

## EVALUATING BIOLOGICAL ETHNOPHARMACEUTICAL ACTIVITIES OF FRUIT AND ROOT EXTRACTS OF *SOLANUM INCANUM* L.

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## ABSTRACT

*Solanum incanum* L. is a plant that belongs to the Solanaceae family, growing in tropical climates all over the world. Despite the extensive use of the root and fruit sap extract of *S. incanum* in the agricultural and ethnopharmaceutical industries in Africa, there is minimal scientific documentation regarding its pesticide bioactive phytochemicals and efficacy. The study investigated the photochemical content (phenolics, flavonoids, saponins, terpenoids and alkaloids) of the methanol, ethyl acetate and hexane crude extracts of the root and fruit of *S. incanum*. The potential radical scavenging, (antioxidant), cytotoxicity, and efficacy activities of all crude extracts were examined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH), propidium iodide (PI) and aphid leaf dip (ALD) assays respectively. The total phenolic, alkaloid, flavonoid, terpenoid and saponin content were highest in the methanol fruit (MFE), ( $3.49 \pm 1.16$  mg GAE/100 mg), steroidal fruit (SAE), ( $0.14 \pm 0.06$  mg ATP/100 mg, hexane fruit (HFE),  $3.62 \pm 1.23$  mg QCT/100 mg, hexane root (Hroot), ( $1.19 \pm 0.47$  mg LIN/100 mg and saponin ( $3.74 \pm 1.17$  mg/100 mg). The aqueous extract of the ethyl acetate fruit had the highest antioxidant activity with an  $IC_{50}$  of  $35.53 \pm 0.53 \mu\text{g} \cdot \text{mL}^{-1}$  ( $p < 0.05$ ). The methanol fruit and steroidal alkaloid extracts exhibited potent cytotoxicity against Vero cells demonstrating the highest cell mortality rate of 98% and 70% respectively at  $100 \mu\text{g} \cdot \text{mL}^{-1}$ . Melphalan which was used as a positive control had a mortality rate of 75% cell at  $100 \mu\text{g/mL}$  concentration. The methanol fruit extract exhibited the highest efficacy against the cabbage aphids with an  $LD_{50}$  of  $13.16 \pm 3.78 \mu\text{g} \cdot \text{mL}^{-1}$  ( $p < 0.05$ ). The results indicated that the fruit and root of *S. incanum* have a variety of phytochemicals that may be considered for use as antioxidants and/or pesticides. By exhibiting the most potent cytotoxic activity against Vero cells, *S. incanum* roots have the greatest potential for the discovery of cytotoxic compounds necessary to develop anticancer drugs. The extracts with high potent efficacy against cabbage aphids need to be investigated further as pesticides to benefit the disadvantaged communities in the Southern African region.

**Key words:** Antioxidant, cytotoxicity, lethal dose, mortality rate, phytochemicals, quantitative analysis

## INTRODUCTION

*Solanum incanum* (also known as the thorn apple, Sodom apple, and bitter apple, Nhundurwa in shona and Intume in IsiXhosa) is widely spread in dry regions or rocky soils in Africa. *S. incanum* has been used since the dawn of civilization in the agricultural, health, and food industries [1, 2, 3, 4, 5] and has drawn increasing attention as a potential source of anti-inflammatory, anti-cancer, antioxidant, anti-obesity, anti-diabetic, cardio-protective, neuroprotective, and hepato-protective compounds [6, 7, 8, 9]. Indigenous people in disadvantaged communities of Southern Africa treat various ailments (sore throats, angina, stomach pains, colic, headaches, painful cutaneous mycotic infections, menstruation) using aqueous extracts from *S. incanum* species [6, 10]. In addition, it is used to treat venereal diseases and skin problems, as well as used to curdle milk for the improvement of flavour in cheese [6].

Several investigations have been performed on local *Solanum* spp. showing a toxic effect on insects or modifying their behaviour [11, 12, 13]. For instance, the insecticidal activity of the methanolic extracts from leaf and seed of *Solanum* spp. *elaeagnifolium* were investigated against three pest species (*Myzus persicae*, *Phthorimaea operculella*, and *Tribolium castaneum*). Seed extract had the greatest effect in causing mortality of 23.6% for peach potato aphids and 34% for the red flour beetle, and inhibiting oviposition (95.9%) and egg hatching (98.6%) for potato tuber moth against *Myzus persicae*, *Phthorimaea operculella*, and *Tribolium castaneum* [11].

*S. incanum* is used by disadvantaged communities in Zimbabwe, Zambia, and Malawi to control pests. Madzimure *et al.* [12] evaluated the efficacy of the aqueous fruit extracts of *S. incanum* against cattle ticks. In their experiment, varying concentrations, 5, 10, 20, and 40 % (w/v) were compared to *Strychnos spinosa* aqueous fruit extract and Tick buster® (Amitraz) spray which was used as a positive control. The *S. incanum*, *Strychnos* and Tick buster® (amitraz) spray Tick buster® (amitraz) spray treatments were allowed to stand for 48 hrs before the analysis of the dead and alive ticks were counted. A higher efficacy ratio was exhibited at a 5 % (m/v) concentration of the fruit of *S. incanum* treatment compared to the 5% (m/v) *Strychnos spinosa* aqueous extract and the Tick buster® (amitraz) spray against cattle ticks [12].

