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Phytochemical screening, cytotoxicity and insecticidal activity of the fish poison plant *Synaptolepis alternifolia* Oliv. (Thymelaeaceae).

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ABSTRACT

Synaptolepis alternifolia is a commonly used fish poison plant in Zimbabwe. Root methanolic extracts of *S. alternifolia* were screened for phytochemicals and tested for cytotoxicity and insecticidal activity. Tests indicated the presence of reducing sugars, terpenoids, flavonoids, saponins, tannins and alkaloids. Cytotoxic effects of the plant were assessed through the Brine shrimp lethality (BSL) bioassay. An LD_{50} of 0.372 mg/ml was found indicating that the root extract were cytotoxic. Insecticidal activity was assessed by the mosquito larvae test using *Culex quinquefasciatus* 3rd and 4th instars. LD_{50} and LD_{90} of the methanol extract after 24 hr was 5.6 mg/ml and 82.5 mg/ml while the LD_{50} and LD_{90} after 48 hr were 0.45 mg/ml and 11.3 mg/ml respectively, showing a higher insecticidal activity after 48 hours of exposure to the extract. The results show a huge potential of *S. alternifolia* as an insecticide.

Keywords: *Synaptolepis alternifolia*, Cytotoxicity, Insecticidal activity.

1. Introduction

In many countries, plant fish poisons also known as piscicides or ichthyotoxins, are commonly used to catch fish. In Africa [1] listed 325 fish poison plant species spread among 71 families and 83 genera with the majority belonging to the family Fabaceae. Different parts of the plant such as bark, roots, fruits, pods, whole plants and stems may be pounded and used as fish poison [2]. The active ingredient is released by mashing the appropriate plant parts and introducing them into usually stagnant water bodies or in slow flowing streams and rivers. The toxins stun, stupefy and eventually kill the fish, which then float on the water surface where they are then easily collected. Fish caught using such poisons are apparently safe for human consumption as there are no obvious associated health risks.

Researchers have identified saponins and rotenones as the toxic phytochemical compounds in fish poison plants and have discovered that these compounds possess other important biological properties, such as insecticidal and anti-cancer activities [3]. These compounds are toxic to fish because they are absorbed directly into the blood stream through the gills whereas in most organisms including humans, mammals and birds these phytochemicals are easily detoxified by the action of digestive enzymes in the gut [4]. Saponins and rotenones are known to function in plant defenses, protecting the plants from phytopathogenic microorganisms, phytophagous mammals and insects [5, 6]. Research studies have established the insecticidal properties of some fish poison plant species in the genera *Derris* and *Lonchocarpus* leading to the discovery of the widely used insecticide rotenone [7]. This has stimulated the search for other plants with similar properties with the aim of developing new organic pesticides. Screening for insecticidal properties has been carried out in several plant fish poison species including *Tephrosia vogelii* [8], *Euphorbia tirucalli* [9], *Neorautanenia mitis* and *Gnidia kraussiana* [10], *Mundulea sericea* [11]. Such studies are invaluable especially now due to increasing public concerns of environmental pollution by the conventional synthetic pesticides and the development of insecticide resistance in some insect groups [12].

Eleven fish poison plants were recorded in Zimbabwe including *Synaptolepis alternifolia* which belongs to the family Thymelaeaceae [1]. *S. alternifolia* known as 'Mutsure' in Shona or 'Snake bite root' in English is distributed in the southern and eastern districts of Zimbabwe, but also known to extend into Mozambique, Malawi and Tanzania [13]. In traditional medicine, the powdered root tubers of *S. alternifolia* are used as a purgative and also as an emetic [14] and in Mozambique [13], reported use of the plant to treat diarrhoea and abdominal pains.

S. alternifolia was described by [13] as a shrub or woody climber less than 10 m high with slender glabrous branches. The bark is dark brown, reddish-brown or black, with horizontal whitish lenticels; sometimes with woody tendrils. Leaves are alternate or opposite with a lamina that is deep green on the upper surface and light green or greyish-green beneath. Flower colour ranges from white to pale yellow, cream or yellowish-cream and flowers occur in terminal or axillary cymes. The ovary has a few hairs at the apex or glabrous and surrounded by hairs at the base. The fruit is yellow to reddish-orange in colour.

The present study evaluates the phytochemical composition of *S. alternifolia* and tests for its cytotoxicity and insecticidal activities.

