

Phytochemical screening, cytotoxicity and anti-inflammatory activities of the Zimbabwean endemic plant *Phyllanthus serpentinicola* Radcl.-Sm. (Phyllanthaceae)

C. Zimudzi, L.F.Gwenhure, N.Kunonga, S. Kativu and J. Jere

Department of biological Sciences, University of Zimbabwe, P.O. Box MP 167, Mt Pleasant, Harare, Zimbabwe.

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ABSTRACT

Stem methanolic extract of *Phyllanthus serpentinicola* was screened for phytochemicals, and tested for cytotoxicity and anti-inflammatory activities. Tests indicated presence of alkaloids, tannins, terpenoids, reducing sugars and flavonoids. Saponins, anthraquinones, and cardiac glycosides were absent. Cytotoxic effects of the plant were assessed through Brine shrimp lethality (BSL) bioassay. An LC₅₀ value of 1.3 µg/ml was found, thus indicating that the stem extract was strongly cytotoxic. Anti-inflammatory activity was assessed by the egg albumen induced hind paw oedema test on Swiss albino mice. The extract significantly ($p < 0.05$) reduced the oedema, thus indicating that *P. serpentinicola* has anti-inflammatory properties.

INTRODUCTION

Genus *Phyllanthus* belongs to family Phyllanthaceae, a recent segregate of Euphorbiaceae *sensu lato*. The genus is widely represented in tropical and subtropical regions of the world. Thirty nine species have been recorded in Zimbabwe, with one, *Phyllanthus serpentinicola*, being endemic to the country (Radcliff-Smith, 1996).

The species is restricted to the serpentine soils of the Great Dyke area of the country. *P. serpentinicola* is a suffrutex, up to 30 cm high, with spirally arranged, simple, obovate leaves which bear inconspicuous flowers in leaf axils. Male flowers are solitary and yellowish green, whilst female flowers are dull green, often pinkish tinged, and form a disk with broad rounded lobes. Phytochemical and pharmacological

evaluation of several *Phyllanthus* species has identified a number of useful biologically active compounds. Phyllanthimide, an alkaloid used as a model for the synthesis of cystostatic agents in treatment of cancer is one of several useful compounds derived from *Phyllanthus* (Calixto *et al.*, 1998). *Phyllanthus* species reportedly have antipyretic, analgesic, anti-inflammatory, antihepatotoxic and antiviral activities (Unander *et al.*, 1990). Phytochemical and pharmacological profiles of *Phyllanthus* show variability according to species and environmental conditions (Calixto *et al.*, 1998).

In southern Africa, *Phyllanthus* species are used to treat a variety of ailments including arthritis, bronchitis, earache, sore eyes, swollen gums, burns and wounds (Bosch, 2008). *P. serpentinicola* has never been phytochemically evaluated nor tested for its cytotoxicity or anti-inflammatory activities. The present study evaluates the phytochemical composition of the species and at the same time tests its anti-inflammation and cytotoxic activities.

* Corresponding Author

C. Zimudzi, Department of biological Sciences, University of Zimbabwe,
P.O. Box MP 167, Mt Pleasant, Harare, Zimbabwe.

Telephone: +263-4-772669975, Fax: +263-4-333407

MATERIAL AND METHODS

Collection of plant material

Fresh stems of *P. serpentinicola* were collected from Mutorashanga Pass along the Great Dyke of Zimbabwe in January 2012. The plant was identified *in situ*. Voucher specimens are deposited at the University of Zimbabwe teaching herbarium. Freshly collected stems were washed in tap water and air-dried for two weeks.

Crude extraction

Dried stems were chopped into pieces and milled into a fine powder by pounding manually with a clean pestle and mortar. Some 2g of ground material was extracted with 100 ml analytical grade methanol as solvent at room temperature for 48 h using the cold maceration method.

The extract was filtered using Whatman filter paper (No: 1), concentrated and evaporated to dryness *in vacuo* at 40°C using a rotary evaporator. The powder extract was re-dissolved in distilled water and stored in a refrigerator at 4°C.

Phytochemical screening

Standard qualitative methods as described by Sowofora (1993) and Tiwari *et al.* (2011) were adopted for phytochemical screening. The crude extract was tested for phytochemical constituents using the following tests and reagents: reducing sugars with Fehlings 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.