There is also a claim that *S. incanum* fruit and root extracts are effective against cabbage aphids [13]. Majority of community vegetable garden farmers in Zimbabwe use *S. incanum*'s fruit sap and root extracts as a cabbage aphid

pesticide since conventional insecticides are expensive and arguably associated with various severe adverse side effects. Despite the extensive uses of the root and fruit sap extract of *S. incanum* in Africa, there is minimal scientific documentation that shows its effectiveness, efficacy, and mode of insecticidal action of the extracts from *S. incanum*. Therefore, this study was carried out to document the biological activities that included the antioxidant, cytotoxicity, and pesticidal properties of the root and fruit of *S. incanum*.

## MATERIALS AND METHODS

### Solvents and reagents

All chemicals and reagents were purchased from Sigma Aldrich (United States of America) unless otherwise stated. Hexane, ethyl acetate, Toluene, petroleum ether, n-butanol, methanol, sodium hydroxide (NaOH), hydrochloric acid, (HCl), and Phosphotungstic,  $H_3PW_{12}O_{40}$  and phosphomolybdic,  $H_3PMo_{12}O_{40}$  acids, Folin-Ciocalteu reagent (FCR), sodium carbonate ( $Na_2CO_3$ ), gallic acid, ( $C_7H_6O_5$  aluminium (III) chloride ( $AlCl_3$ ), and quercetin ( $C_{15}H_{10}O_7$ ). Vero cells were purchased from Cellonex, South Africa. Dulbecco's Modified Eagle Medium (DMEM) and Foetal Bovine Serum (FBS) were purchased from GE Healthcare Life Sciences (Logan, UT, USA). PBS with and without  $Ca^{2+}$  and  $Mg^{2+}$  and trypsin were purchased from Lonza (Walkersville, MD, USA). Bis-benzamide H 33342 trihydrochloride (Hoechst 33342) and propidium iodide (PI) were purchased from Sigma (St. Louis, MO, USA).

### Plant material

The plant material that comprised ripe fruits and roots of *S. incanum* were collected from their natural habitat (bushes surrounding Mkoba village in Gweru, Zimbabwe), based on the ethno-pesticidal and medicinal information provided by the local community who were using the plant for various medicinal and pesticidal activities. The collection of the specimen was done on the 5<sup>th</sup> of May 2018. Each collected fruit was cut into half, to enable the separation of the seeds and the pericarp. The pericarp and the roots were dried in the sun, pulverised then transported to Nelson Mandela University, South Africa as powdered biomass. Plant authentication was performed by Tony Dold, a curator and taxonomist at Selmar Schonland Herbarium (GRA) in Makhanda, Eastern Cape, South Africa, where a specimen of *S. incanum* with voucher number Zivanayi012 (GRA) was deposited.

### Extraction

Solvent extraction methods described by Gupta *et al.* [14] and Ushir *et al.* [15] were utilized in this study. The fruit biomass (546 g) and the root biomass (148 g)



were extracted sequentially with 100% methanol, ethyl acetate, and hexane in biomass to solvent ratio of 1:3 (w/v) using the maceration method for 72 hrs to obtain crude extracts of fruit and root. The extracts are labelled as methanol fruit extract (MFE), methanol root (Mroot), ethyl acetate fruit crude (EFE), hexane fruit (HFE), ethyl acetate root (Eroot) and hexane root (Hroot). Each extract was filtered under vacuum using a Buchner funnel, and the solvent was concentrated using rotary evaporation (Buchi, Switzerland) at 50 °C. All extracts were stored at 4 °C until the time needed for the analysis. The steroidal alkaloid extraction (SAE) was carried out as follows: the coarse powdered dry fruit (200 g) of *S. incanum*, was transferred to a 5 L round bottom flask and was defatted with 300 mL petroleum ether to yield a greenish-yellow oil. The oil was filtered off and the residue was then refluxed with 32% v/v conc. HCl, water, and toluene in 1:2:3 ratios for 5 hours. The reaction mixture was subsequently made alkaline with 1M NaOH, to a pH of 10, then refluxed again for 2 hours. After refluxing, the upper pale-yellow toluene layer was siphoned out and the lower aqueous layer was extracted with toluene three times. The toluene layer was acidified with 25% v/v acetic acid and the mixture was allowed to stand for an hour. The toluene layer was then filtered off and the acetic acid layer was made alkaline with 25% v/v NH<sub>4</sub>OH to a pH of 10. The mixture was briefly heated and then cooled at room temperature to precipitate steroidal glycoalkaloids. The precipitate was filtered off and washed with cold water and dried in air. The SAE was stored at 4 °C until the time needed for the analysis.

### Quantitative analysis

Spectrophotometric analysis was used to estimate the quantitative analysis of the extracts via the use of calibration curves of selected standards of the major phytochemicals under this study. The total alkaloid content was assessed using the bromocresol green (BCG) method with atropine standard as described by Gupta *et al.* [14] and Ushir *et al.* [15] with little modification. Atropine was used as the reference standard to generate the calibration curve, as indicated in Table 1. The absorbance of the yellow BCG - atropine complex in chloroform as well as the extracts was measured in triplicates at 470 nm against a blank- chloroform, using SHIMADZU 3100 UV-VIS spectrophotometer. The total alkaloid content was estimated and expressed in milligram atropine equivalent per 100 mg of extract (mg ATP/100 mg extract).