2. Materials and Methods

2.1 Collection of plant materials

The roots of *S. alternifolia* were collected from the Redza Mountain in Zaka district, Masvingo province in early January 2013. Identity of the species was confirmed by botanists in the Department of Biological Sciences, University of Zimbabwe where voucher specimens were deposited in the University herbarium.

2.2 Crude extraction

Fresh rootstocks were oven dried at 50 ^{o}C for 48 hours and then ground to a fine powder using a mortar and pestle. Phytochemical extraction followed the cold maceration extraction procedure [15] as follows: Some 50 gm of powdered material was extracted with 1L of methanol solvent at room temperature in a constantly agitated incubator for 48 hr. The extract was then filtered through Whatman paper no. 42 (125 mm) and the filtrate was collected and concentrated to a thick oily paste at 70 °C using a rotary evaporator. The extract was stored in universal bottles refrigerated at 4 °C.

2.3 Phytochemical screening

Standard qualitative methods as described by [16] were adopted for phytochemical screening. The crude extract was tested for phytochemical constituents using the following tests and reagents: reducing sugars with Fehling's test, anthraquinones with Borntrager's test, terpenoids with Salkowski test, flavonoids with ammonia and sulphuric acid, saponins with foam test, tannins with Ferric Chloride test, alkaloids with Mayer's and Dragendorff's tests and cardiac glycosides with Keller- Killian's test.

2.4 Brine shrimp lethality assay

The cytotoxicity of the crude extract was assessed on brine shrimp nauplii (*Artemia salina*) according to the brine shrimp lethality assay [17]. To prepare the artificial sea water, 12 gm of sodium chloride were dissolved in 1 L of distilled water and the pH was adjusted to 8.5 using 40 % sodium hydroxide. About 2 gm of brine shrimps were hatched in 1 L of sterile sea water in a flask. The cysts were kept under bright light and were constantly agitated and aerated using an aquarium pump. The nauplii hatched within 48 hours at room temperature. The crude extract was dissolved in 1% aqueous dimethyl sulfoxide (DMSO) in artificial sea water to obtain extract concentrations varying from 0.125 mg/ml to 2.0 mg/ml. Ten shrimp nauplii were transferred to each vial. The experiments were performed in triplicate for each concentration. Potassium dichromate (5 mg/ml) and 1% DMSO in sea water were used as positive and negative controls respectively. After 24 hours the vials were examined against a lighted background using a hand held magnifying glass and the number of nauplii that survived in each vial was counted.

2.5 Determination of insecticidal activity

The insecticidal activity of the crude extract was assessed using the Mosquito larvae test following the methods described by [12]. The larvae for the experiments were collected from species of *Culex quinquefasciatus* mosquitoes at Hatcliff Sewage Works in Harare. Larvae were scooped from the sewage ponds and transported to the laboratory in 500 ml glass bottles with perforated lids. The larvae were kept warm on bench tops close to the windows and fed with crushed dog biscuits. Bioassays on the mosquito larvae were done following the methods recommended by [18]. The 3rd and 4th instar larvae were used for the assay and were exposed to test concentrations of 0.125, 0.25, 5, 10 and 20 mg/ml of methanol crude extracts. For all the larvicidal experiments 10 larvae were placed in plastic exposure bowls, with depth and width of 5 cm and 10 cm respectively. Three replicates were performed for each concentration. The number of dead larvae at the end of 24 hr and 48 hr were recorded and the mortality percentage values were calculated.

2.6 Data analysis

Mean and standard deviations were calculated using Microsoft Excel 2007. The cytotoxicity and pesticidal activity data were analysed using linear regression analysis. The regression equations obtained after fitting the trend lines to the data points, were used to calculate the lethal concentrations resulting in 50% mortality (LD_{50}) of the brine shrimp or mosquito larvae.

3. Results and Discussion

3.1 Phytochemical screening

The extract tested positive for reducing sugars, terpenoids, flavonoids, tannins, saponins and alkaloids and negative for anthraquinones and cardiac glycosides (Table 1). These groups of secondary metabolites are similar to those extracted in closely related species of the Thymelaeaceae like in *Aquilaria malaccensis* [19], *Stelleropsis antonina* [20], *Gnidia glauca* [21] and *Synaptolepis kirkii* [22]. Humans have over the years exploited secondary metabolites for their beneficial role in a diverse array of applications including as pharmaceuticals, food, flavors, colours, dyes, poisons and perfumes. Secondary metabolites are referred to as natural products when they have a biological effect on other organisms [23]. The three main groups of natural products i.e. terpenes, alkaloids and phenolic compounds, are represented in the *S. alternifolia* crude extract; hence such a plant is expected to have a number of biological activities.

