) of the virus in infected plants (Figure 1). As a result of applying different extracts treatments, significant differences were observed between samples. As compared with the infected controls, these significant differences were in plant height and fresh weight. Forty days after inoculation, the significant differences of plant height (36.9%) occurred in infected plants pre-treated with CQE in comparison with the control (mock+TMV), followed by those pre-treated with RCE and AAE (31.6 and 28.3%, respectively) (Figures 1-a and -b). The plants treated with CQE were heavier (44.4%) than those treated with RCE and AAE (38.1 and 43%, respectively). As shown in Figure 1, there were no significant differences in height and fresh weight between these three groups. The growth parameters of infected plants pre-treated with extracts increased variably compared to the control (mock+TMV).

Phytotoxicity assay of each extract separately showed various symptoms on tobacco leaves including leaf spotting, leaf

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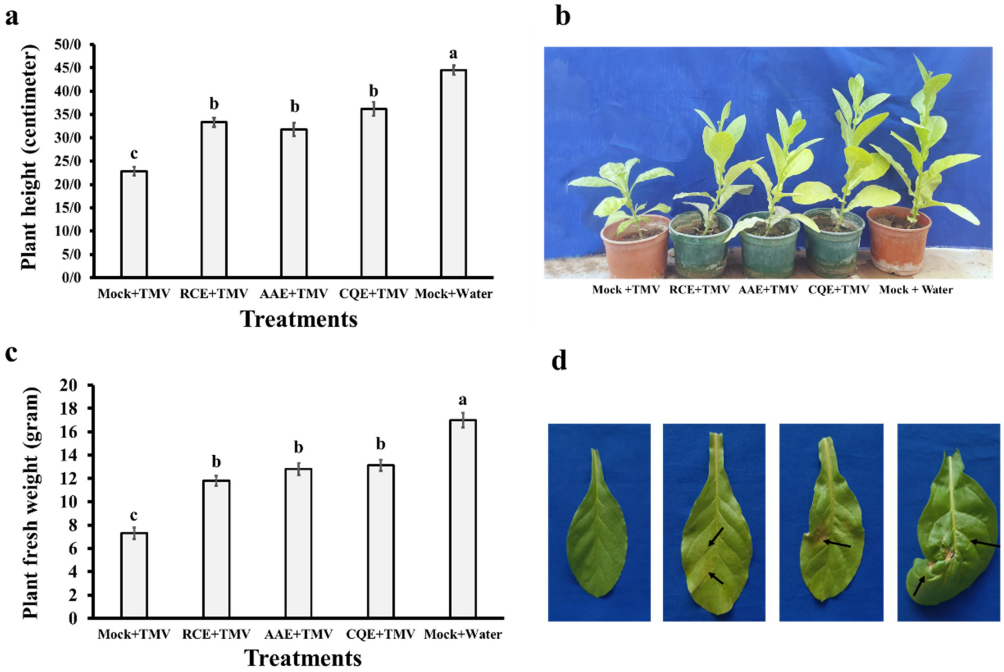

Figure 1. The effects of three plant extracts, *Rhus coriaria* (RCE), *Ailanthus altissima* (AAE), and *Chenopodium quinoa* (CQE) on growth parameters of TMV-infected tobacco plants at 40 days post-inoculation. (a) Plant height; (b) Phenotypical modification of tobacco plants in response to five different treatments;(c) Plant fresh weight; (d) Visual symptoms of phytotoxicity (Arrow) in leaves of tobacco sprayed with mock (distilid water+2% DMSO) and extracts of *Chenopodium quinoa, Rhus coriaria* and *Ailanthus altissima*, respectively, from left to right, 7 days after exposure to treatments. Data are expressed as the mean±SE (error bars) of three independent experiments. Different letters indicate statistically different values ($P< 0.05$, Tucky test).

deformation, and scorch or dead tissue at concentrations of 20, 30, and 40 mg mL^{-1} (Figure 1-d). In addition, phytotoxicity was observed in the leaves treated with all three extracts; though phytotoxicity intensity was higher in the leaves treated with AAE extract than in the other two extracts.

EC_{50}

Among the crude extracts with the highest antiviral activity, the EC_{50} values of CQE, RCE, and AAE were evaluated by local lesion assay. CQE exhibited the most inhibitory activity (EC_{50}, 1.64 mg mL^{-1}) on TMV, followed by RCE and AAE showed EC_{50} values 2.82 and 4.42 mg mL^{-1}, respectively (Figure 2).

