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SCREENING OF*SECURIDACA LONGIPEDUNCULATA*ROOTEXTRACTS FOR ACTIVITY AGAINSTNEWCASTLE DISEASE VIRUSANDBACTERIAL ISOLATES FROM UPPER RESPIRATORY TRACT OF HUMAN

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Abstract

Securidacalongipedunculata refereed in Hausa language of the northern Nigeria as "Sanya, uwarmagunguna"literally meaning the mother of drugs, is used in the treatment of variety of ailments. Over the years, Newcastle disease (ND) has defied all control measures. The disease has remained at the forefront of infectious diseases afflicting poultry production after avian influenza, despite the continuous global use of million doses of ND vaccine annually. Upper respiratory tract infections are highly prevalent worldwide, and there are currently no medications or herbal remedies that have been conclusively demonstrated to shorten the duration of such infections in human. Hence, this study was carried out to determine the antiviral and antibacterial activities of Securidacalongipedunculata root extracts. The plant root was extracted with soxhlet apparatus using methanol and petroleum ether as the extraction solvents. The extracts were subjected to phytochemical screening using standard procedures. Antiviral activity of the extracts was determined using embryonated chicken eggs treated with 100EID_{50}/ 0.1 ml Newcastle Disease Virus (NDV) pre- treated with S. longipedunculata root extracts at concentrations of 5mg/ml to 40mg/ml based on the toxicity result and embryo survival was observed daily. The root extracts of the plant were tested against upper respiratory tract bacteria isolates of human using agar well diffusion method. The mean diameter zones of inhibition (ZI) were measured and results recorded in millimeter (mm). The result of phytochemical screening revealed the presence of some secondary metabolites of pharmacological significance. Antiviral assay showed that embryo survival was directly proportional to increasing extract concentration, just as increase in extract concentration was directly proportional to virus death. Root methanol extract showed slight activity against NDV at 20mg/ml with percentage mortality of 60 while the petroleum ether extract did not show any activity. Antibacterial activity test showed that increase in the concentrations of root extracts is directly proportional to the increase in the zone diameter of inhibition of the bacteria. The petroleum ether extract showed highest activity on Proteus sp.

at 120µg/ml with ZI of 30mm and least activity onStreptococcuspyogenes with 17mm ZI. The methanol extract exhibited activity on Streptococcuspyogenes only at 120µg/ml with ZI of 19mm. These findings have clearly demonstrated that, S. longipedunculataroot extracts have antiviral and antibacterial activity.

Keywords: NDV = Newcastle Disease Virus, EID50 = Egg Infective Dose of virus in 50% embryonated chicken eggs, ZI = Zone of Inhibition, mm = millimeter.

INTRODUCTION

*Securidacalongipedunculata*is a semi-deciduous shrub or small tree that grows up to 12 meter (36 feet) tall. The ethnomedicinal uses of *Securidaca longipedunculata* varies immensely. The common English names are: violet tree, fiber tree and Rhodesian violet. In Northern Nigeria, it is called Sanya, Uwar Magunguna in Hausa language, literally translated the mother of all drugs, a tribute to its very numerous medicinal uses (Dapar*et al.,* 2007). The most commonly used plant part is the root used in the treatment of a variety of ailments including coughs, headache, fever, malaria, tuberculosis and sexually transmitted diseases and many diseases in different geographical areas.

Newcastle disease (ND) is a viral disease of birds caused by a filterable virus Newcastle Disease Virus (NDV) which belongs to the family paramyxoviridae (Alexander, 1997). It is a peracute, acute and sometimes subclinical contagious disease of poultry (Health *et al*., 1991). Newcastle disease is considered among the most important disease of poultry and outbreaks with mortality of up to 100% is common (Alders and Spreadbrow, 2001; Sa'idu and Abdu, 2008) and can also cause conjunctivitis in humans, but the condition is generally very mild and self – limiting (OIE, 2012).
Chukwudi*et al*., (2012), reported a prevalence of 3.2% for NDV in clinically healthy chickens in Nsukka area, Nigeria. Manchang*et al*., (2004), reported a higher incidence rate (68.4%) of ND during the dry season against 34.6% in the rainy season and higher rate in the young (20.7%) against 12.1% in the adult. This disease is endemic, causing huge economic loses to farmers and hampering growth of poultry industries in Nigeria.

Upper respiratory tract infections (URTIs) are common acute infections involving the nose, paranasal sinuses, pharynx, larynx, trachea, and bronchi. It is usually identified by the community as common cold. According to the findings of Meneghetti (2006) and Abed and Boivin (2006),URTIs are the most common acute illness found in an outpatient setting which have a wide range of clinical manifestation that may vary from the common cold (mild and self-limiting) to a life threatening disease, such as epiglottitis.