The total phenolic content of each extract was assessed using the single electron transfer mechanism of the Folin-Ciocalteu reagent as described by Ushir *et al.* with minor modifications [15]. The standard gallic acid (GAE) was used to generate the calibration curve, as indicated in Table 1. The absorbance of the standard was measured at 760 nm and recorded against reagent blank- methanol. The total

phenolic content was estimated and expressed in milligram gallic acid equivalent per 100 milligrams of extract (mg GAE/100 mg extract).

The total flavonoid content of each extract was estimated using the aluminium-chloride colorimetric assay described by Gupta *et al.* [14] with slight modification. Quercetin was used as the reference standard to generate the calibration curve as indicated in Table 1. The total flavonoid content was estimated and expressed in milligram quercetin equivalent per 100 milligrams of extract (mg GAE/100 mg extract).

The total terpenoid content method was adopted from Ghora *et al.* [16] with minor modifications. The total terpenoid content of the extracts was estimated using the linalool calibration curve as indicated in Table 1. The absorbance was measured against a blank at 538 nm. The total terpenoid content of each sample was estimated and expressed in milligram linalool equivalent per 100 milligrams of extract (mg LIN/100 mg extract).

For the estimation of saponins, El Aziz *et al.* [17] method was adopted. An estimated quantity of the saponins was carried out by dissolving 2.0 g of each crude extract sample in 50 mL of 20% aqueous ethanol. The solution was heated under refluxed for 4 hours at 55 °C. The resulting mixture was filtered and then concentrated to 50% of the original volume, extracted with diethyl ether. The aqueous layer was recovered while the ether layer was discarded. The aqueous extract was purified by adding 60 mL n-butanol and then washed with 10 mL of 5% aqueous NaCl (aq).

The solution was dried and the saponin content was calculated as follows:

$$\% \text{ Saponins} = \frac{\text{Mass of saponin}}{\text{Mass of the sample}} \times 100 \quad (1)$$

### Antioxidant activity test

The antioxidant activities of the *S. incanum* crude extracts were validated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. The DPPH radical scavenging activity was estimated based on the method used by Veerapagu *et al.* [18]. Ascorbic acid was used as a standard. Test samples were dissolved separately in appropriate solvents to a concentration of  $1.0 \text{ mg} \cdot \text{mL}^{-1}$ . Series of crude sample dilutions to make concentrations of 25; 50; 75; 100; 125; 150; 175 and  $200.0 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$  were prepared. Assays were performed in vials by adding  $2 \text{ mL}$  of  $1.0 \times 10^{-6} \text{ mol} \cdot \text{dm}^{-3}$  DPPH solution. The mixture was shaken gently and left to stand for 30 min

in the dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using a SHIMADZU UV-3100 UV-VIS spectrophotometer. Methanol was used as the blank. Ascorbic acid was used as a reference (control) antioxidant compound and was prepared the same way as the above crude samples. Each analysis was done in triplicate. The percentage of the DPPH scavenging effect was calculated using the equation below.

$$\text{DPPH \% scavenging effect} = \frac{A_c - A_s}{A_c} \times 100 \quad (2)$$

$A_s$  – Absorbance of the sample

$A_c$  – Absorbance of the DPPH solution without any antioxidant

Results were expressed as mean  $\pm$  SD. The  $IC_{50}$  values were calculated using Microsoft Excel 2016 version.

### Cytotoxicity test

#### Sample preparation

Test extracts were reconstituted in dimethyl sulfoxide (DMSO) to give a final concentration of 100 mg·mL<sup>-1</sup>. Samples were sonicated if solubility was a problem. Samples were stored at 4°C until required.

#### Treatment protocol

The African green monkey kidney cell line and Vero cells were used for cytotoxicity screening. They were maintained at 37°C in a humidified incubator with 5% CO<sub>2</sub> in 10 cm culture dishes. The complete growth medium consisted of DMEM supplemented with 10% FBS. Cells were seeded into 96 well microtiter plates at a density of 3000 cells/well using a volume of 100 µl in each well. The microtiter plates were incubated at 37°C, 5% CO<sub>2</sub>, and 100% relative humidity for 24 hrs prior to the addition of test samples to allow for cell attachment [19].

Cells were treated with each extract (50, 100, and 200 µg/mL) and melphalan as the positive control (10, 20, and 40 µM), diluted in a culture medium. One hundred microliter aliquots of the diluted extract in a fresh medium were used to treat cells which were further incubated for 48 hrs.

The treatment medium was aspirated from all wells and replaced with 100 µL of Hoechst 33342 nuclear dye (5 µg·mL<sup>-1</sup>) and incubated for 20 mins at room temperature. Then, cells were stained with propidium iodide (PI) at 100 µg·mL<sup>-1</sup> to enumerate the proportion of dead cells within the population. Cells were imaged immediately after the addition of propidium iodide (PI) using the ImageXpress

Micro XLS Widefield Microscope (Molecular Devices) with a 10x Plan Fluor objective and DAPI and Texas Red filter cubes. Nine image sites were acquired per well which is representative of roughly 75% of the surface area of the well.

### Data Quantification

Quantification of live and dead cells for the screening assay was performed using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices) and acquired images were analysed using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module. Acquired data were transferred to an EXCEL spreadsheet and data was analysed and processed. Each analysis was carried out in quadruplicates.