Brine shrimp lethality test

The cytotoxicity activity of the crude extract was assessed on brine shrimp nauplii (*Artemia salina*) according to brine shrimp lethality bioassay (Meyer *et al.*, 1982). Artificial sea water was prepared by dissolving 12 g sodium chloride in 1 L of distilled water and adjusting the pH to 8.5 using 40 % sodium hydroxide. Some 2 g of brine shrimp eggs were hatched in 1L of sterile sea water in a flask. The cysts were kept under bright light, and were continuously agitated and aerated using an aquarium pump. The nauplii hatched within 48 h at room temperature.

The methanol crude extract was dissolved in 1% aqueous dimethyl sulfoxide (DMSO) in artificial sea water to obtain concentrations of extract varying from 100 mg/ml to 0.25 mg/ml. Some 50 µl of each solution was transferred, to clean sterile vials containing 5 ml of aerated seawater. Ten shrimp nauplii were transferred to each vial. The experiments were performed in triplicate for each extract concentration. Potassium dichromate (5mg/ml) and 1% DMSO in seawater were used as positive and negative controls, respectively. After 24h, the vials were examined against a lighted background using a hand held magnifying glass, and the number of nauplii that survived in each vial were counted.

Evaluation of anti-inflammatory activity

Animals

Adult Swiss albino mice of both sexes were used for these experiments. They were obtained from the Animal House at Central Veterinary Laboratories in Harare, Zimbabwe. The animals were housed in cages, and were allowed free access to standard pellets and water. All procedures used in the study comply with guidelines on animal care provided by the University of Zimbabwe Ethics Committee on the use of animals.

Anti-Inflammatory Test

The test was carried out using a phlogistic agent – induced mouse hind paw oedema as a model of acute inflammation (Winter *et al.*, 1962). Fresh egg albumen was used as the phlogistic agent. Adult Swiss albino mice of either sex were fasted for 14 h and then divided into 5 groups of six mice each. Each group received a different treatment. The extract (100,200 and 300 mg/kg) was administered intra-peritoneally using a syringe 1h before inducing inflammation. The negative control group received 10 ml/kg distilled water and the positive control 100 mg/kg of Acetic salicylic acid (ASA). Inflammation was induced by injection of 0.1ml of fresh egg albumen into the subplantar surface of the right hind paw of the mice. Paw oedema was measured by wrapping a piece of cotton thread round the paw and measuring the circumference with a ruler (Okokon *et al.*, 2012). The measures were determined at 1, 2 and 3 h following egg albumen injection.

Data analysis

Results were analyzed using Microsoft Excel statistical package (2007). Cytotoxicity data were analysed using linear regression analysis. All values were expressed as mean ± SEM. A one-way ANOVA was applied to determine the significance of the difference between the control groups and mice treated with the extracts. A Student's t-test was used to compare the treatments and controls, and results were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening

Table 1 shows the results of the phytochemical screening. The extract tested positive for alkaloids, tannins, terpenoids, reducing sugars and flavonoids, and negative for saponins, anthraquinones, and cardiac glycosides. The observed phytochemical profile of *P. serpentinicola* is similar to that of other *Phyllanthus* species (Calixto *et al.*, 1998).

Screening for phytochemicals in plants is important as a first step in elucidating the pharmacological properties of a plant species. The phytochemicals identified in *P. serpentinicola* belong to large diverse groups with varied pharmacological activities. Alkaloids are known to exhibit anti-inflammatory, cytotoxic antibacterial, analgesic and anti cancer activities (Roberts and Wink, 1998). Flavonoids and tannins are phenolic compounds that are known to have antimicrobial, anti-inflammatory, cytotoxic,

antiallergic, analgesic and antioxidant properties (Cowan, 1999). Terpenoids are reported to have anti-inflammatory, anti cancer, antimalarial and antibacterial properties (Govindappa *et al.*, 2011).

Table 1. Results of phytochemical screening tests on methanol extract of *P. serpentinicola*

Test	Observation
Alkaloids	+
Cardiac Glycosides	-
Tannins	+
Antraquinones	-
Terpenoids	+
Saponins	-
Reducing sugars	+
Flavonoids	+

Key - = absent and + = present

Table 2. Effect of stem methanolic extract of *P. serpentinicola* on fresh egg albumen-induced inflammation in mice (means \pm SD, n=6, * p <0.05).

