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Antiviral activity of crude extracts of *Synadenium glaucescens* (Pax) against infectious bursal disease and fowlpox virus

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The effect of crude extracts from different morphological parts of *Synadenium glaucescens* against infectious bursal disease virus (IBDV) and fowlpox (FP) virus using an *in ovo* assay were investigated. Viable 9 days embryonated chicken eggs were challenged with viral strains then treated with *S. glaucescens* extracts at concentration of 0.2 mg/ml. Un-inoculated group were saved as negative control and groups inoculated with virus and diluent saved as positive controls. The treatments were observed daily and embryo weights were measured 5 days post-inoculation. Embryo survival and mean embryo weight were significantly higher ($P \leq 0.001$) in groups treated with *S. glaucescens* extracts than the positive control. More than 50% of the extract prevented death and deformation of embryo and formation of pock lesions in embryos. Furthermore, the treatments with ethanolic extract of the root bark demonstrated significantly higher mean embryo weight compared to other extract for both viruses ($P \leq 0.001$). The mean embryo weights from eggs challenged with infectious bursal disease virus and fowlpox virus treated with the extract were 6.3 ± 2 and 5.9 ± 0.5 g, respectively. These findings demonstrate potential and feasibility of using *S. glaucescens* extracts for treatment of the viral diseases. Furthermore, it validates the ethnoveterinary exploitation at community level.

Key words: Chicken, gumboro, Fowlpox disease, liyugi, mvunjakongwa and viral infection

INTRODUCTION

Controlling diseases in tropical and sub-tropical countries like Tanzania is a continuing battle. In developing countries like Tanzania where poverty is an issue of serious concern, farmers have opted on traditional treatment and control of both human and animal diseases. This has been taken as a means of buffering up the lack of access and financial resource to afford buying the commercial vaccines and drugs. Traditional plants preparations for decades have been deployed and are increasingly been reported locally as effective to control and treat different kind of diseases including viral diseases affecting chicken. The use of plant extracts for control of viral diseases in rural Tanzania is not uncommon (Buza and Mwamuhehe, 2001).

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Table 1. Grouping and treatment allocation for the in ovo assay.

Group (G): n = 5	Treatment
G1 to G15	IBDV+extract+DMSO; 15 different extracts (SP/SD/SE 1-5)
G16 to G30	FPDV+Extract+DMSO; 15 different extracts (SP/SD/SE 1-5)
G31	IBDV alone (V-)
G32	FPVD alone (V-)
G33	IBDV+DMSO (VS+)
G34	FPDV+DMSO (VS+)
G35	Untreated embryonated chicken egg ECE (V-)

Synadenium glaucescens (euphorbiaceae) is been deployed by communities in Tanzania for ethnomedical (Chhabra et al., 1984) and ethnoveterinary purposes (Mabiki et al., 2011; Wickama et al., 2006; Wickama et al., 2004). The plant is known as Liyugi in Bena/Hehe language and Mvunjakongwa in Swahili language. The water extract of the leaves and stems of *S. glaucescens* have demonstrated antimolluscicidal activity (Kloo*s* et al., 1987) and weak inhibition of electrically induced contractions of the guinea-pig ileum (Rukunga et al., 1990). The viral diseases IBD and FP are among serious challenges for development of poultry industry in Tanzania (Yongolo et al., 2002). Despite the reports on the use of the plant in controlling various diseases, there is no report on the use of *S. glaucescens* in controlling both infectious bursal disease (IBD) and fowlpox disease (FPD). The aim of the study was to investigate the effectiveness of extracts from different morphological parts of *S. glaucescens* against infectious bursal disease virus (IBDV) and fowlpox disease virus (FPDV). Findings from this research provide valuable information on the usefulness of the plant against IBD and FPD in chicken.

MATERIALS AND METHODS

Plant collection and processing

Aided with local informants and botanist, the fresh plants were collected in Njombe region, Njombe district in southern higherlands of Tanzania. The samples were identified and confirmed by a botanist and voucher specimen was stored in the herbarium at the Botany Department, College of Natural and Applied Sciences of the University of Dar es Salaam (UDSM) in Tanzania, with specimen's number HOS/FM 3672. The roots, stems and leaves of *S. glaucescens* were cleaned and mechanically separated to get five parts; (1) the root bark, (2) root wood, (3) stem bark, (4) stem wood and (5) leaves. The samples 1 to 5 were air dried and pulverized to a particle size of 1 mm for use during extraction.

