WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 8, 263-269. Research Article ISSN 2277– 7105

# INVITRO SCREENING OF THE METHANOLIC ROOT EXTRACT OF *SECURIDACA WELWITSCHII* OLIV FOR ANTHELMINTIC ACTIVITY

**Onah Cosmas Chuks*[1], Asuzu Isaac Uzoma[1], Anaga Aruh Ottah[1], Chuks Leonard Nnaedozie[1], Odeh Julius Okwoche[2] and Omale Simeon[3]**

[1]Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

[2]Department of Pharmacology & Toxicology, Faculty of Veterinary Medicine, University of Abuja.

[3]Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos.

Article Received on
12 June 2016,

Revised on 04 July 2016,
Accepted on 26 July 2016

DOI: 10.20959/wjpr20168-6801

***Corresponding Author**
**Dr. Onah Cosmas Chuks**
Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

## ABSTRACT

The use of ethnomedicinal plants in folk medicine predates advances in pharmacological knowledge and investigative procedures. Extract of *Securidaca welwitschii* Oliv is subjected to this pharmacological study to establish the pharmacological basis for the use by local healers in deworming people. Secondary metabolites produced by plants are responsible for the medicinal properties of plants generally. Phytochemical analysis of *S. welwitschii* indicates that it has tritherpenes. Terpenoids exhibit anthelmintic activities and may be responsible for the anthelmintic effect of *S. welwitschii*. Heligmosomoides polygyrus is a popular model for the study of helminths and it belongs to the same superfamily as *Haemonchus* species. The latter is the most important helminth of ruminants. The methanolic root extract of *S. welwitschii* produced concentration dependent levels of mortality on the third stage larvae of *H. polygyrus*. The highest concentration (1 mg/ml) produced 87.2% mortality of the larvae. The effect was significantly different ($p<0.05$) from the effect of other concentrations and the control. The lowest concentration (0.031 mg/ml) exerted 7% mortality of the larvae.

**KEYWORDS**: *Securidaca welwitschii*, Root extract, *Heligmosomoides polygyrus*, anthelmintic.

## INTRODUCTION

Ethnomedicinal plants were used in folk medicine for over five millennia for relief from illness in developing and developed world alike.[1] In developing and developed world, high percentage of the population either depends on or had used alternative/ complimentary medicine.[2] Despite the wide use of plants in various forms, only a small percentage of the estimated 250,000 – 500,000 plant species has been evaluated phytochemically and pharmacologically which has attracted universal attention.[3-5] World Health Organisation (WHO) highly recommended herbal based traditional remedies because of their safety, easy availability, low cost in the treatment of various diseases.[6] Taking steps to achieve standardization of formulations from plants is of importance in order to properly guide the use of plant products. Ethnopharmacologists improve ethnomedical systems of people by testing their indigenous medicine for efficacy and toxicity in order to improve their safety. Many important plant-derived drugs were discovered through such works in ethnopharmacology.[7] Presently, chemotherapeutic agents are the major means of controlling helminthosis however parasitic resistance against major anthelmitics is a significant problem.[8] Local herdsmen and other people who keep animals as means of livelihood in Nigeria are using herbal remedies successfully.[9] *Securidaca welwitschii* is locally used in Eastern Nigeria for deworming people without any report of toxicity yet there is no scientific work on the plant. The objectives of this study are to scientifically conduct systematic investigation to establish the pharmacological basis of the folkloric use of decoction of the root of *Securidaca welwitschii* among people in parts of Eastern Nigeria. The findings could be beneficial to both the traditional livestock farmers and Veterinarians as it could alleviate problems associated with endermic helminthosis in our environment.