Analysis of Secondary Metabolites

Mass spectrometry analysis was performed on the hydroethanolic (ethanol 80%) extracts of CQE, RCE, and AAE (Table 3) and showed higher antiviral activity than the other plant extracts. In particular, CQE showed a rich profile of saponins and anthocyanins, with a high amount of oleanolic acid. The main anthocyanins content of CQE was petunidin 3-*O*-glucoside (11.09 mg g^{-1}), cyanidin pentoside (9.12 mg g^{-1}), and 7-*O*-methylated anthocyanins (8.56 mg g^{-1}). In AAE, with the highest amount of the phenolic compounds, high concentrations were found of epicatechin, ferulic acid, and galloylglucose. Compared to other analyzed extracts, RCE demonstrated a

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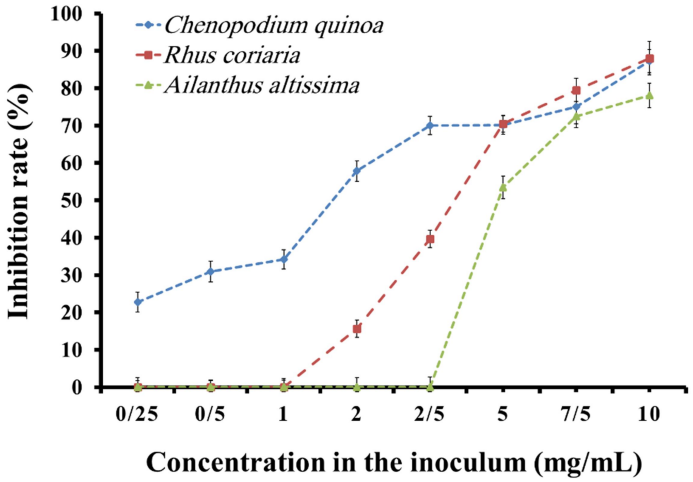

Figure 2. EC_{50} values of selected extracts against tobacco mosaic virus using local lesion bioassay in *N. glutinosa*. Error bars show the standard error of the average of three independent experiments with three replicates.

rich profile of phenolic compounds, with a significant concentration of ferulic acid, *p*-coumaric acid, sinapic acid, and isorhamnetin (Table 3).

DISCUSSION

Identifying a large number of valuable biogenic substance with insecticidal, antimicrobial, fungicidal, nematocidal, and antiviral activity in different plant species suggests an excellent opportunity for designing a novel biorational pesticides (Gonçalves *et al.,* 2021; Kaur and Chandi, 2021; Pushpa *et al.,* 2013). Therefore, owing to the limitation of the existing management of plant viruses, there is a global tendency to identify antiviral agents and search for biogenic product alternatives for the purpose of controlling viruses (Jing *et al.,* 2012; Sharma *et al.,* 2021).

In the present study, 60 plant extracts were screened for their antiviral activity against TMV as an experimental model, and their anti-TMV inhibitory effects were compared with that of MAP30 protein *in vivo* (Table 1). Extracts from six species, *C. quinoa*, *A. altissima, R. coriaria, Salvia officinalis, Stachys benthamiana, and Ziziphus spina-christi,* had significant inhibition against TMV replication. Among these six plants, *R. coriaria* (Ashoori *et al*., 2020), *S. officinalis* (Ghorbani and Esmaeilizadeh, 2017), *C. quinoa* (Pereira *et al*., 2020), *and Z. spina-christi* (Owayss *et al*., 2020) have been reported to have antimicrobial activity and have already been used for the prevention and treatment of some human and animal microbial infections. However, few studies have explored the antiviral activity of plant extracts, except that on *A. altissima* demonstrated potent antiviral activity (Ni *et al.,* 2019). The results of local lesion assays support the possibility of using *A. altissima* extract.

This study is the first report of potential antiviral activity of crude extracts of *S. benthamiana, S. officinalis, R. coriaria, C. quinoa,* and *Z. spina-christi* against plant viruses. Notably, *R. coriaria*, *C. quinoa,* and *A. altissima* extracts showed the highest inhibitory activity. Besides, as compared to the controls, the investigated growth parameters in systemic infection of TMV showed the potentiality of *R. coriaria*, *C. quinoa,* and *A. altissima* extracts to decrease TMV infection. In addition, results indicated

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Table 3. Mass spectrometry analysis of the secondary metabolites from the hydroethanolic extracts of three plants, *Rhus coriaria* (RCE), *Ailanthus altissima* (AAE), and *Chenopodium quinoa* (CQE).[a]