MATERIALS AND METHODS

Collection and processing of plant part

Fresh rootsof *Securidacalongipedunculata* were collected directly the plant tree from Doguwa Local Government Area of Kano State, Nigeria during early morning hours. The plant roots were identified at Department of Plant Biology, Bayero University, Kano. The roots were then processed by washing

with clean water, air dried under shade and ground into powder using an electric blender as described by Mukhtar and Tukur(1999).

Collection of virus and 9- day old embryonated chicken eggs for the antiviral assay

A velogenic strain of NDV was obtained from department of viral research, National Veterinary Research Institute, Vom, Jos, while embryonated chicken eggs were obtained from poultry division, National Veterinary Research Institute, Vom, Jos, Nigeria.

Collection of bacteria and confirmation

Six (6) isolates of bacterial specie (*S. aureus*, *S. pneumoniae*, *S. pyogenes*, *K. pneumoniae*, *Proteus* sp. and *P. aeruginosa*) were obtained from Department of Microbiology, Aminu Kano Teaching Hospital, Kano State.

The bacteria were further subjected to confirmatory tests using biochemical procedures described by Cheesbrough(2006).Optochin sensitivity, catalase and bile solubility tests were done for *Streptococcus pneumoniae*. *Streptococcus pyogenes* was confirmed using catalase and bacitracin sensitivity tests. Methyl Red, indole and citrate tests were done for *Proteus* sp. *Pseudomonas aeruginosa* was confirmed by catalase, oxidase, methyl red and citrate tests. Catalase and coagulase tests were done to confirm *S. aureus* and *K. pneumoniae* was confirmed using catalase, oxidase, methyl red, indole, citrate, and urea tests and also hydrogen sulphide and gas production were observed.

Extraction of plant materials

A procedure by Redfern*et al*., (2014) was followed for extraction using Soxhlet apparatus. One hundred grams (100g) of each of the powdered plant material was extracted separately using methanol and petroleum ether as extraction solvents.

Phytochemical screening

Phytochemical screening was carried out using the method described by Trease and Evans (1989) for the presence of alkaloids, resins, tannins, saponins, glycosides, flavonoids, anthraquinone, and phenols (Poongothai*et al*., 2011).

Determination of EID_{50} (egg infective dose of NDV in 50% of embryonated chicken eggs)

The Egg infective dose (EID_{50}) of the virus was determined according to the method of Young *et al*., (2002). Sterile Bijou bottles were labelled 10^{-4} to 10^{-9}. Sterile phosphate buffered saline, PBS (0.9ml) was added to each bottle. A ten – fold serial dilution was carried out by adding 0.1ml of NDV to first bottle (10^{-4}) and mixed thoroughly. From the content of first bottle, 0.1ml was transferred asceptically to second bottle (10^{-5}) and mixed thoroughly. This transfer was done into the bottles until the last one (10^{-9}) when 0.1ml of the content was discarded into a discard jar. Embryonated eggs were divided into eight (8) groups of five (5) for inoculation of the dilutions. All groups were inoculated via allantoiccavity. First group were inoculated with 0.2ml of contents of 10^{-4}, group two were inoculated with 0.2ml of contents of 10^{-5}. Same inoculation was done to other groups with corresponding dilutions of virus up to six (6) which was inoculated with 10^{-9} of mixture. Uninfected control (PBS only) as well as virus control were included. Infected eggs were sealed and incubated in a humid chamber at 37^{o}C for 24 hrs. They were candled after 24hrs for unspecific death while percentage

mortalities after 48hrs and 72hrs was recorded. From this, 100 EID_{50}/ 0.1ml of the virus stock was made for the experiment.

Haemmagglutination test

This was done to confirm the presence of the virus in the virus suspension. A drop of 5% chicken red blood cell was mixed thoroughly with a drop of the virus stock on a clean slide for formation of agglutination (Young *et al*., 2002).

Inoculation of eggs

The method used by Chollom*et al*., (2012) was adopted. Nine day old embryonated chicken eggs were labelled according to the extracts and concentrations that were used. A set of plastic egg trays were thoroughly cleaned with Virkon® (Day – Impex limited, United Kingdom), the eggs were swabbed with 70% alcohol in cotton wool and transferred into the cleaned trays for inoculation.