*Securidaca welwitschii* Oliv (family- *Polygalacaae*) is a large shrub often climbing to a great height (10-25 m) with smooth and glabrous branches. It is found in evergreen forest, and rain forest of East Africa, West Africa, Central and South Africa.[10-11] In Eastern Nigeria, the decoction of the root of *S. welwitschii* Oliv is used to deworm people, while in Gabon the sap of the lianous stem is used for eye trouble.[11] Chemically, five new tritherpene saponins were discovered in *S. welwitschii.*[12] Glucose, galactose, xylose, arabinose, fucose, rhamnose made up the sugar component.[12]

A trichostrongylid nematode of laboratory mice, *Heligmosomoides polygyrus* is generally popular as a model for the study of nematode infection.[13] The prevalence of the infection in

mice could reach 100% at the age of three weeks. The intensity of the infection increases to peak in 10-15 weeks old mice and remains throughout life which may be up to 8 months.[14] *Heligmosomoides polygyrus* and *Haemonchus* spp belong to the same super family *Trichostrongyloidea*. *Haemonchus* spp is the most important worm infection in ruminants.[15] *Heligmosomoides polygyrus* has a direct life cycle involving both free living and parasitic stages[16], just like *Haemonchus* spp. These features make this worm, an acceptable model even for the study of chronic gastrointestinal helminthosis.

## MATERIALS AND METHODS

### Collection, identification, and extraction of plant materials.

The plant was obtained in Eastern Nigeria (Nsukka) within latitude 6.8567 and Longitude 7.3958. It was identified by a plant taxonomist, ( Ozioko, Alfred) of Botany Department in University of Nigeria as *Securidaca welwitschii*. Adulteration was strictly avoided during collection, after collection and during storage as the identified roots were washed thoroughly under running water, then rinsed with distilled water. The roots were then cut into pieces and dried under shade with elaborate ventilation until there was no difference in weight. The dry pieces were pulverised into coarse powder using clean pestle and mortar.

Extraction with the solvent was done through cold maceration. The coarse plant material (500g) was weighed on an analytical weighing balance (Mettler $H_3O$, Switzerland) then loaded into a glass bottle containing 80% of the analytical grade of methanol (Fulks, Germany). The content was left in the bottle for 48 hours, with regular shaking at 2 hours intervals. The extract was filtered using Whatmann No. 1 filter paper and concentrated in a hot air oven at 40$^{o}$C. The percentage yield was calculated.

### Culturing of *H. polygyrus* larvae.

The modified method of Chris JCJ et al [14] was used. Donor mice were infected with stage three larvae ($L_3$) of *H. polygyrus* and the infection was established after 14 days. Faeces passed freshly by donor mice were collected in a clean container over a period of about one hour. The pellets were scraped into a 50 ml tube. The faecal pellets were mashed into a smooth paste with spatula. The 50 ml tube containing the faecal slurry was filled with distilled water and centrifuged at 250 r.p.m. for 2 minutes. The supernatant was aspirated down to the pellet. The procedure was repeated twice. The faecal material was re-suspended and poured into double layer gauze to remove much of the faecal material while most of the nematode eggs pass through the gauze. The tube was filled again with distilled water and

centrifuged as above. The supernatant was aspirated leaving the pellet. The remaining sediment was poured into 5 layer No. 1 filter paper placed in a moist culture dish. The dish was placed in a refrigerator (4°C) for 7 days. The dish was then tilted and sprayed gently with distilled water and then centrifuged at 250 r.p.m. for 2 minutes. The supernatant was aspirated and the centrifuging was done twice then the larvae were stored at 4°C until when the larvae was needed.

**Determination of effect of *S. welwitschii* extract (SWE) on larvae of *Heligmosmoides poly gyrus* (*in-vitro* model), and determination of lethal concentration 50 ($LC_{50}$).**

Micro liter bucket was used for the experiment. Five replicates of 6 concentrations (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, 0.031 mg/ml respectively) of SWE in 0.1 ml of distilled water were prepared. Each replicate contained 50 $L_3$ of *H. polygyrus* placed in 0.1 ml distilled water. The content of each well was 0.2 ml distilled water. This was refrigerated at $4^0$C for 48 hours. The content of each well was in turn examined under light microscope to determine the number of larva that died during incubation. Dead larvae remained inactive for up to 5 seconds according to Martin and Le Jambre.[17] The $LC_{50}$ was determined by plotting the concentration of SWE (x-axis) against the percentage mortality of *H. polygyrus* larvae (y-axis). The results are summarized in the table and figure below.