Extraction of crude extracts

The five dried and pulverized samples 1 to 5 each were soaked sequentially in solvents with increasing polarities [that is, petroleum spirit (P), dichloromethane (D) and ethanol (E)] twice each for 72 h for each solvent. After filtration, the extract was dried using rotary evaporator to obtain 15 crude extracts. The extracts were stored at 4°C before being used for antiviral tests. Extracts for use were dissolved in dimethyl sulphuroxide (DMSO) to make a concentration of 200 µg/ml. The extracts were coded with two letters, S denoting *Synadenium* followed by letter (P, D or E) denoting solvent type used and number 1 to 5 denoting the plant part.

Antiviral screening

Test organisms

The local strains of IBDV and FPDV were supplied by the Bacteriology and Mycology Laboratory, Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture. *In ovo* assay following the procedure by Sally (2002) and Senne (1998), with slight modification, was used to test the antiviral potential of *S. glaucescens* extracts. Embryonated chicken eggs (ECE) which were 9 days old, were checked for viability by candling before being used. The ECE were randomized into 35 groups (n = 5) and allocated as shown in Table 1. During inoculation, a hole was made through the egg shell just above the air sac to allow vertical inoculation of 0.1 ml of the inoculum into the chorialallantoic fluid. The first 15 groups were treated with 15 inocula made by mixing 0.9 ml of 0.2 mg/ml of different extracts and 0.1 ml of IBDV, and the second 15 groups were treated with 15 inoculum made by mixing 0.9 ml of 0.2 mg/ml different extracts and 0.1ml of FPV.

A group of un-inoculated ECE (V-) served as negative control, two groups of ECE inoculated with 0.1 ml virus suspension only (V+) served as a positive control, and a group of ECE inoculated with 0.9 ml diluents (dimethylsulphoxide) and virus (VS+) served as positive control to study the effect of solvent. After inoculation, the inoculated site was sealed with paraffin wax then the eggs were kept at 4°C (refrigerated) for one hour. The eggs were then incubated at 37°C with the air sac uppermost. Embryos survival was monitored daily by checking the embryo movements, blood vessels and time of embryo death through candling of eggs. Five days post-inoculation, the eggs were chilled and growing embryos were observed for growth and weight change. The assessments of antiviral activity were based on survival of the embryo, mean embryo weight (MEW), formation of pock lesions on eggs and embryo for the eggs infected with FPV and deformation of embryos observed in chicken embryonated eggs infected with IBDV.

Statistical analysis

Data collected were analyzed using CoStat Version 6.400 (CoHort Software, USA). The weights for different groups were reported in

MEW (%) ± standard deviation at 95% confidence interval. The differences of MEW were further analyzed by one-way analysis of variance (ANOVA) and significance was reported at $P \leq 0.05$. Comparison of means was performed by Tukey-Kramer test.

RESULTS

Observed time of embryo death, embryo deformation and Pox lesions formation

Death time and embryo conditions during incubation and during harvest are as shown in Table 2. More than 50% of the extracts prevented death, prevented deformation of embryo and also prevented formation of pock lesions. A hundred percent death was recorded in the two positive controls of IBDV (EVS+ and EV+) as well as in embryos treated with *Synadenium* dichloromethane 1 and 2 (SD1, SD2) and *Synadenium* ethanol 2 (SE2) extracts within five days of incubation in IBDV treatment group. In all dead embryos, there was deformation of embryo and pox lesion formation except for SD1 and SD2 groups.

Mean embryo weights of embryonated chicken eggs challenged with IBDV treated with extracts

There was a significant difference between MEW treated with different extracts ($P \leq 0.001$) in ECE challenged with IBDV, as indicated in Figure 1. The MEW in the positive control was significantly lower ($P < 0.001$) than that of the negative control and most of the extracts. There was no significant difference between the positive controls and the four extracts; SE2, SD1, SD4 ($P > 0.001$). The MEW of treated ECE ranged between 1.3 to 6.3.2 g. Generally, the embryos treated with petroleum ether extracts demonstrated higher MEW well above 4 g, followed by ethanolic extract and dichloromethane extracts which were the lowest. The results further indicated that MEW of the ECE treated with ethanolic extract SE1 were significantly higher ($P \leq 0.001$) 6.3 ± 0.2 g compared to all treatment groups.

Mean embryo weights of embryonated chicken eggs challenged with FPDV treated with extracts

Figure 1 shows the data about the effect of different extracts on the weights of FPDV infected embryos. The data shows that there was a significant difference between MEW of different extracts ($P \leq 0.001$) in ECE. The MEW in the positive control was significantly lower ($P \leq 0.001$) than that of the negative control and majority of the extracts except SP3, SP5, SE2, SD2 and SD1. The MEW of treated ECE ranged between 1.3 ± 0.1 to 6.3 ± 0.2 g. Generally, the embryos treated with ethanolic extract demonstrated higher MEW well above 4 g, followed by and dichloromethane extracts that recorded the lowest MEW. The results further indicate that MEW of the ECE treated with ethanolic extract SE1 were significantly higher ($P \leq 0.001$). The extract SE1 recorded MEW of 5.98 ± 0.5 g compared to all treatment groups.