Terpenes which include terpenoids and saponins are the most numerous and structurally diverse plant natural products and have been exploited by humans in the pharmaceutical and food.

Industries [24]. Important terpenoids include camphor, methanol and

the insecticide pyrethrin. Alkaloids have historically been used as sources of drugs, teas, poultices and poisons [25]. Some important alkaloids include the arrow poison strychnine, morphine, caffeine, quinine and colchicine. Phenolic compounds which include the tannins and flavonoids in this case are mainly associated with antioxidant properties of plant extracts [19]. Our study concurs with similar studies showing presence of saponins, alkaloids and tannins in fish poison plants [4]. These phytochemicals are known to suffocate the fish by destroying the respiratory organs or interfering with the biochemical respiratory pathway thereby causing death or forcing the fish to the surface to gulp for air [26].

Table 1: Results of phytochemical screening tests on methanol extract of *S. alternifolia*

Phytochemical constituents	Observation
Reducing sugars	++
Anthraquinones	-
Terpenoids	++
Flavonoids	++
Saponins	+
Tannins	++
Alkaloids	+
Cardiac glycosides	-

3.2 Brine shrimp lethality assay

Plant toxicity evaluation is important in assessing the safety of use of any plant product. The plant's intrinsic toxicity and the effects of overdose can be determined. The brine shrimp cytotoxicity assay is widely considered as a convenient method for preliminary assessment of toxicity since the brine shrimp is highly sensitive to a variety of chemical substances [27]. It has been successfully used for the detection of toxicity by fungal toxins, food additives, plant extract, heavy metals, cyanobacteria toxins, pesticides and dental materials [28]. Table 2 shows the effect of extract concentration on shrimp mortality.

Table 2. Effect of stem methanolic extract of *S. alternifolia* on brine shrimp.

Extract concentration (mg/ml)	Shrimp mortality (%)
0.125	3.33±5.8
0.25	36.7±5.8
0.5	70.0±10.0
1	93.3±5.8
2	100

The data shows that shrimp mortality increases with increase in concentration of the extract (Figure 1). Mortality was 100% in the potassium dichromate standard and there was no mortality in the negative control.

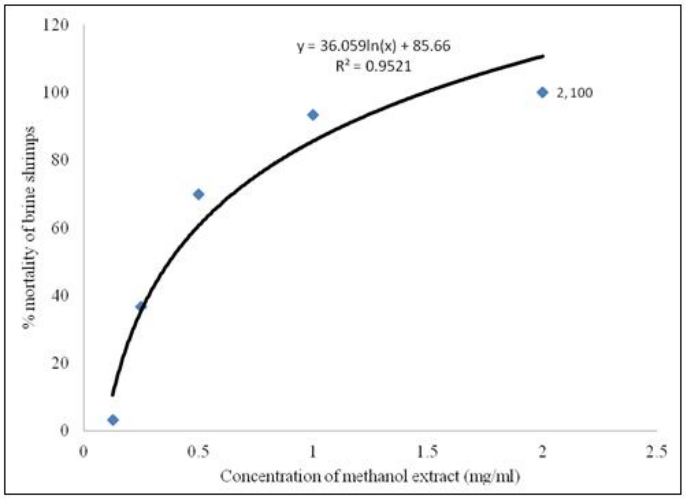

Fig 1: Cytotoxic effects of *S. alternifolia* methanol extract on brine shrimps.

The equation on Figure 1 was used to calculate an LD_{50} of 372 µg/ml. In evaluating plant crude extracts for cytotoxicity [17] recommended that extracts with LD_{50} values less than 1000 µg/ml be considered cytotoxic. In agreement with this [29], classified cytotoxicity as weak when LD_{50} is between 500 and 1000 µg/ml, moderate when LD_{50} is between 100 and 500 µg/ml and strong when LD_{50} is less than 100 µg/ml. According to these benchmarks, the LD_{50} of 372 µg/ml of the *S. alternifolia* crude extract is considered moderately cytotoxic. This indicates the presence of cytotoxic compounds responsible for the observed toxicological activity. Previous studies have implicated alkaloids, flavonoids, saponins and tannins as the cytotoxic agents [30]. All these compounds have been observed in this study and could be responsible for this activity. It is known that *Artemia salina* toxicity test results have a correlation with rodent and human acute oral toxicity data [31]; therefore the current observations could be interpreted to mean that *S. alternifolia* may be harmful to humans when they consume fish killed by the plant. This calls for dose adjustment amongst the communities using the plant extracts since the results show that the degree of lethality is directly proportional to the concentration of the extracts.