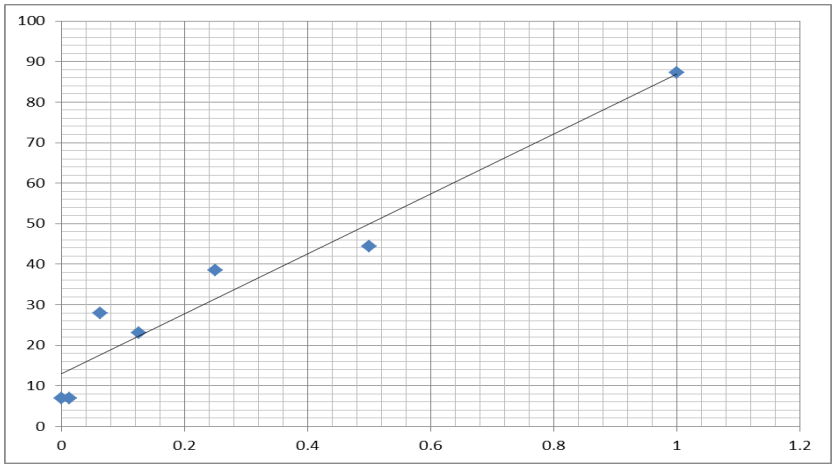
## RESULTS AND DISCUSSION

**The *in vitro* effect of SWE on $L_3$ of *H. polygyrus***

| Treatment Group | SWE Concentration (mg/ml) | Mean* Percentage Mortality |
|---|---|---|
| 1 | 1.000 | $87.2 \pm 18.50^{a}$ |
| 2 | 0.500 | $44.4 \pm 11.67^{b}$ |
| 3 | 0.250 | $38.6 \pm 7.02^{b}$ |
| 4 | 0.125 | $23 \pm 3.75^{bc}$ |
| 5 | 0.063 | $28 \pm 9.27^{bc}$ |
| 6 | 0.031 | $7 \pm 3.05^{c}$ |
| 7 | Control (distilled water) | $7 \pm 2.94^{c}$ |

*$LC_{50}$ was determined to be 0.45mg/ml as shown in the figure below. The different superscripts $^{abc}$ in the mean column indicate significant difference between the mean percentage mortality of the treatment groups; $p<0.05$*

**Mean ± STD error based on 5 observations*




*$LC_{50}$ of SWE on mice therefore is 0.45mg/ml*

***X axis-*** *Concentration of SWE in mg/ml*

***Y axis-*** *Percentage Mortality*

SWE produced concentration dependent percentage mortality on the larvae ($L_3$) of *Heligmosomoides polygyrus* ($p<0.05$). The highest concentration of SWE (1mg/ml) induced 87.2% mortality. This effect was significantly different ($p<0.05$) from both the control and the other concentrations. The second concentration (0.500mg/ml) affected the mortality of 44.0% of the larvae which was not significantly different from the effect of 0.250 mg/ml, 0.125mg/ml, and 0.063 mg/ml respectively. The second concentration 0.500mg/ml and the third, 0.250mg/ml produced percentage mortality which was significantly different ($p<0.05$) from the sixth concentration (0.031) but not from the fourth (0.125 mg/ml) and fifth (0.063 mg/ml) concentration respectively. This interesting concentration dependent effect must be traceable to specific components of the extract. Gaoussou T. et al found that *S. welwitschii* has tritherpenes.[12] The observed potential anthelmintic property may be traceable to the tritherpenes. Crude extracts, as deduceable from literatures are rich in phytochemicals or secondary metabolites produced by plants for self defence or in response to microbial infection.[1] These secondary metabolites have interesting biological activities and have given plants different pharmacological attributes necessitating scientific evaluation.[17] Further work on the crude extract of *S. welwitschii* to separate the components of the crude extract will confirm the secondary metabolite or combination of phytochemicals responsible for the potential anthelmintic activities observed.

## CONCLUSION

The findings in the present study shows that *S. welwitschii* has metabolite(s) which demonstrated anthelmintic activity against *H. polygyrus* (*in-vitro* model). This study therefore

establishes the pharmacological basis for use of the plant by local healers as anthelmintic. It will be helpful also to conduct in-vivo tests with SWE in an effort to establish further pharmacological ground for the folkloric use of *S. welwitschii* as anthelmintic. Full separation of the component will also prove useful towards introducing an anthelmintic for both animal and human use.

