Antiviral Activity of *Aloe pirottae* A. Berger Root Extracts Against Influenza A and B Viruses, Picornaviruses and Dengue Virus: An Endemic Plant Species of Ethiopia

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Abstract. This study is aimed to evaluate the antiviral studies of *Aloe pirottae* root extracts in Vitro on Influenza A and B viruses, Picornaviruses, and Dengue virus. During this study, crude methanol extract (MeOH) and its organic solvent fractions including n-hexane (HxF), chloroform (CHF), ethyl acetate (EAF) and n-butanol (BuF) of *Aloe pirottae* root in different concentrations were prepared and evaluated for a cytopathic effect (CPE) inhibitory assay for influenza virus and picornavirus and an immunofluorescence assay (IFA) for dengue virus. In vitro test revealed that *Aloe pirottae* root extracts have shown important effects in reducing both A and B influenza viruses, HRV B14 and EV-71(H) in MDCK cells, and DENV-2 replication in Vero cells. EAF exhibit more potential inhibitory activity with selectivity index >7.2 >17.8 and >8.5 than positive controls AMT with selectivity index not determined (ND), >47.6 and ND and RBV with selectivity index >2.9, >5.3 and >5 against PR8, HK and Lee, respectively. EAF exhibits the strongest anti-HRV B14 and anti-EV-71(H) activity in MDCK cells. Crude MeOH extract, EAF and BuF at both concentrations exhibit strong inhibition of DENV-2 induced CPE where as CHF and HxF at 20 μg/ml exhibit strong inhibition of DENV-2 production. The findings show significant inhibitory effect of *Aloe pirottae* root on influenza A and B viruses, HRV B14 and EV-71(H), and DENV-2 replication.

Keywords: Antiviral · Influenza A and B virus · Picornavirus · Aloe pirottae

1 Introduction

Viruses are generally classified in to DNA viruses and RNA viruses. They are responsible for causing number of diseases in both animals and plants. Influenza virus, Picornavirus

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such as rhinovirus and enterovirus-EV68 are examples of the causative agents of respiratory diseases [1, 2] and Dengue virus is one of the causative agents of acute febrile disease [3]. Despite the tremendous progress in human medicine, viral diseases, like SARS, acute febrile disease, acquired immunodeficiency syndrome (AIDS), and hepatitis are still the main cause of mortality in mankind. Emergence of latest drug-resistant viruses like influenza viruses and the lack of promising drugs and vaccines for several viral infections such as dengue viruses, rhinoviruses and enteroviruses have urged a requirement to developing new and effective chemotherapeutic agents to treat viral diseases. Crag and his colleagues have acknowledged that approximately 60% of the anti-tumor and anti-infective agents that are commercially available and utilized in clinical trials today are of natural products in origin [4]. Medicinal plants possess many active compounds, with high chemical diversity and biochemical specificity and offer major opportunities for identifying novel lead structures that are active against a broad range of assay targets. During this study *Aloe pirottae an* endemic medicinal plant of Ethiopia was selected for antiviral studies based on ethno pharmacological knowledge and native healers prescribing folk medicines. This plant features a history of traditional usage and wasn't studied for antiviral activity. *Aloe pirottae* Berger belongs to the family Aloaceae [5] and natively used as a folk medicine for the treatment of inflammation, viral, bacterial and fungal infections, malaria, ulcer, gastro-intestinal parasites, gallstone, eye diseases, constipation, burns, dermatitis, snake bite and as a insectifuge [6]. The target of this work is to assess the anti-viral activity of crude MeOH extract and its four organic solvent fractions of *Aloe pirottea* root against influenza virus strains A/Hong Kong/8/68 (H3N2, HK), A/Puerto Rico/8/34 (H1N1, PR8) and B/Lee/40 (Lee), picornavirus strains: rhino/HRV B14 and HRV A16; entero /EV 68, EV 71(H), CB3, PV3 using Cytopathic effect (CPE) reduction assay and for dengue/DENV-2 strain using an Immunofluorescence assay (IFA) method.