Toxicity assay

This was carried out to check the toxicity of the extracts on the chicken embryos. Nine day old embryonated chicken eggs were divided into six groups of five. Group one (1) to four (4) were swabbed with 70% alcohol using cotton wool, punched with eggshell punch and immediately injected with needle attached to 1ml syringe. By keeping the needle and syringe vertical, the needle was placed through the punched hole and penetrated into the allantoic cavity (approximately 16mm) with the extracts at concentrations of 80, 60, 40, and 20mg/ml in that order. The needle was then withdrawn from the egg and the hole was sealed with melted wax. Group five (5) were inoculated with 0.2ml phosphate buffered saline (diluent control) while group six (6) were not injected with any extract (negative control). The eggs were sealed with molten wax and incubated at 37^0C for 24hours. This was done for each of the extract. Embryo survival was observed after 24hours (Chollom*et al*., 2012).

Antiviral assay

This was carried out to check the efficacy of the extracts using the procedure of Chollom*et al*. (2012). Group one (1) to four (4) of embryonated eggs were swabbed and placed in the bio - safety cabinet where they were punched and immediately inoculated with the virus via allantoic route, sealed with molten wax and incubated at 37^0C. After 24 hours, the methanol extract at final concentrations of 20, 15, 10 and 5mg/ml while petroleum ether extractat final concentrations of 10, 20, 30 and 40mg/ml were then injected in that order. Group five (5) were inoculated with 0.2ml of 100EID_{50}/ 0.1 ml standard NDV (virus control), group six (6) were inoculated with 0.2 ml extract (extract control). Group seven (7) were inoculated with 0.2ml phosphate buffered saline (diluent control) while group eight (8) were not inoculated with anything (uninoculated control). The eggs were sealed with molten wax and incubated at 37^0C for 72 hours. This was done for each extract. Embryo survival was observed daily for 3 days.

Standardization of bacterial suspension

To standardize bacterial suspension, the bacterial isolates were first cultured on nutrient agar (Blood agar for *S. pneumoniae* and *S. pyogenes*) and incubated at 37^0C for 24 hrs. Enough loopful of the

overnight culture was taken and emulsified in a tube containing 2ml normal saline until turbidity matched with 0.5 McFarland standard (Cheesebrough, 2006).

Antibacterial assay

The antibacterial assay was done using agar well diffusion method described by Nester *et al*. (2004) was used. Blood Agar was prepared for *S. pneumoniae* and *S. pyogenes*. Mueller Hinton Agar was also prepared as specified by the manufacturer for the remaining bacteria. The media were autoclaved and poured aseptically into sterile Petri dishes and allowed to gel. A loopful of the standardized bacterial suspension was streaked evenly on each agar plate.

Stock solution (240µg/ml) of the root, leaf, and stem extracts of *S. longipedunculata* was separately prepared by dissolving 0.00024g of the extracts into 2mls dimethylsulphoxide (DMSO) to obtain the concentration of 240µg/ml. From this, the working concentrations of 120µg/ml, 60µg/ml, 30µg/ml and 15µg/ml were made. Then 0.1 ml of each crude extract was inoculated into four wells (6mm diameter) bored with a sterile cork borer in each plate. Dimethylsuphoxide (0.1ml) was inoculated in the fifth well on the plate to serve as negative control and 0.1ml of commercially prepared ciprofloxacin (30µg/ml) was inoculated in the sixth well to serve as positive control. The plates were allowed to stand for 30 minutes on the table for pre- diffusion of the extracts, after which they were incubated at 37^0C for 24 hours. The antibacterial activity of the extracts was determined after incubation by measuring the mean diameter of zones of inhibition produced by each of the extracts against the bacterial species and results were recorded in millimeter (mm).

Result

Methanol extract of the root of *Securidacalongipedunculata* yielded the highest extracts with weight of 10.3grams whereas, extract from root petroleum ether yielded the least extract with 9.2grams. All extracts differed in their colour before and after evaporation of the respective solvents. The extracts also varied in their consistency after evaporation. Methanol extract which was yellow in colour before evaporation became yellowish brown hard solid after evaporation while the yellow petroleum ether extract before evaporation remained yellow but powdered solid after evaporation as shown in Table 1.

The egg infective dose in 50% of the embryonated eggs (EID_{50}) of Newcastle disease virus was found to be at dilution 10^{-9}. This is shown in Table 2.

In the antiviral assay, the toxicity result showed that all the extracts were relatively toxic. Methanol extract was more toxic in which the highest concentrations of this extrac the embryonated eggs survived was 20mg/ml. Meanwhile, 40mg/ml concentrations of the petroleum ether extract was the highest concentration of this extract the embryonated eggs survived. At 80mg/ml concentration of methanol extract, the embryonated eggs were not survived after 24hours. Also, only one embryonated egg survived the concentrations of petroleum ether extract at 60mg/ml. This is shown in Table 3.