Type of phytochemicals	Compound	MW	Formula	RT (min)	CQE (mg g^{-1})	AAE (mg g^{-1})	RCE (mg g^{-1})
Phenolic compounds	Ferulic acid	194	$C_{10}H_{10}O_4$	3.15	0.25	5.83	2.19
	Gallic acid	170	$C_7H_6O_5$	3.36	0.41	1.64	ND
	Chlorogenic acid	354	$C_{16}H_{18}O_9$	11.14	ND	1.84	1.16
	Galloylglucose	332	$C_{13}H_{16}O_{10}$	17.26	0.32	4.92	0.77
	Ellagic acid	302	$C_{14}H_6O_8$	22.16	0.11	4.11	0.89
	Vanillic acid	168	$C_8H_8O_4$	25.25	0.32	2.76	1.16
	p-Coumaric acid	164	$C_9H_8O_3$	27.40	0.06	1.22	1.55
	Syringic acid	198	$C_9H_{10}O_5$	42.16	ND	ND	ND
	Sinapic acid	224	$C_{11}H_{12}O_5$	44.29	0.44	ND	1.84
Flavonoids and their glycosides	Quercetin	302	$C_{15}H_{10}O_7$	23.48	0.24	0.04	ND
	Rutin	610	$C_{27}H_{30}O_{16}$	24.43	ND	1.19	1.12
	Catechin	290	$C_{15}H_{14}O_6$	27.36	0.02	ND	ND
	Apigenin	270	$C_{15}H_{10}O_5$	30.30	0.03	0.96	0.15
	Epicatechin	442	$C_{22}H_{18}O_{10}$	21.71	ND	24.03	1.36
	Luteolin glucoside	448	$C_{21}H_{20}O_{11}$	29.74	ND	1.12	ND
	Quercetin 3-O-galactoside	626	$C_{27}H_{30}O_{17}$	30.02	0.53	3.50	1.66
	Isorhamnetin	316	$C_{16}H_{12}O_7$	34.17	ND	0.51	1.14
	kaempferol	286	$C_{15}H_{10}O_6$	41.31	0.02	ND	ND
Anthocyanins	Petunidin 3-O-glucoside	479	$C_{22}H_{23}O_{12}$	6.51	11.09	0.74	0.62
	Malvidin O-glucoside	493	$C_{23}H_{25}ClO_{12}$	8.25	5.15	1.48	1.36
	Cyanidin 3-O-β-dgalactopyranoside	449	$C_{21}H_{21}O_{11}^+$	9.31	6.84	0.19	0.77
	Cyanidin 3-O-galactoside	449	$C_{21}H_{21}O_{11}^+$	11.36	7.39	0.63	0.32
Saponins and their aglycones	Ginsenoside	801	$C_{42}H_{72}O_{14}$	3.22	ND	0.21	0.02
	Notoginsenoside R4	1241	$C_{59}H_{100}O_{27}$	3.61	ND	0.36	ND
	Floranotoginsenoside	1095	$C_{53}H_{90}O_{23}$	3.95	1.05	ND	0.95
	Oleanolic acid	456	$C_{30}H_{48}O_3$	4.15	9.12	ND	ND
	Notoginsenoside R3	933	$C_{48}H_{82}O_{19}$	4.29	ND	0.17	0.25
	Hederagenin	472	$C_{30}H_{48}O_4$	4.49	5.3	ND	ND
	Gypenoside XIII	755	$C_{41}H_{70}O_{12}$	4.66	ND	1.32	0.38
	Malonyl-vinaginsenoside	1033	$C_{51}H_{84}O_{21}$	4.83	ND	ND	ND
	Yesanchinoside	1093	$C_{53}H_{88}O_{23}$	5.11	ND	0.71	0.37
	Serjanic acid	500	$C_{31}H_{48}O_5$	5.26	2.07	ND	0.61
	Quinquenoside	819	$C_{42}H_{74}O_{15}$	5.34	0.11	ND	ND
	Ginsenoside Mc	755	$C_{41}H_{70}O_{12}$	5.47	0.02	1.65	0.05
	Phytolaccagenic acid	516	$C_{31}H_{48}O_6$	5.53	3.51	ND	0.4

[a] ND: Not Detected); MW: Molecular Weight, RT: Retention Time. Retention time was calculated as the time from injection to detection.

that treatment with extracts reduced the symptoms caused by TMV compared with the control group.

Assay of EC_{50} value highlighted the strong antiviral activity of *C. quinoa* extract compared to other extracts. The experimental results indicate that these plant species might potentially be sources of natural antivirus or might be used to develop new antiviral agents. Differences in the effects of crude extracts could be attributable to the diversity of plant samples, structural diversity of biogenic substances and the relative concentrations of bioactive components in the extracts (Shen *et al.,* 2007). Exploring various lead compounds, there has been a strong interest in natural product research, which may be used as models for developing new biorational compounds in the pharmaceutical and agrochemical industries. Due to restrictions on antiviral activity screening techniques, there is an immediate need for developing new approaches and methodologies to reduce the screening costs and time. In particular, integrating standard methods with computer-based techniques would be very useful. With

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the reduced number of possible bioactive compounds by in-silico methods, *in-vitro* and *in vivo* assays would further evaluate these components efficacy (Siqueira *et al.,* 2020).