2 Materials and Methods

2.1 Chemicals and Reagents

The commercially available standard antiviral agents used in this study are ribavirin (RBV) and amantadine hydrochloride (AMT) obtained from Sigma (St Louis, MO), arbidol hydrochloride (ARB) from AK Scientific, Inc. (Mountain View, CA) and oseltamivir carboxylate (OSV-C) from US Biological (Swampscott, MA). Rupintrivir was synthesized in-house by I. Y. Lee. All solvents used for preparation of the extracts and purifications are HPLC-grade and obtained from Korea Research Institute of Chemical Technology, South Korea.

2.2 Plant Material

The plant material (root) of *Aloe pirottae* (Voucher No. A004/2017) was collected from Addis Ababa in November 2017 (see Fig. 1). The plant was identified and authenticated by Prof. Teshome Soromesa of the Biology Department, Addis Ababa University. The herbarium sheet of the specimen is preserved in the department of Biology, Addis Ababa University, Ethiopia.

2.3 Preparation of Plant Extract

The roots of *A. pirottae* were cleaned and cut into small pieces and air-dried at room temperature under shade. Then it was grounded to fine powder and was soaked in methanol in 3:1 ratio for 3 days. After 3 days methanol was squeezed out using rotary evaporator under reduced pressure at below 34 °C. This solid or semisolid material is named the crude methanol extract (MeOH). The MeOH is suspended in distilled water in the ratio of 1:10. This water suspension in separating funnel extracted each for 12 h three times with the water immiscible organic solvent in increasing polarity (n-hexane, chloroform, ethyl acetate and n-butanol) and shaken in 30-min interval. Whenever, the quantity of the organic solvent used was an equivalent as that of the water layer. The aqueous layer was extracted with the organic solvent in the order of increasing polarity and the organic layer was dried in the rotary evaporator. Each time, the collected organic solvent layer was combined and evaporated by rotary evaporator to dryness at temperature between 30–33 °C, and the small amounts of wet extracts from each solvent were then lyophilized by using vacuum dryer.

Fig. 1. *Aloe pirottae* Berger

2.4 Cells and Viruses

Influenza virus strains A/Puerto Rico/8/34 (H1N1) (PR8), Lee and A/Hong Kong/8/68 (H3N2) (HK) were obtained from ATCC. Influenza A viral PR8 and HK strains are propagated in 10-day-old chicken embryos at 37 °C for 3 days and influenza B virus (Lee) by infection of MDCK cells under serum-free conditions. Madin-Darby canine kidney (MDCK) cells and C6/36 mosquito cells (ATCC, Manassas, VA) were grown in minimum essential medium (MEM; Gibco/ Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Invitrogen) at 37 °C and 28 °C, respectively [7]. Picorna viruses were grown in RD cells. The cells maintained at 37 °C, 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) [8]. The RD-SCARB2 (RDS) cell line stably over expressing hSCARB2 was cultured in 10% FCS-DMEM supplemented with puromycin (0.5 μg/ml; Clontech, Mountain View, CA, USA). Stocks stored at −80 °C until use, as described in the literature. Picornaviruses were provided by the Bio and Drug Discovery Division, Korea Research Institute of Chemical Technology, Korea. DENV-2 (New Guinea C strain) was purchased from the National Collection of Pathogenic Viruses, Culture Collections of Public Health England (Salisbury, Great Britain) and propagated in C6/36 cells. DENV viral titters were quantified by focus-forming assay on Vero76 cells [9].

2.5 Antiviral Assay

Cytopathic Effect (CPE) Reduction Assay: CPE reduction assay was used for influenza and picornavirus antiviral assay**.** In the CPE reduction assay, MDCK cells are seeded in 96-well plates and either mock-infected or infected with virus at a multiplicity of infection (MOI) of 0.001 50 plaque-forming units (PFU) of given virus per well [7]. After incubation for 1 h at 35 °C, the medium was removed, and test and standard chemicals were added, which were serially diluted in MEM containing 2 μg/ml TPCK-trypsin (Sigma). On day 3 post-infection (p. i), the cell viability was measured after treatment with fluorescein diacetate (FDA; Sigma), as described by Kim et al. [10] and Schols et al. [11]. The 50% cytotoxic concentration (CC_{50}) and the 50% effective concentration (EC_{50}) values were calculated using Soft Max Pro Software (Molecular Devices, Sunnyvale, CA). The selectivity index (S.I.) is that the ratio of CC_{50} to EC_{50}.