The result of the antiviral activity of *Securidacalongipedunculata* root extracts revealed that, methanol extract exhibited slight activity only at 20mg/ml concentration with mortality rate of 60%. This

indicates that only 40% of the embryonated eggs were susceptible to the extract at that concentration. Moreover, the petroleum ether extract did not reveal any activity on the embryonated eggs at all concentrations (20mg/ml, 15mg/ml, 10mg/ml and 5mg/ml). This showed that mortality rate of the eggs at these concentrations is 100% (Table 4).

The outcome of the antibacterial assay of the crude extracts showed that, petroleum ether extract revealed activity against *Streptococcus pyogenes*, and *Proteus* sp. with highest activity exhibited against *Proteus* sp. at 120µg/ml concentration. At this concentration, the zone of inhibition was 30mm which is similar to the zone of inhibition exhibited by ciprofloxacin (positive control) at 30 µg/ml. However, the methanol extract revealed the least in which it exhibited activity against *Streptococcus pyogenes* only, with zone of inhibition of 19mm at 120µg/ml concentration of the extract. This is shown in Table 5.

The result of phytochemical screening revealed the presence of bioactive components tested. Alkaloids, resins, tannin, saponin, flavonoids and phenols were present in all the extracts with anthraquinone present in much quantities. Glycoside was only present in the methanol extract (Table 6).

Table 1: Physical appearance and weights of S. longipedunculata root extracts

	Physical appearance		
Extract	**Before evaporation of extract**	**After evaporation of extract**	**Weight/grams**
A	Yellow Liquid	Yellowish brown hard solid	10.3
B	Yellow Liquid	Yellow Powdered solid	9.2

Key: A = *S. longipedunculata* root methanol extract, B = *S. longipedunculata* root petroleum ether extract.

Table 2: EID_{50} of Newcastle disease virus after 72 hours

Dilution	**No. of eggs with live embryo**	**No. of eggs with dead embryo**	**% Mortality (%)**
10^{-4}	1	4	80
10^{-5}	1	4	80
10^{-6}	1	4	80
10^{-7}	1	4	80
10^{-8}	2	3	60
10^{-9}	3	2	40

Table 3: Toxicity assay of *S. longipedunculata*root extracts on Newcastle disease virus

(No. of eggs inoculated = 5)

Extract(s)	Conc. (mg/ml)	No. of eggs with dead embryo after 24h	No. of eggs with live embryo after24h
A	20	0	5
	40	2	3
	60	3	2
	80	5	0
B	20	0	5
	40	0	5
	60	3	2
	80	4	1
Dc		0	5
Nc		0	5

Key: A = *S. longipedunculata* root methanol extract, B = *S. longipedunculata* root petroleum ether extract, Dc: Diluent control, Nc: Negative control, h = Hour.

Table 4: Antiviral activity of *S. longipedunculata* root extracts against NDV

Extract	Conc. (mg/ml)	No. of eggs	Mortality 24h	48	72h	% Mortality after 72h
A	5	5	0	5	0	100
	10	5	0	5	0	100
	15	5	0	3	2	100
	20	5	0	0	3	60
Ec		5	0	0	5	0
B	10	5	0	5	0	100
	20	5	0	5	0	100
	30	5	0	5	0	100
	40	5	0	3	2	100
Ec		5	0	0	0	0
Vc		5	5	0	0	100
Dc		5	0	0	0	0
Nc		5	0	0	0	0

Key: Vc: virus control, Ec: extract control, Dc: diluent control, Nc> negative control, h=Hours

Table 5: Antibacterial activity of *S.longipedunculata* root extracts against bacterial isolates from upper respiratory tract of human

Concentrations (µg/ml) / Zone diameter (mm)										
Organisms	**A**				**B**					
	120	**60**	**30**	**15**	**120**	**60**	**30**	**15**	**+c**	**-c**
S. aureus	0	0	0	0	0	0	0	0	45	0
S. pyogenes	19	0	0	0	17	0	0	0	17	0
S. pneumoniae	0	0	0	0	0	0	0	0	18	0
K. pneumonia	0	0	0	0	0	0	0	0	45	0
P. aeruginosa	0	0	0	0	0	0	0	0	18	0
Proteus sp.	0	0	0	0	30	26	21	18	30	0

Key: A = *S. longipedunculata* root methanol extract, B = *S. longipedunculata* root petroleum ether extract, +c = Positive control, -c = Negative control

Table 6: Phytochemical constituents of *S.longipedunculata* root extracts

	Extracts	
Compounds	**A**	**B**
Alkaloids	+	+
Resins	+	+
Tannin	+	+
Glycoside	+	-
Saponin	+	+
Flavonoids	+	+
Anthraquinone	+++	+
Phenols	+	+

Key: A = *S. longipedunculata* root methanol extract, B = *S. longipedunculata* root petroleum ether extract.