### Efficacy assays (Determination of the lethal dose (LD<sub>50</sub>) of the crude extracts) Sample preparation

The cabbage leaves infested by aphids were harvested from a garden. Live aphids on each leaf were counted using a magnifying lens and recorded. Series of crude sample dilutions of concentrations of 25, 100, 200, and 300 mg·mL<sup>-1</sup> were prepared.

### Treatment protocol

Assays were performed by dipping the infected cabbage leaf cutting in the differently prepared aliquots for 5 seconds before placing it on a piece of tissue paper and then placed in a petri dish of diameter 5.5 cm with moistened cotton. The assessment was carried out using a magnifying glass by counting the dead cabbage aphids after 24 hours. Those cabbage aphids which had no leg movement were considered dead. Each experiment was carried out in triplicate and results were expressed as Mean ± SD (N = 3) at a 95% confidence level. The cabbage aphid mortality in the treatments was corrected with that in the negative control according to Abbott's formula [20]. Each analysis was carried out in triplicates.

$$CAM = \frac{\%MT - \%MC}{100 - \%MC} \times 100 \quad (3)$$

- CAM - Corrected % cabbage aphid mortality
- %MT is the percentage mortality of the treatment
- %MC is the percentage of mortality due to the solvent (Negative control)

A Probit transformed technique was used to determine the lethal doses (LD<sub>50</sub>).

## RESULTS AND DISCUSSION

The study revealed that *S. incanum* fruit and root possessed various phytochemicals linked to *S. incanum*'s antioxidant, cytotoxicity, and pesticidal bioactivities. The acaricidal properties of *S. incanum* have been attributed to the presence of phytochemicals such as alkaloids, polyphenolic compounds, and terpenoids [5]. The presence of phenolic compounds in abundance in the fruit and root of *S. incanum* extracts confirms the high antioxidant activity observed in the DPPH assay. Literature search reveals that polyphenolic compounds serve as antioxidants to protect and defend and enhance the wound healing process through skin regeneration processes in animals which is probably the reason why *S. incanum* is associated with wound healing in different African communities [2, 12, 13, 21].

### Quantitative analysis

The yield and the physical characteristics of extracted crudes are reported in Table 2. The ethyl acetate fruit (EFE) crude extract produced a black soft solid with the highest percentage yield of 17.8 g and the hexane fruit (HFE) crude extract was a dark green solid and had the lowest yield of 10.4 g.

The validation parameters for estimating the quantities of the types of phytochemicals in the extracts are reported in Table 1. The (LOQ) values for all three standard curves showed that the method presented for the different total content estimates was detectable and quantifiable under conditions used as low as  $8.08 \times 10^{-4}$  mg GAE, without suffering an alteration of equipment's intrinsic factors. The recovery rate for the quantitative analysis ranged between 99.2% and 99.9% for all the experiments.

The total alkaloid content estimation was based on the formation of a yellow alkaloid-bromocresol green (BCG) complex and the absorbance of this complex is proportional to the concentration of alkaloids present [14, 15]. The total alkaloidal estimation was then calculated using the atropine standard calibration curve, indicated in Table 2. The quantity estimation of the total phenolic content of the extracts was based on the fact that in an alkaline medium, phenols reduce the mixture of phosphotungstic and phosphomolybdic acid present in the Folin-Ciocalteu reagent to a blue-coloured tungsten and molybdenum oxide chromophore which is proportional to the concentrations of phenolic compounds present in the extracts [15]. The quantity of the phenolics was estimated using the Gallic calibration curve (Table 1).

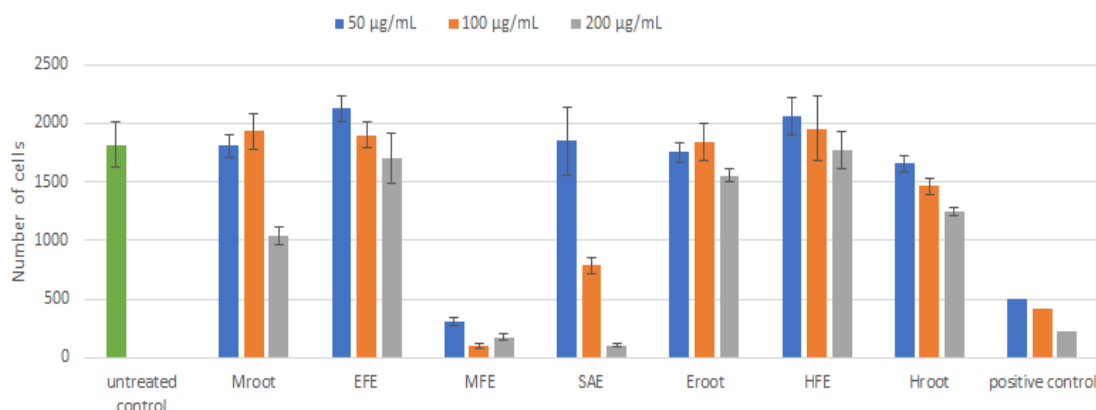
The total flavonoids content of the extracts was measured based on the formation of a yellow flavonoid -  $Al^{3+}$  whose intensity is proportional to the concentration of the flavonoids in the sample, which was calculated from the Quercetin calibration curve (Table 1) [14]. The total terpenoids in the crude extract were based on the reaction of the terpenoid with a mixture of chloroform and concentrated sulphuric acid to form a red brick precipitate [13]. The concentration of the terpenoid is directly proportional to the intensity of the brick red colour on the UV-spectrophotometer. The total phenolics were calculated using the linalool calibration curve (Table 1). The estimation of the saponins was based on the gravitational analysis in which the saponin in the crude extract was dissolved in n-butanol and the solution was precipitated to recover the saponin [17, 22].