Immunofluorescence Assay (IFA): The assay was used for DENV-2 antiviral assay. Vero cells were seeded on 96-well plates for DENV antiviral assay. After overnight incubation, cells were inoculated with DENV-2 at a multiplicity of infection (MOI) 0.2 for 2 h at 37 °C. Crude extracts were added at two different concentrations (20 μg/ml and 100 μg/ml). An immunofluorescence assay (IFA) used to detect dengue infection is optimized for the dengue high-throughput content imaging assay. Briefly, DENV-infected cells are detected by probing anti-DENV E (4G2) monoclonal antibody and Alexa Fluor 488 (A488)-conjugated goat anti-mouse IgG (H+L) (Invitrogen Molecular Probes, USA) as secondary antibody [7]. Cell nuclei counterstained with 5 μg/ml 4', 5-diamidino-2-phenyl indole (DAPI, Sigma-Aldrich, USA). After washing, digital images are acquired using Operetta® high content imaging system (Perkin Elmer, USA). The digital images were taken from 4 different fields of each well at 20X magnification. The percentage of inhibition was derived by using the formula; [1-(A488-positive cells/total cells)] × 100%.

3 Results and Discussion

There are reports on the antiviral properties of some Aloe species, however there is no reported work and data on antiviral activity of *Aloe pirottae*. Therefore, this is often the primary study of the antiviral activity of *Aloe pirottae* on human influenza A (PR8, BB, and KR; A/H3N2: HK) and B (Lee), viruses, picornaviruses HRV B14, HRV A16, CB3, PV3, EV68, EV 71(H) and DENV-2.In the anti-influenza virus assay, HxF, CHF and EAF exhibited strong antiviral activity against PR8, HK and Lee viral-induced cytopathic effect. Crude MeOH extract showed strong antiviral activity against PR8 and Lee affording 76% and 54% cellular protection, respectively. The most effective antiviral activity was obtained with CHF and EAF, which afforded complete cell protection against influenza A and B viral induced CPE (Table 1).

In cell cytotoxic effect assay, EAF exhibited significantly potent antiviral activity against PR8, HK and Lee with SI values of >7.2, >17.8 and >8.5, respectively, which was nearly 2.5-, 3.5- and 2- fold stronger than the positive control antiviral agent RBV against PR8, HK and Lee, respectively (Table 2). A literature survey indicated some

Table 1. Inhibition by *A. pirottea* root crude Methanol extract and its organic solvent layers of influenza PR8, HK and Lee viral replication. Results are expressed as percent of cell protection relative to control (100%).

S. no	Conc.	20 μg/ml			100 μg/ml		
	Extract/virus	PR8	HK	Lee	PR8	HK	Lee
1	MeOH	41%	44%	21%	76%	25%	54%
2	HxF	67%	73%	76%	−21%	−9%	−7%
3	CHF	117%	60%	97%	17%	24%	33%
4	EAF	69%	33%	65%	117%	100%	126%
5	BuF	10%	18%	12%	22%	6%	28%

extracts of Chinese herbs exhibiting anti-influenza activities in vitro, like Polygonum Chinese methanol extract inhibited PR8, HK and Lee viruses with SI values of 5.5, 7.8 and 5.4; EAF with SI values of 6.4, 13 and 5.9, respectively [12]. Some purified compounds inhibited PR8, HK and Lee viruses such as gallic acid (SI = 5.1, 6.3 and 6.5), ellagic acid (>3.7, >4.8 and >3.8), β-sitosterol (1.1, 1.0 and -), methyl gallate (16.6, 17.5 and 15.5) and caffeic acid (>7.9, >9.4 and >20.4). This study revealed that extracts of *A. pirottae* root showed higher anti-influenza A and B activity than reference antiviral reagent RBV and AMT. Therefore, these plant extracts exhibited significantly potent anti-influenza A and B activities and further study needed to isolate active principles and the exact mechanism underlying behind the activity.