3.3 Insecticidal activity

The Mosquito larvae assay has been used by a number of researchers to test for the insecticidal properties of plant extracts [32]. Table 3 shows the mortality of the mosquito larvae after 24 hr and 48 hr exposure at different concentrations.

Table 3: Mortality of *Culex quinquefasciatus* larvae at different *S. alternifolia* root extracts concentrations. (N=3±S.D).

Extract concentration (mg/ml)	Mean mortality (%)	
	After 24hrs	After 48hrs
0.5	13±5.8	40±10.0
1	17±5.8	50±10.0
5	20±0.0	53.3±5.8

10	23±5.8	70±10.0
20	60±10.0	96.7±5.8

Mortalities of mosquito larvae were observed to increase with increase in concentrations of the extract and time of exposure, with higher mortalities being observed after 48 hr compared to 24 hr (Figure 2). Twenty-four hours after exposure, the calculated LD_{50} and LD_{90} were 5.6 mg/ml and 82.5 mg/ml, respectively. When the exposure time was increased to 48 hours, the LD_{50} and LD_{90} were 0.453 mg/ml and 11.28 mg/ml respectively, showing that the extracts exerted maximum insecticidal activity after 48 hours exposure. This result is in agreement with that of [33, 34]. The low LD_{90} value recorded after 48 hr shows that the extract can be applied in small amounts during mosquito larviciding, an economic aspect which makes the plant very useful as a potential insecticide. The small quantities of extract applicable will also assist in preventing the possible insecticide resistance which is common when large doses of the insecticide are used.

In their reviews of plants with insecticidal properties [35] and [36] listed LD_{50} values for plant extracts of more than 150 plant species. Most of these values are much higher than the LD_{50} values obtained in this study for *S. alternifolia*. Furthermore of more the 40 species where methanol was used as the solvent [36] only four species i.e. *Atlanta monophylla, Moringa oleifera*, *Ocimum gratissimum* and *Solenostemma argel* had lower LD_{50} values than those observed in *S. alternifolia*, showing its huge potential as an insecticide.

Phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics have previously been implicated in the insecticidal properties of plants [37]. Insecticidal effects of plant extracts vary not only according to plant species and age, mosquito species, and geographical varieties but also due to solvents used during extraction[35, 38] reviewed the mechanism of action of plant secondary metabolites on insects and documented several physiological disruptions such as inhibition of acetyl cholinesterase, sodium and potassium ion exchange disruption, inhibition of cellular respiration, blockage of calcium channels, disruption of morphogenesis and alteration in the behavior and memory of the cholinergic system.

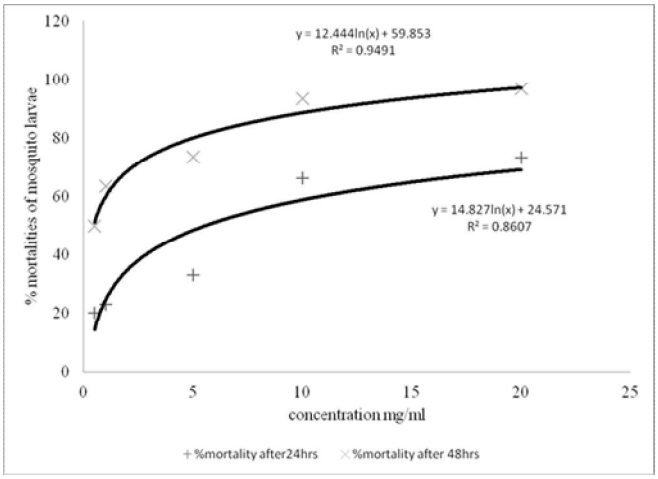

Fig 2: Percentage mortalities of *Culex quinquefasciatus* mosquito larvae at different concentrations.

4. Conclusions

Cytotoxicity has been proved in the *S. alternifolia* crude extracts signaling a public health risk to communities that consume fish poisoned by this plant. However, the effects of cooking that the fish are subjected to before consumption needs further evaluation as the toxic phytochemicals may be thermolabile. Additionally, the extracts showed great potential as an insecticide but firm conclusions can only be arrived at after a more rigorous analysis involving different insect species and life history stages.

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