The results of quantitative analysis showing the phytochemical content of each plant part are summarised in Table 3. The alkaloid content was highest in the steroidal crude extract, phenolic content was highest in the methanol root, flavonoids in the hexane fruit, terpenoids and saponins in the hexane root at  $0.14 \pm 0.06$  mg ATP /100 mg crude;  $3.72 \pm 1.28$  mg GAE /100 mg crude;  $3.62 \pm 1.23$  mg QCT /100 mg crude;  $1.71 \pm 0.47$  mg LIN /100 mg crude and  $0.374 \pm 1.17$  mg /100 mg crude respectively. The results of quantitative analysis showing the phytochemical content of each plant part are summarised in Table 3.

The estimated quantity of phenolic compounds in the Mroot of *S. incanum* ( $3.72 \pm 1.28$  mg GAE/100 mg sample) was comparable to the total content of phenolic compounds found in the roots of *S. nigrum* ( $3.01 \pm 1.11$  mg GAE/100 mg sample) and *S. torvum* ( $2.94 \pm 0.86$  mg GAE/100 mg sample) when a similar standard was used [23].

### Cytotoxicity

Cytotoxicity increased with the increase of the concentration as shown by the decrease in the number of the live Vero cells from 50 to 200  $\mu\text{g} \cdot \text{mL}^{-1}$  for each crude extract (Figure 1). The percentage of live cells after 48 hrs (measured in quadruplicates) ranged between  $5 \pm 2.21\%$  and  $80 \pm 9.45\%$  at a concentration of 100  $\mu\text{g} \cdot \text{mL}^{-1}$ . The methanol fruit extract (MFE) extract exhibited the most toxicity followed by the steroidal alkaloid extract (SAE) with percentage values of  $5 \pm 2.21$  and  $22 \pm 5.62\%$  live cells after 48 hours at 100  $\mu\text{g} \cdot \text{mL}^{-1}$ .



**Figure 1: Cytotoxicity of *S. incanum* crude extracts against Vero cells. Melphalan was used as positive control at 10, 20 and 40 µM. Data points represent the mean  $\pm$  SD of quadruplicate (4) determinations**

The presence of the alkaloids in the MFE and SAE supports the medicinal function of *S. incanum* as an anticancer drug. This study also revealed that the MFE and SAE extracts exhibited high cytotoxicity properties. Cytotoxic chemicals are linked with anticancer treatment [24]. The findings in this study agree with Al Sinani *et al.* [1], who concurred that glycoalkaloids isolated from *Solanum* species are cytotoxic against certain cancer cell lines. For example, solamargine causes human hepatoma cell death (Hep3B) by apoptosis;  $\alpha$ -solasonine from *S. crinitum* and *S. jabrense* has a cytotoxic effect on leukaemia cells; chaconine,  $\alpha$ -solanine, tomatine, and their derivatives inhibit human colon (HT29) and liver (HepG2) cancer cells from growing;  $\beta$ -2-solamargine from *S. nigrum* has a toxic effect on the cell lines: HT-29 (colon), HCT-15 (colon), LN C P (prostate), PC-3 (prostate), T47D (breast and MDA-MB-231 (breast) [25; 26].