In the anti-picornaviruses assay, EAF exhibit strong antiviral activity with cell viability 50% and 41% against HRV B14 and EV 71 (H) induced CPE. CHF exhibit strong antiviral activity with cell viability 46% against EV 71 (H) induced CPE. HxF exhibit moderate antiviral activity with cell viability 41% against EV68 induced CPE (Table 3). None of the extract/fractions at both concentrations had afforded >10% cellular viability against HRV A16, CB3 and PV3 replication. A literature survey indicated some extracts of Chinese herbs exhibiting anti- EV 71 (H) and coxsackievirus A16 (CVA16) activities in vitro, such as water extract of *H. cordata thumb* inhibited the CPE and plaque formation induced by EV71 and coxsackievirus A16 (CVA16), in Vero cells with an EC_{50} of 125.92 μg/mL. The *H. cordata thunb* extract (125 μg/mL) lowered the 50% viral RNA yield [13]. Geniposide, a primary component of *Fructus gardenia*, protected over 80% of cells against EV71 infection at a concentration of 3 mg/ml [14]. Pure compounds dihydroxy flavone, kaempferol, quercetin, hesperetin, and hesperidin isolated from Chinese herbal medicines *Chrysanthemum morifolium* reduced 80% of EV71-induced CPE at a concentration of 50 μM [15]. This study reveals that extract/fractions of *Aloe pirottae* root exhibited weak antiviral activity compared to some of Chinese herbal medicines against EV68, HRV B14 and EV 71 (H) induced CPE.

In the anti-DENV-2 assay (Table 4), the result demonstrate that when the cell was treated with 20 μg/ml of MeOH, HxF, CHF, EAF and BuF, infectivity by DENV-2 was reduced by 89%, 58%, 69%, 76% and 95% compared with that in untreated cells, respectively. MeOH, EAF and BuF at 20 μg/ml and 100 μg/ml exhibited significant protection

Table 2. Antiviral activity of HxF and EAF of *A. pirottea* root against influenza A and B viruses infecting MDCK cells[a]

	Toxicity CC^{b}_{50}(μg/ml)	Antiviral activity (EC^{c}_{50}μg/ ml)			Selectivity index[d]		
		Flu A	Flu A	Flu B	Flu A	Flu A	Flu B
		H1N1	H3N2	-	H3N1	H1N2	H3N2
		PR8	HK	Lee	PR8	HK	Lee
HxF	19.6	7.9	6.5	6.4	2.48	3.01	3.06
EAF	>100.0	13.9	5.7	11.7	>7.2	>17.8	>8.5
AMT	>100.0	>100.0	2.1	>100.0	ND[e]	>47.6	ND
RBV	>100.0	35.0	18.8	20.0	>2.9	>5.3	>5.0
OSV-C	>100.0	0.5	<0.0055	0.6	>204.1	>200.00	>175.4

Solubility: Good, a The data presented are the means ± standard deviations (SD) for three independent experiments. b The CC_{50} is the 50% cytotoxic concentration, which is defined as the compound concentration that produces cellular toxicity of 50%. c The EC_{50} is the 50% effective concentration, which is defined as the compound concentration that reduces the replication of influenza viruses by 50% in the CPE reduction assay. d Selectivity index, CC_{50}/EC_{50}. The 50% cytotoxic (CC_{50}) and 50% effective (EC_{50}) concentrations are calculated from concentration-effect curves after linear regression analysis.

Table 3. Inhibition by *A. pirottea* root crude methanol extracts and organic solvent fractions of picornaviruses replication. Results are expressed as percentages of cellular viability relative to control (100%)[c].

Solvent	HRV B14		HRV A16		CB3		PV3		EV68		EV 71(H)	
	100[a]	20[b]	100	20	100	20	100	20	100	20	100	20
Me	0	0	0	0	0	−1	0	−1	−1	−1	−5	−1
HxF	1	3	−2	0	0	−1	−1	0	41	−6	4	−3
CHF	1	1	−1	0	0	−1	0	−1	−12	−11	46	−5
EAF	50	0	10	1	0	−1	0	−1	−13	4	41	4
BuF	1	0	0	0	0	−1	−1	−1	−1	1	−2	6
Sample conc.in μM	5	1	5	1	5	1	5	1	5	1	5	1
Rupintrivir	104	100	105	102	107	99	103	102	106	99	73	104

a, b concentration in μg/ml, c Results were expressed as the mean value of three independent experiments. The absorbance of the control group was defined as 100%. Sample results are compared with Rupintrivir.