Discussion

The methanolic extract of the root yielded more extract than the petroleum ether extract. All the extracts yielded a variety of compounds in varying amounts but the methanolic extract contained all the compounds tested which implies that the solvent had the highest extraction capacity. This is in agreement with the study done by Muanda*et al*. (2010).

The result of the EID_{50} indicated that, the titre of Newcastle disease virus that was able to infect fifty percent of the experimental eggs was found to be at dilution 10^{-9} which is similar to the work of Chollom*et al*.(2012).

In the antiviral assay, toxicity study showed that the methanolic root extract has greater toxicity in which at concentration of 40mg/ml, only three (3) out of five (5) embryos survived at which petroleum ether extracts exhibited zero mortality at the same concentration. This shows that the methanol extract has greater toxicity than the petroleum ether extract. This is in line with the findings of Auwal*et al*. (2012) on albino rats. Acute toxicity evaluation of the root extracts classified it as toxic to the experimental eggs at concentration above 40mg/ml.

Antiviral assay of *S. longipedunculata* confirms that the root methanolic extract has a slight antiviral property against ND virus. This was revealed by slight inhibition of virus growth at 20mg/ml with sixty percentage mortality whereas, the petroleum ether extract revealed 100% mortality. This is not strange as there was no available literature on the antiviral property of the plant root and the best concentration that could kill the virus could be above 40mg/ml which is toxic to the embryo. These findings are scientific and relevant judging from the performance of the control groups. The virus control was potent enough to cause embryonic death within 24hrs post inoculation just as extract, diluents and uninoculated controls did not interfere with embryo survival signifying the acceptability of the outcome of the tests groups. According to available literature, this is the first animal virus to be confirmed susceptible to the plant.

The bacterial assay revealed activity of the crude extracts against Gram positive (*S. pyogenes*) and Gram negative (*Proteus* sp.) bacteria with high degree of activity in petroleum ether extract which yielded the highest zone of inhibition (ZI) of 30mm at concentration of 120µg/ml against *S. pyogenes* which is similar to the ZI of the control drug (ciprofloxacin) at concentration of 30µg/ml. Furthermore, the methanolic extract exhibited ZI of 19mm at concentration of 120µg/ml. These findings correspond to earlier reports by Ndamitso*et al*. (2013) and Musa *et al*. (2013) but in contrast with the report of Adebayo and Osman (2012) where they recorded ZI of 15mm by the ethanol extract of the root bark of *S. longipedunculata*at a concentration of 100mg/ml.

The result of phytochemical screening of the root extract of *S. longipedunculata* showed the presence of alkaloids, resins, glycosides, flavonoids, saponins, tannins, anthraquinoneandphenols. These were found to be present in larger quantities. Also, glycoside was found be in appreciable quantity but only in methanol extract. The reason may be due to the greater solubility of the compounds in the extraction solvents used.

The presence of these chemical constituents in the root of *S. longipedunculata* is an indication that this plant if properly screened would yield drugs of plant origin with pharmacological significance. This is better supported by the fact that, the plant family (*Polygalaceae*) to which *S.longipedunculata* belongs, is known to be involved in ethnomedicine in the management of some ailments notably epilepsy (Mathias, 1982). They are also used as anti-snake venom and as purgative (Chhabra*et al*., 1991). Moreover, the presence of alkaloids, glycosides, flavonoids, saponins, tanninsand phenols in this plant is in compliance with an already documented literature which stated that, plants belonging to families *Polygalaceae*, *Moraceae*, and *Cannabinaceae* are known to contain glycosides, triterpenes, resins and higher fatty acids in their stem and root bark (Evans, 1996).

Conclusion

It is evident that *S. longipedunculata*is a very important medicinal plant used extensively for various purposes, according to the literature review. From the result obtained in this study, the methanolic extract has greater antiviral activity while petroleum ether extract exhibited the highest antibacterial activity. These findings have clearly demonstrated that, the root of *S. longipedunculata*is an important part of the plant that have antiviral and antibacterial activity.

Recommendations

In view of the results observed in this study, it is recommended that;

- There is the need to identify the active compounds in the root extracts of the plant.
- There is also a need to investigate the mechanism of activity of the compounds occurring in the plant root extracts.

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