Treatment	Dose(mg/kg)	Paw Oedema (cm)		
		1h	2h	3h
Distilled water	10ml/kg	1.47 \pm 0.12	1.58 \pm 0.08	1.73 \pm 0.15
PSE	100	1.90 \pm 0.13*	1.81 \pm 0.1*	1.77 \pm 0.10
PSE	200	1.87 \pm 0.08*	1.76 \pm 0.09*	1.68 \pm 0.073
PSE	300	1.85 \pm 1.17*	1.72 \pm 0.14*	1.62 \pm 0.13
ASA	100	1.95 \pm 0.04*	1.86 \pm 0.07*	1.79 \pm 0.06

Key: PSE-*Phyllanthus serpentinicola* extract; ASA-Acetosallylic acid

Brine shrimp lethality test

The brine shrimp mortality was 100% in the Potassium dichromate standard and in all extract concentrations above 8 μ g/ml. These data have been excluded from Figure 1 which shows the mean percent mortality of the brine shrimp nauplii against the logarithm of the sample concentrations. The mortality of brine shrimps increased with increasing concentration of extract. The regression equation on Figure 1 was used to calculate an LC₅₀ value of 1.3 μ g/ml.

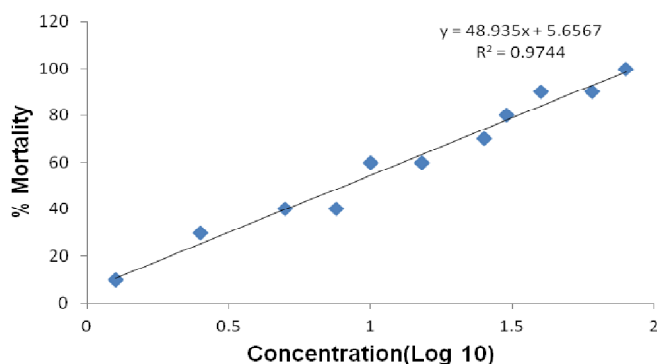


Fig. 1: Mortality of brine shrimp nauplii in different *P. serpentinicola* stem extract concentrations.

Standard brine shrimp lethality bioassay stipulates that LC₅₀ values less than 1000 μ g/ml are considered bioactive in toxicity evaluation of plant extracts (Meyer *et al.*, 1982). Based on this benchmark, the *P. serpentinicola* extract is strongly cytotoxic since the LC₅₀ value was less than 1000 μ g/ml. Cytotoxicity of the extract is attributed to presence of alkaloids, tannins, flavonoids and terpenoids (Sandhar *et al.*, 2011). The LC₅₀ value of 1.3 μ g/ml

for *P. serpentinicola* is comparable to that for *Phyllanthus engleri* (0.47 μ g/ml) where cytotoxicity has been attributed to presence of triterpenoid phyllanthol (Bosch, 2008). *P. engleri* is a poison plant commonly encountered in suicide cases in Zimbabwe (Bosch, 2008). Thus, care should be exercised in using *P. serpentinicola* as a medicinal plant.

Anti-inflammation activity

Egg albumen induced oedema is a widely accepted model for the evaluation of anti-inflammatory effect of plant extracts. Table 2 shows the activity of *P. serpentinicola* extract against egg albumen induced paw oedema in mice. Control mice produced a local hind paw oedema that increased progressively throughout the three hour period. Pretreatment by *P. serpentinicola* extract significantly (p <0.05) reduced the oedema in the first two hours, but no significant differences between the treatments and control were observed after three hours (p >0.05). The standard showed a similar pattern to the treatments. Egg albumen inflammation is biphasic, and the high anti-inflammatory activity of the extract may be a response to the initial acute phase of inflammation. The first phase occurs within two hours of administering the phlogistic agent, and is due to the release of histamine or serotonin, and the second phase of oedema is due to the release of prostaglandin (Oyekunle and Idowu, 2012). Results of the present study indicate that the methanol extracts of *P. serpentinicola* significantly reduced paw oedema in mice. The anti-inflammatory mechanism of action may be due to blocking of the release of these chemical mediators.

The anti-inflammatory activity *P. serpentinicola* is attributed to the phytochemical constituents of the plant extract which include terpenoids, tannins, flavonoids and alkaloids. Plants containing these chemical classes of compounds have been reported to possess potent anti-inflammatory properties that act through inhibiting prostaglandin pathways (Oyekunle and Idowu, 2012).

CONCLUSIONS

The present study has demonstrated that stem methanolic extract of *P. serpentinicola* exhibits anti-inflammatory and cytotoxicity activities. Caution should; however, be exercised in the use of this species for medicinal purposes because of its high toxicity as evidenced by the LC₅₀ values of 1.3 μ g/ml. The results also suggest that the plant contains bioactive constituents that are responsible for the observed activities. The results from this study suggest that *P. serpentinicola* like other *Phyllanthus* species can be useful for the management of pain.

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