### Antioxidant activity

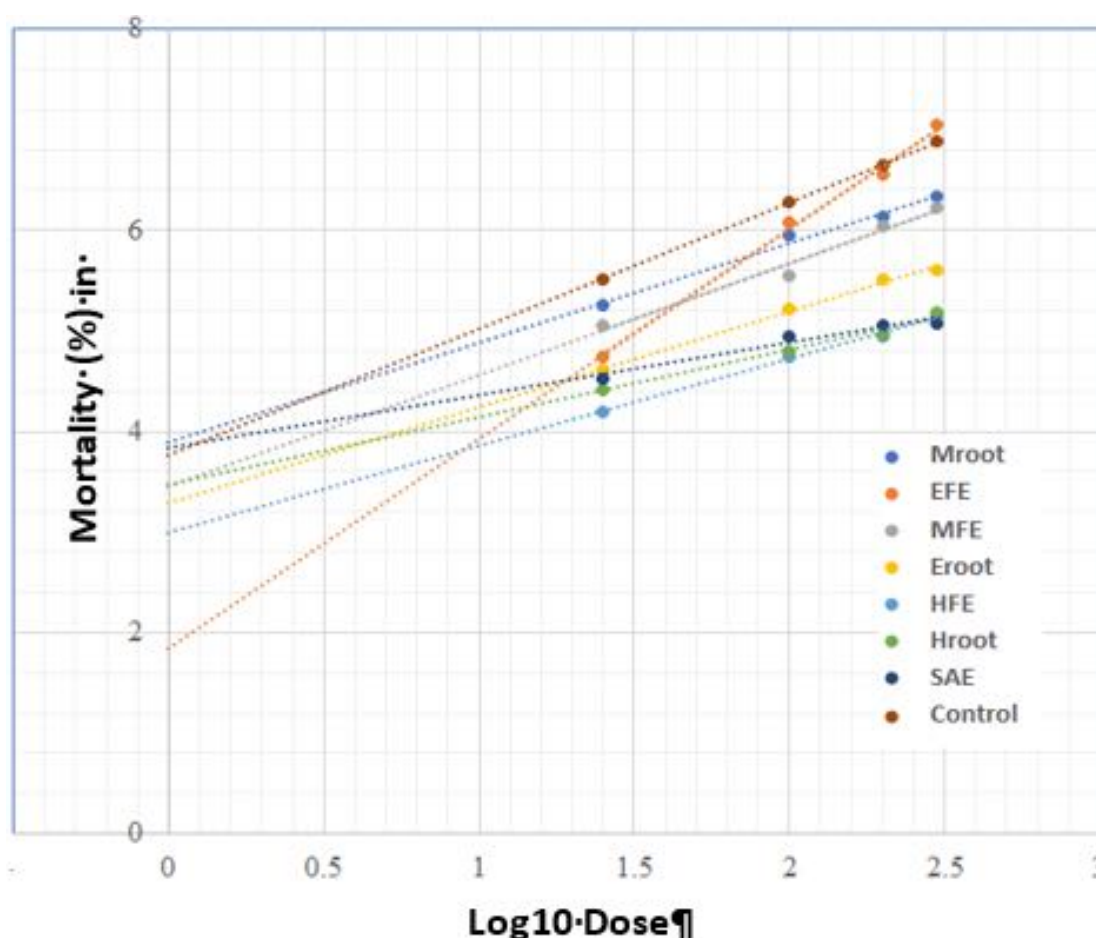
The percentage inhibition values obtained from the experiment were Probit transformed to obtain linear graphs to enable the calculation of the  $IC_{50}$  for each extract [22]. The inhibition concentration ( $IC_{50}$ ) are reported in Table 4. The  $IC_{50}$  values of the crudes ranged from  $35.53 \pm 0.53$  to  $193.77 \pm 0.45 \mu\text{g} \cdot \text{mL}^{-1}$ . A lower  $IC_{50}$  value implied that the sample's potency in scavenging the DPPH free radicals was high. Eroot, Hroot, and EFE had low  $IC_{50}$  values of  $35.53 \pm 0.53$ ,  $39.63 \pm 0.54$ , and  $39.93 \pm 0.21 \mu\text{g} \cdot \text{mL}^{-1}$  respectively. The positive control (ascorbic acid) which recorded an  $IC_{50}$  of  $34.86 \pm 0.25 \mu\text{g} \cdot \text{mL}^{-1}$ .

Antioxidants play a vital role in reducing oxidative processes and harmful effects of ROS in the human body [23]. Since the crude extracts from *S. incanum* showed high antioxidant activity, it can be concluded that they are also possible candidates as antioxidants in the human body and explains why other communities in Africa and India use some of the *Solanum* species as food.

*Solanum* species are recognised for their alkaloid content and anti-growth effects. Alkaloids are also antioxidants, and antidiabetic making them potential drugs for treating neurodegenerative disorders such as Huntington's disease, Parkinson's disease, epilepsy, schizophrenia, and Alzheimer's disease [27]. In *S. melongena*,  $\alpha$ -solamargine,  $\alpha$ -solanine, solasonine, and solasodine have been reported in fruit, root, and peel [28]. Other species of therapeutic interest, such as *S. torvum* have shown the content of solasodine, solasonine, and solamargine as well [28]. *S. nigrum* presents steroidal glycoalkaloids, such as solasodine,  $\alpha$  and  $\beta$ -solamargine,  $\beta_2$ -solasonine, solasonine, solamargine, and 12 $\beta$ , 27-dihydroxy solasodine [9]. The presence of the alkaloids, in the fruit and root of *S. incanum* support the use as ethnomedicines in the communities.

#### **Efficacy tests (Lethal dose-LD<sub>50</sub> for the crude extracts**

Table 5 reports the percentage aphid mortality rates at different concentrations of 25; 100; 200 and 300 mg·mL<sup>-1</sup> which were obtained after 24 hours. The methanol fruit (MFE) and steroidal alkaloidal (SAE) showed a high percentage mortality rate which was above 80% at 100 mg·mL<sup>-1</sup>. A Probit transformation technique [29] was used to transform the percentage of aphid mortalities into regression curves (Figure 2) to enable the determination of the LD<sub>50</sub>.



**Figure 2: The Probit transformed mortality percentages obtained from the regression analysis of the percentage mortalities. The linear graphs were used to calculate the LD<sub>50</sub>**

A summary of the lethal doses of the crude extracts is presented in Table 6. The MFE indicated a high lethal dose against the cabbage aphids followed by the steroidal extract with a lethal dose (LD<sub>50</sub>) of  $13.16 \pm 3.78 \text{ mg} \cdot \text{mL}^{-1}$  and  $24.82 \pm 1.13 \text{ mg} \cdot \text{mL}^{-1}$  respectively. The LD<sub>50</sub> of the MFE and SAE were comparable to that of the control used (Bambazonke) which had an LD<sub>50</sub> of  $10.19 \pm 1.15 \text{ mg} \cdot \text{mL}^{-1}$ .