cells against the cytopathic effect of DENV-2 by 82 and 89%, 65 and76%, and 78 and 95%, respectively. HxF and CHF at 100 μg/ml exhibit complete inhibition of DENV-2

replication. In general, the higher concentration was more effective against virus than cells (treatment mode) and the lower concentration was more effective against virus infection (protective mode). A literature survey indicates extracts of plants exhibiting anti- DENV-2 activities. The investigation conducted with *Cladosiphonfucoidan* [16] demonstrated that when the virus was treated with 10μg/ml of fucoidan, infectivity by dengue virus serotype 2 was reduced by 80% compared with that in untreated cells and the determined IC_{50} corresponded to 4.7 μg/ml. Glabranine and 7-O-methylglabranine showed significant inhibitory activity and presented 70% DENV-2 infection inhibition at 25 μmol/l [17]. The cyclohexenylchalcone derivatives pandurantin A and 4-hydroxypanduratin A inhibited DENV-2 by about 27.1 and 52.0% at 40 μg/ml, 66.7 and 78% at 80 μg/ml, 92.2 And 97.3% at 240 μg/ml, and 99.8 and 99.6% at 400 μg/ml, respectively. Pinocembrin was inhibiting by about 60% at μg/ml concentration [18].

Table 4. Inhibition by *A. pirottea* root crude methanol extract and organic solvent fractions of DENV-2 virus. Results are expressed as percentages of cellular viability and viral inhibition relative to control (100%)[a].

S. no	Conc./extracts	100 μg/ml		20 μg/ml	
		% viability	% inhibition	% viability	% inhibition
1	MeOH	82%	−3%	89%	−9%
2	HxF	1%	100%	58%	44%
3	CHF	0%	100%	69%	16%
4	EAF	65%	22%	76%	7%
5	BuF	78%	2%	95%	−16%

[a]Results were expressed as the mean value of three independent experiments

Methyl gallate which was purified from the methanol extract of *Quercus lusitanica* inhibited 98% of DENV-2 NS2B/3 protease at 0.3 mg/ml [19]. Squalamine discovered within the tissues of the dog fish shark (*Squalus acanthias*) was evaluated in vitro on dengue virus infection of human endothelial cells (HMEC-1). At the concentration of 40 μg/mL, dengue infection was inhibited by 60%. The infection was completely suppressed at 100 μg/ml [20]. This study revealed that extracts of *Aloe pirottea* root exhibited stronger anti-DENV-2 activity than extracts of many herbs and the active compounds of natural products in above previous reports. Therefore, these plants extracts exhibited significantly potent anti-dengue virus activities and merit further study to isolate active principles along with testing in vitro and in vivo and the exact mechanism behind their activity.

4 Conclusions

This work shows that MeOH extracts and organic solvent fractions of *A. pirottae* root in vitro exhibit strong anti-influenza A and B, moderate anti-picornavirus and strong

anti-dengue-2 activity. The fact that the resulted broad spectrum antiviral activity demonstrates that traditional knowledge of medicinal plant usage and the utility of using ethno botanical leads is an efficient way of identifying biological antiviral activity. Strong anti-influenza A and B virus activity was demonstrated for extracts of *A. pirottae* root, which was stronger than the positive control antiviral agent RBV *A. pirottae* root significantly inhibited DENV-2 production as well as DENV-2 induced CPE and increased the cell viability of Vero cells. This data suggests that the extract and solvent fractions of this plant contains potent components utilized as wide-spectrum antiviral agents and applied to development of a unique herbal medicine. Therefore, further analysis has to be compelled to isolate the bio-active molecules and the exact mechanism underlying behind the activity.