DISCUSSION

This study is the first to report the activity of *S. glaucescens* extracts against IBDV and FPV using chicken embryo model. A hundred percent death of embryo within three days post inoculation implies that the viruses were virulent. It is clear from Table 2 that treatment with extracts prevented death of embryo or prolonged survival of embryo compared to positive control. Furthermore, it prevented embryo deformation in ECE challenged with IBDV and formation of lesions in ECE challenged with FPDV. Treatment with extract SD1, SD2 and SE2 indicated toxicity to the embryo and possibly viral strains. This is indicated by early mortality of the embryos with neither deformation nor pox lesion. The fact that these were toxic to the virus, they can be used in biosecurity measure as natural disinfectants for decontamination purposes.

The mean embryo data in Figures 1 and 2 records higher MEW in most of the ECE treated with extracts which indicates the ability of extract to prevent effects of viral strains on the growing embryo. The effects of the extract is then linked to increase of MEW of infected embryos and thus extract differed significantly in their effect on the viral strains by demonstrating different MEW of the treated embryo for each treatment. The continuation of embryo growth unveiled by increase in weight and organ formation in ECE challenged FPDV implies that the extracts could potentially interfere the viral replication cycle either by blocking one point of propagation mechanisms inside the cells, prevent the invasion mechanism or kill the virus in the Inoculum.

The significant difference in MEW for different extracts could be attributed to the diversity of compounds in the extracts. Each extract had a different degree of inhibitory activity and specificity against the virus and/or its essential enzymes. A diversity of antiviral agents with diversified mechanism is reported in plants (Jasmin et al., 2003; Mohamed et al., 2010). Different researchers have reported the attempts to use plant to inhibit the effects of virus on cells with positive and negative results.

Simon et al. (2007) and Esimone et al. (2007) reported negative results in *in vitro* screening of antiviral activity of more than 9 plant species from the Brazilian flora and Nigeria against IBDV and none of them were active. However, several species are reported to have the potential to inhibit the effect of IBDV and FPDV on cells

Table 2. Embryo deaths as observed from day 1 to day 5 post inoculation with IBDV and FPDV.

Inoculum		Time of embryo death (in days)					Other observations[a,b]
Extract in DMSO	Virus	Day 1	Day 2	Day 3	Day 4	Day 5	
SP1	IBDV						No embryo deformation
	FPV	0	0	0	0	1	Pock lesions
SP2	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	No pock lesions
SP3	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	1	Pock lesions
SP4	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	No pock lesions
SP5	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	Pock lesions
SD1	IBDV	3	2	0	0	0	No embryo deformation
	FPV	4	1	0	0	0	No pock lesions
SD2	IBDV	3	2	0	0	0	No embryo deformation
	FPV	3	1	1	0	0	No pock lesions
SD3	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	No pock lesions
SD4	IBDV	0	0	0	0	1	No embryo deformation
	FPV	0	0	1	2	0	Pock lesions
SD5	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	No pock lesions
SE1	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	No pock lesions
SE2	IBDV	1	3	1	0	0	Embryo deformation
	FPV	1	2	2	0	0	Pock lesions
SE3	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	No pock lesions
SE4	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	No pock lesions
SE5	IBDV	0	0	0	0	0	No embryo deformation
	FPV	0	0	0	0	0	No pock lesions
EV+	IBDV	0	0	2	2	1	Embryo deformation
	FPV	0	0	0	0	2	Pock lesions

EVS+	IBDV	0	0	3	2	0	Embryo deformation
	FPV	0	0	0	0	2	Pock lesions

[a]Embryo deformation = included any observed haemorrhage from ruptured blood vessels, perforation in membrane/skin and deformed shape of the embryo.[b]Spot like dots formed on chorio-allantoic membranes/egg shells due to infection with FPV.