The occurrence of various secondary metabolites in the fruit and root of *S. incanum* correlates with the pesticidal activity claims made by the Southern African disadvantaged communities. The MFE and the SAE extracts indicated a high efficacy with the LD<sub>50</sub> of  $13.16 \pm 3.78 \text{ mg} \cdot \text{mL}^{-1}$  and  $24.82 \pm 1.13 \text{ mg} \cdot \text{mL}^{-1}$  respectively. Various types of research on *S. incanum* plants have identified secondary plant metabolites, such as alkaloids, glycoalkaloids, terpenoids, organic acids, alcohols, and phenolic compounds as promising sources of bioactivity [1,

11, 30]. Alkaloids and glycoalkaloids being the most abundant phytochemicals in the *Solanum* genus possess a wide range of pesticide activity. Alkaloids inhibit the acetylcholinesterase enzyme (AChE) from transmitting impulses, disrupting the biological membranes, disturbing metabolism, and causing acute toxicity [1, 13, 31]. Terpenes make the mid-gut malfunction, disturb the metabolism process, and repel insects from the crop [32].

Phenolic compounds generally disrupt the biological membranes and affect the feeding habit of the pests [33]. The biological activity of glycoalkaloids, tropane alkaloids, and steroidal alkaloids isolated from *Solanum* species against pests is based on the inhibition of acetylcholinesterase (AChE) [13].

## CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

This study reveals that *S. incanum* has an abundance of phytochemicals that have cytotoxic, antioxidant and pesticidal properties. The MFE and SAE extracts had the highest cytotoxicity, antioxidant activity, and efficacy in the aphid mortality test. This investigation can serve as a guide to chromatographic isolation and purification of new single compounds with pesticide potential which can serve as chemical ingredients to manufacture new pesticides.

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Conflict of interest: The authors declare that they have no conflict of interest and have not reviewed or written a paper like this one before.

**Table 1: The validation parameters obtained for the different standard curves of atropine, quercetin, Gallic acid, and linalool**

| Parameter                            | Standard         |                 |                      |                      |
|--------------------------------------|------------------|-----------------|----------------------|----------------------|
|                                      | Atropine         | Quercetin       | Gallic acid          | Linalool             |
| Recovery (%)                         | 99.24            | 99.9            | 97.9                 | 99.8                 |
| SD                                   | 3.88             | 8.438           | 7.85                 | 6.11                 |
| SE of intercept ( $\times 10^{-2}$ ) | 3.32             | 15.5            | 19.4                 | 0.082                |
| SD intercept ( $\times 10^{-2}$ )    | 8.14             | 37.9            | 43.3                 | 0.184                |
| Mean                                 | 6                | 6               | 5                    | 6                    |
| LOD ( $\times 10^{-2}$ ) mg/mL       | 1.32*            | 0.072*          | 0.000268*            | 13.8*                |
| LOQ ( $\times 10^{-2}$ ) mg/mL       | 0.03996*         | 0.22*           | 8.08*                | 42*                  |
| Accuracy mg/mL                       | 99.24 $\pm$ 3.88 | 99.9 $\pm$ 8.44 | 97.89 $\pm$ 7.85     | 99.8 $\pm$ 6.11      |
| Gradient                             | 0.055            | 0.017           | 0.02                 | 0.45                 |
| Intercept                            | 0                | 0               | 0.0024               | 0.113                |
| Linearity range/ mL                  | 0.2-1.2          | 0.5-3           | 1-5                  | 0.012-0.062          |
| Correlation (R)                      | 0.996            | 0.990           | 0.99                 | 0.99                 |
| Variance (R <sup>2</sup> )           | 0.992            | 0.980           | 0.98                 | 0.98                 |
| Linear equation                      | y = 0.0556x      | y = 0.0173x     | y = 0.0214x + 0.0024 | y = 0.0196x + 0.0032 |

\*At 95% confidence level

**Table 2: Yields and physical characteristics of crude extracts of *S. incanum***

| Crude Name          | Code  | Mass of biomass (g) | Mass of crude (g) | Colour           | The texture of the crude extract |
|---------------------|-------|---------------------|-------------------|------------------|----------------------------------|
| Methanol fruit      | MFE   | 546                 | 13.7              | dark green solid | Oily Sticky                      |
| Ethyl acetate fruit | EFE   | 546                 | 17.8              | Black solid      | soft                             |
| Hexane fruit        | HFE   | 546                 | 11.2              | Dark green solid | oily                             |
| Methanol root       | Mroot | 148                 | 10.4              | dark brown solid | Hard solid                       |
| Hexane root         | Hroot | 148                 | 14,2              | Brown solid      | Hard                             |
| Ethyl acetate root  | Eroot | 148                 | 12.9              | Brown solid      | hard                             |
| Steroidal alkaloids | SAE   | 112                 | 2.61              | Brown solid      | sticky                           |