**ACKNOWLEDGEMENT**

The authors are grateful to the Department of Veterinary Parasitology and Entomology of University of Nigeria, Nsukka for permitting us to use their laboratory facilities.

**FUNDING:** The World Bank through the step B innovators of tomorrow award project funded this work in full.

**REFERENCES**

1. Kiran CN, Santosh F, Hamid AK, May FDCB, Mohd. FA, Asif IC. In-Vitro screening of Hibiscus Cannabinus L. Leaves extracts for antibacterial activity. World Journal of Pharmaceutical Research. 2016; 5(6): 369-378.
2. Sumya A, Mohammad SHK, Md. SIA, Belkis F, Md. SH, Fahmida AT, Mosarrof H, Md. NU, Md. MR. Antibacterial activity of methanol leaves extract of Andrgraphis Paniculata and PASS prediction for antibacterial effect and ADME/T property analysisof its isolated compounds. 2016; 5(6): 141-152.
3. Mukherjee S, Jagtap SD, kuvalekar AA, Kale YB, Kulkarni OP, Hasulkar AM et al. Demonstration of the potential of Hibiscus cannabilus Linn. Flowers to manage bone stress related disorders and free radical DNA damage. Indian Journal of Natural Products and Resources. 2010; 1(3): 322-327.
4. Kiran CN, Asif IC, Nishaidevi, Ugandar RE. Antimicrobial potential of petiole extracts of Apium graveolens L. World Journal of Pharmacy and Pharmaceutical sciences. 2015; 4(4): 216-231.
5. Asif ICM, Gunjan M, Chellappan KD. Antimicrobial activity of Tinospora crispa root extracts. Int. J. Res. Ayu.Pharm. 2012; 3(3): 417-419.
6. Lalitha N. Protecting Traditional knowledge in SSiddha system of medicine. Journal of Intellectual Property Rights. 2013; 18: 272-282.
7. Heinrich M, Edwards S, Moerman DE, Leonti M.Ethnopharmacological field studies: A critical assessment of their conceptual basis and methods. Journal of Ethnopharmacology. 2009; 124(1): 1-17.

8. Burkill HM. The useful plants of west Tropical Africa. URL:http://plants.jstor.org/stable/10.5555/al.ap.upwta(26 June 2016).
9. Dapar LPM, Aguiyi CJ, Wannang NN, Gyang SS, Tanko MN. The histopathology effect of Securidaca longipediculata on heart, liver, lungs, and kidney of rats. African Journal of Biotechnology. 2007; 6(5): 591-595.
10. Prof. Oliver. Flora of Tropical Africa, 1; 125(1868).
11. Jorge Paiva. Flora of Tropical East Africa, 2007; 1.
12. Gaussou T, Anne-Claire MO, Tomofumi M, Chiaki T, Thomas P, Clement D, Marie-Aleth LD. Presengenin Gycosides from S. welwitschii . Helvetica Chimica Acta. 2010; (93): 2237-2244.
13. . Martin PJ, Le Jambre LF. Larval paralysis as an in vitro assay levamisole and morantel tartarate resistance in Ostertagia. 1979. Vet. Sc. Comm. 3. 159
14. Chris JCJ, Eliane R, Yvonne H, John RG, Gillian CDJ, Henry JM, and Rick M. Cultivation of Heligmosomoids polygyrus : An immunomodulatory nematode parasite and its secreted products. Journal of visualized experiments. 2015; (98): 52412.
15. Nwosu CO, Ogurinade AF, Fagbemi BO.Clinicopathological studies of Haemonchus contortus infection in red Sokoto (Maradi) goats.Nig. J. Exp. Applied Biology. 2001; 2: 157-164.
16. Soulsby EJL. Helminths, Athropods andProtozoa of Domesticated Animals. 1982; 7th Ed. Baillre Tindall, London.
17. Helgi II, Pratima T, Karl FH, Ashley HE, Nicole BR, Xavier P, Djurre H de J, Martin Z, Duygu Y, Kartherine H, Thorsten M,Hugh CH Jr, Carl B, Siewert JM, Armagan K, Jon TS, and Olaf SA. Phytochemicals perturb membranes and promiscuously alter protein function. ACS Chem. Bio., 2014; 9: 1111788-1799.