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References

1. Carter, J., Saunders, V.A.: Virology: Principles and Applications, pp. 103–114. Wiley, Hoboken (2007)
2. Melnick, J.L.: Enteroviruses, polioviruses, coxsackieviruses, echoviruses and newer enteroviruses. Fields Virol. **3**, 655–712 (1996)
3. Mukherjee, A.K., Basu, S., Sarkar, N., Ghosh, A.C.: Advances in cancer therapy with plant based natural products. Curr. Med. Chem. **8**, 1467–1486 (2001)
4. Cragg, G.M., Newman, D.J., Snader, K.M.: Natural products in drug discovery and development. J. Nat. Prod. **60**, 623–639 (1997)
5. Demissew, S., Nordal, I.: Aloes and Lilies of Ethiopia and Eritrea, pp. 8–42 (2010)
6. Belayneh, A., Asfaw, Z., Demissew, S., Bussa, N.: Medicinal plants potential and use by pastoral and agro-pastoral communities in Erer Valley of Babile Wereda, Eastern Ethiopia. J. Ethnobiol. Ethnomed. **8**(1), 42 (2012). https://doi.org/10.1186/1746-4269-8-42
7. Kim, M., et al.: Inhibition of influenza virus internalization by (−)-epigallocatechin-3-gallate. Antiviral Res. **100**(2), 460–472 (2013). https://doi.org/10.1016/j.antiviral.2013.08.002
8. Wang, C.Y., et al.: Eupafolin and Ethyl acetate fraction of Kalanchoe gracilis stem extract show potent antiviral activities against Enterovirus 71 and Coxsackievirus A16. Evid. Based Complement. Alternat. Med. (2013)
9. Cos, P., Maes, L., VandenBerghe, D., Hermans, N., Apers, S., Vlietinck, A.J.: Ethnopharmacology – vol. II - Plants and Plant Substances Against AIDS and Others Viral Diseases (2015)
10. Kim, M., et al.: In vitro inhibition of influenza, A virus infection by marine microalga-derived sulfated polysaccharide p-KG03. Antiviral Res. **93**, 253–259 (2012)
11. Schols, D., Pauwels, R., Vanlangendonck, F., Balzarini, J., De Clercq, E.: A highly reliable, sensitive, flow cytometric/fluorometricassay for the evaluation of the anti-HIV activity of antiviral compounds in MT-4 cells. J. Immunol. Methods **114**, 27–32 (1988)
12. Tran, T.T., Kim, M., Jang, Y., et al.: Characterization and mechanisms of anti-influenza virus metabolites isolated from the Vietnamese medicinal plant *Polygonum chinense*. BMC Complement. Altern. Med. **17**, 162 (2017). https://doi.org/10.1186/s12906-017-1675-6

13. Lin, T.Y., et al.: Anti-enterovirus 71 activity screening of chinese herbs with anti-infection and inflammation activities. Am. J. Chin. Med. **37**(1), 143–158 (2009)
14. Lin, Y.J., et al.: Inhibition of enterovirus 71 infections and viral IRES activity by Fruct-us gardeniae and geniposide. Eur. J. Med. Chem. **62**, 206–213 (2013)
15. Tsai, F.J., et al.: Kaempferol inhibits enterovirus 71 replication and internal ribosome entry site (IRES) activity through FUBP and HN RP proteins. Food Chem. **128**(2), 312–322 (2011)
16. Kroeger, A., Nathan, M.B.: Dengue: setting the global research agenda. Lancet **368**, 2193–2195 (2006)
17. Sánchez, I., Garibay, F.G., Taboada, J., Ruiz, B.H.: Antiviral effect of flavonoids on the dengue virus. Phytother. Res. **14**, 89–92 (2000)
18. Kiat, T.S., Pippen, R., Yusof, R., Ibrahim, H., Khalid, N., Rahman, N.A.: Inhibitory activity of cyclohexenylchalcone derivatives and flavonoids of fingerrot, Boesenbergia rotunda (L.), towards dengue-2 virus NS3 protease. Bioorg. Med. Chem. Lett. **16**, 3337–3340 (2006)
19. Rahman, N.A., Hadinur, B., Muliawan, S., Rashid, N.N., Muhamad, M., Yusof, R.: Studies on Quercus. lusitanic extracts on DENV-2 replication. Deng. Bull. **30**, 260–269 (2006)
20. Zasloff, M., et al.: Squalamine as a broad-spectrum systemic antiviral agent with therapeutic potential. Proc. Natl. Acad. Sci. USA **108**, 15978–15983 (2011)