**Table 3: The results of the total estimates of the phytochemicals in different crude extracts of *S. incanum***

| Crude | Alkaloids<br>mg ATP<br>/100 mg<br>sample | Phenolics<br>mg GAE /100<br>mg sample | Flavonoids<br>mg QCT<br>/100 mg<br>sample | Terpenoids<br>mg LIN /100<br>mg sample | Saponins<br>mg/100 mg<br>sample |
|-------|------------------------------------------|---------------------------------------|-------------------------------------------|----------------------------------------|---------------------------------|
| MFE   | 0.11±0.56                                | 3.49±1.16                             | 1.18±0.93                                 | 1.16±0.79                              | 0.79±0.15                       |
| Mroot | 0.03±0.44                                | 3.72±1.28                             | 0.99±0.07                                 | 0.64±0.08                              | 2.82±0.68                       |
| EFE   | 0.05±0.11                                | 3.53±1.05                             | 0.34±0.16                                 | 1.03±1.03                              | 3.3±1.15                        |
| Eroot | 0.08±0.24                                | 3.14±1.22                             | 0.41±0.10                                 | 0.36±0.14                              | 2.67±1.01                       |
| HFE   | 0.03±0.06                                | 2.52±1.09                             | 3.62±1.23                                 | 1.06±0.44                              | 2.02±0.27                       |
| Hroot | 0.06±0.03                                | 2.84±0.87                             | 2.35±1.01                                 | 1.19±0.47                              | 3.74±1.17                       |
| SAEF  | 0.14±0.06                                | 0.19±0.11                             | 0.33±0.15                                 | 0.1±0.07                               | 0.42±0.17                       |

\*Each experiment was carried out in triplicate and results were expressed as Mean ± SD (N = 3) at 95% confidence level

**HFE:** Hexane fruit extract

**EFE:** Ethyl acetate fruit extract

**Mroot:** Methanol root

**SAE:** Steroidal alkaloid fruit extract

**MFE:** Methanol fruit extract

**Hroot:** Hexane root

**Eroot:** Ethyl acetate root

**Table 4: The inhibitory concentration (IC<sub>50</sub>) of the fruit and root crude extracts of *S. incanum***

| The Crude Extract        | Code          | IC <sub>50</sub> (µg·mL <sup>-1</sup> ) |
|--------------------------|---------------|-----------------------------------------|
| Ethyl acetate fruit      | EFE           | 35.53±0.53                              |
| Hexane fruit             | HFE           | 149.93±1.19                             |
| Methanol fruit           | MFE           | 44.7±0.37                               |
| Ethyl acetate root       | Eroot         | 39.63±0.54                              |
| Hexane root              | Hroot         | 39.93±0.21                              |
| Methanol root            | Mroot         | 59.67±0.37                              |
| Steroidal alkaloid fruit | SAE           | 193.77±0.45                             |
| -                        | Ascorbic acid | 34.86±0.25                              |

\*Each experiment was carried out in triplicate and results were expressed as Mean ± SD (n=3) at 95% confidence level

**Table 5: The percentage aphid mortality rate at different concentrations measured after 24 hours**

| Crude extract code | Concentration (mg·mL <sup>-1</sup> ) |             |             |             |
|--------------------|--------------------------------------|-------------|-------------|-------------|
|                    | 25                                   | 100         | 200         | 300         |
| EFE                | 52.3±6.33a                           | 71.7±7.99a  | 85.3±11.33a | 90.3±9.01a  |
| Mroot              | 32.3±4.12c                           | 48.7±8.24c  | 52.3±4.44b  | 53.3±9.82c  |
| EFE                | 40.7±3.33a                           | 86.6±9.20a  | 94.3±10.66a | 98.7±12.56a |
| Eroot              | 35.3±6.67c                           | 58.3±7.75b  | 70.7±7.63a  | 73.3±9.63b  |
| HFE                | 22.3±5.67c                           | 40.7±6.78c  | 48.3±8.78b  | 56.7±7.56cc |
| Hroot              | 28.3±4.33c                           | 42.3±5.14c  | 49.3±5.92b  | 57.7±8.66   |
| Control            | 70.7±10.64b                          | 90.3±10.65d | 85.7±12.33a | 90.3±10.54a |

- Data are reported as the mean of 3 replicates ±SEM
- In a column showing mortalities, values denoted by the same letter are not significantly different by LSD at  $p \leq 0.05$
- In a row showing mortalities, values denoted by different letters are significantly different by LSD at  $p \leq 0.05$

\*\*Positive control

\*Negative control

**Table 6: The Lethal dose LD<sub>50</sub> values of the *S. incanum* crude extracts against cabbage aphids using an aphid-leaf dip method**

| Crude extract       | Code    | *LD <sub>50</sub> (mg · mL <sup>-1</sup> ) |
|---------------------|---------|--------------------------------------------|
| Steroidal alkaloid  | SAE     | 24.82±1.13a                                |
| Methanol fruit      | MFE     | 13.16±3.78b                                |
| Ethyl acetate fruit | EFE     | 32.64±5.44a                                |
| Ethyl acetate root  | Eroot   | 62.3±11.68c                                |
| Hexane fruit        | HFE     | 95.92±14.21d                               |
| Hexane root         | Hroot   | 169.04±21.25e                              |
| Methanol root       | Mroot   | 194.9±16.84e                               |
| -                   | Control | 10.19±1.55e                                |

\*The results are expressed as (mean ± SD), N = 3

\*\*If the letters next to the LD<sub>50</sub> are different, it shows that the values are significantly different by LSD ( $p \leq 0.05$ )

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