Besides, we investigated chemical components present within the hydrohalic extract by LC-ESI-MS analysis (Table 3). The major components of *R. coriaria*, *C. quinoa,* and *A. altissima* extracts were ferulic acid (2.19 mg mL^{-1}), petunidin 3-*O*-glucoside (11.09 mg mL^{-1}), and epicatechin (24.03 mg mL^{-1}), respectively. The extracts of *C. quinoa* demonstrated a rich profile of saponins and anthocyanins. In the extracts of *A. altissima and R. coriaria,* a significant concentration of phenolic compounds including ferulic acid, galloylglucose, epicatechin, *p*-coumaric acid, sinapic acid, and isorhamnetin were observed (Table 3). Flavonoid glycosides from *Clematis lasiandra* demonstrated obvious antiviral activities against TMV with multiple modes of action (Li *et al.,* 2021). Three triterpene saponins were isolated from a MeOH extract of the leaves of *Ilex oblonga,* and showed potent antiviral activities against TMV (Wu *et al.,* 2007). Although several reports show that compounds of epicatechin, ferulic acid, galloylglucose, and oleanolic acid have antiviral properties (Behrendt *et al.,* 2017; Wang *et al.,* 2017; Su and D'Souza, 2013), when working with crude extracts, constituents responsible for bioactivity are often unknown. Therefore, to improve the efficacy of bioactive substances, they should be comprehensively characterized, and the identities of components contributing to the biological activity must be investigated. (Caesar and Cech, 2019). Therefore, to compare the profile of these metabolites among the evaluated plants, chemical analysis would be essential. This chemical profiling could provide clues to link the detected bioactivity with the natural abundance of some metabolites.

CONCLUSIONS

In this study, we have demonstrated the antiviral activity of 60 medicinal plants against TMV. Six of these plant samples showed potent antiviral activity against TMV. *R. coriaria, C. quinoa,* and *A. altissima* extracts demonstrated the most potent antiviral effect amongst the selected plant. To isolate and characterize the bioactive constituents responsible for the antiviral activity of these plant extracts, some further research is required. To the best of our knowledge, this is the first report of the potential antiviral activity of *Stachys benthamiana*, *Salvia officinalis*, *Rhus coriaria*, *Chenopodium quinoa,* and *Ziziphus spina-christi* extracts against the TMV. Therefore, these plant extracts can be considered good candidates in developing natural virucides.

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فعالیت ضد ویروسی سه گونه گیاهی *Chenopodium quinoa*، *Rhus coriaria* و *Ailanthus altissima* بر روی ویروس موزاییک تنباکو

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چکیده

تحقیقات بر روی ترکیبات طبیعی، فرصت‌های جدیدی برای کنترل موثر و پایدار بیمارگرهای ویروسی گیاهان فراهم نموده است. در این پژوهش، ما عصاره هیدرواتانولی ۶۰ گونه گیاهی از ۲۲ خانواده مختلف را آماده و فعالیت ضد ویروسی آن‌ها را بررسی نمودیم. از میان عصاره‌های گیاهی تهیه شده، عصاره‌های *Rhus coriaria*، *Chenopodium quinoa* و *Ailanthus altissima* ویروس موزاییک توتون را به خوبی مهار نمودند. عصاره *C. quinoa* با نمایش EC_{50} در سطح ۱/۶۴ میلی گرم بر میلی لیتر بالاترین تاثیر را در کاهش آلودگی ویروس موزائیک توتون داشت. عصاره‌ی گیاهان *R. coriaria* و *A. altissima* به ترتیب با مقادیر EC_{50} معادل ۲/۸۲ و ٤/٤٢ میلی گرم بر میلی لیتر در مقایسه با *C. quinoa* در غلظت‌های بالاتری ویروس موزائیک توتون را مهار نمودند. نتاج بررسی فعالیت ضد ویروسی عصاره‌ها بر روی ویروس موزائیک توتون در میزبانان سیستمیک نشان داد که هر سه عصاره به طور معنی داری در کاهش علائم ویروس در گیاهان توتون موثر بودند. تجزیه و تحلیل شیمیایی عصاره *C. quinoa* پروفایل غنی از ترکیبات ساپونین و آنتوسیانین را نشان داد در حالی که عصاره‌های *R. coriaria* و *A. altissima* بیشتر دارای ترکیبات فنولی بودند. نتایج این مطالعه نشان داد که عصاره گیاهان *C. quinoa*، *R. coriaria* و *A. altissima* فعالیت ضد ویروسی قابل توجهی دارند و می توانند به عنوان منابع با ارزش برای کشف ترکیبات ضد ویروس جدید مورد استفاده قرار گیرند.

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