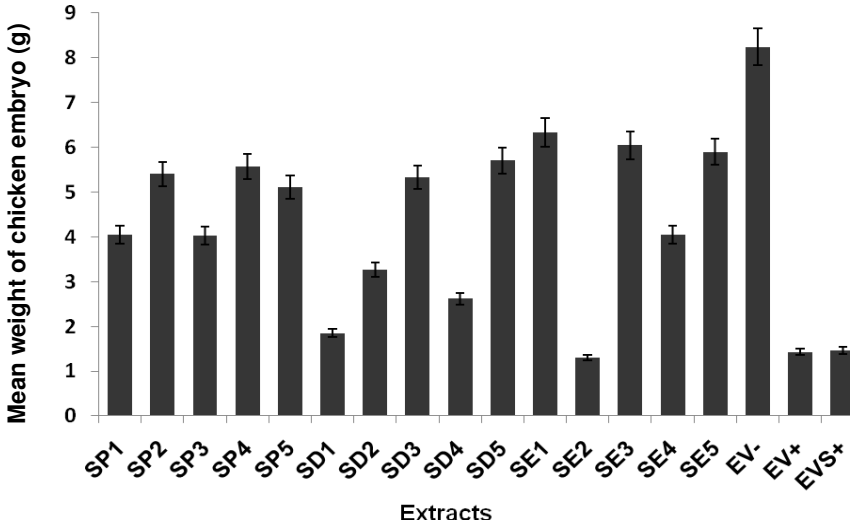

Figure 1. Mean embryo weights following inoculation of embryonated chicken eggs (ECE) with IBDV treated with different concentrations of crude extracts of *S. glaucescens*. Where S = synadenium, P = petroleum ether, D = dichloromethane, E = ethanol, No. 1, 2, 3, 4, 5 = rootbark, root wood, stem bark, stem wood and leaves, respectively. EV+ = positive control group of eggs inoculated with; EVS+ = positive control group of eggs inoculated with IBDV and DMSO, EV- = negative control group of un-inoculated eggs.

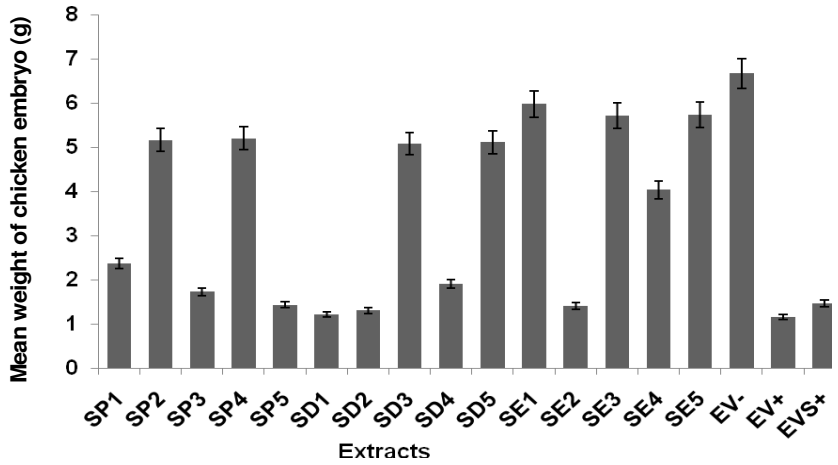

Figure 2. Mean embryo weights following inoculation of embryonated chicken eggs (ECE) with FPDV treated with different concentrations of crude extracts of *S. glaucescens*. Where S = synadenium, P = petroleum ether, D = dichloromethane, E = ethanol, No. 1, 2, 3, 4, 5 = rootbark, root wood, stem bark, stem wood and leaves, respectively, EV+ = positive control group of eggs inoculated with FPDV; EVS+ = positive control group of eggs inoculated with FPDV and DMSO, EV- = negative control group of un-inoculated eggs.

as reported by Meenakshi et al. (2009) on the *in vitro* activity of extracts from *Moringa oleifera*, *Holarrhena antidysenterica*, *Synzium aromaticum*, *Allium sativum*, *Piper nigrum* and *Azadirachta indica* against IBDV. Extracts from leaves of *Acacia arabica* and *Eugenia jambolana* are reported to have inhibited the replication of goat pox virus *in vitro* (Bhanuprakash et al., 2008). Mechanisms of the different compound responsible for the reaction and their mechanism is well explained by Jasmin et al. (2003).

The polar extract ethanolic SE1 recorded higher MEW in treating both ECE challenged with IBDV and pox, it prevented death, deformation and formation of pox lesions in ECE challenged with both IBDV and FPDV. In other studies, the polar extracts of *S. glaucescens* have demonstrated molluscicidal activity (Kloos et al., 1987). The activity of this extract could be attributed by the antiviral agents such as those with sugar moiety and other group of compounds reported to have antiviral activity which were previously reported in various extracts of *S. glaucescens* (Rukanga et al., 1990; Neuwinger, 1994; Jasmin et al., 2003).

Conclusion

These studies have demonstrated for the first time the antiviral potential of the extracts of *S. glaucescens*. The results indicate clearly that the plant extracts from S*. glaucescens* contains antiviral chemical constituents which can act against both IBDV and FPDV. These findings validate the ethnoveterinary uses of the plant and demonstrate a high potential and feasibility of using *S. glaucescens* extracts for treatment and control of IBD and FPD, especially in rural areas where conventional disease management options are limited. This study stands as a stepping stone towards further research on antiviral drug search from *S. glaucescens.*

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