NJBAS

Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (December, 2019), 27(2): 136-141
DOI: http://dx.doi.org/10.4314/njbas.v27i2.18

ISSN 0794-5698

Brine Shrimp Cytotoxic and *Lemna minor* Phytotoxic Evaluations of the Methanol Extracts of the Leaves, Stem and Root Barks of *Sacrocephalus latifolius*

E. O. Ikpefan
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University Abraka
[E-mail: ikpefanemmanuel@delsu.edu.ng]

ABSTRACT

This work was aimed at evaluating the probable toxicity of the methanol extract of the leaf, stem bark and root of *Sacrocephalus latifolius* on *Artemia nauplii* and fronds of *Lemna minor*. The powder samples of the three morphological parts were independently extracted with soxhlet extractor apparatus using 95% methanol and were dried with aid of ritory evaporator at 40°C. The extracts were subjected to biological activities involving brine shrimp cytotoxicity and *Lemna minor* test for phytotoxicity at 10-100 µg /mL respectively. The experiment was done in replicates of three. Concentration-dependent cytotoxic and phytotoxic activities were recorded for the extracts. The results revealed that extract of the leaves demonstrated significant cytotoxic and phytotoxic activities over the other parts of the plant. At 10µg/mL, the three extracts recorded little or no cytotoxic activity. However, at the maximum concentration of 1000 µg /mL, cytotoxicities of 66.67 and 3.33% were recorded for extracts of the leaf and root bark respectively, while the extract of the stem bark showed no activity. However, the LC_{50} of the leaf was 467.74µg /mL, and that of the other extracts were observed to be >1000µg /mL. The results for phytotoxicity followed a similar trend with the extracts of the leaf and root bark recording 52.96 and 23.33 % phytotoxicities respectively at 1000 µg /mL. Having shown a higher activity over the other extracts, the leaf extract of *S. latifolius* could serves as a natural alternative pesticide and weedicide.

Keywords: *Sacrocephalus latifolius*, phytotoxicity, cytotoxicity, fronds, *Artemia nauplii, Lemna minor*

INTRODUCTION

The plant *Sacrocephalus latifolius* (Sm.) E. A. Bruce (family, Rubiaceae) has over the years been reported to have numerous medicinal uses and applications. Previously known as *Nauclea latifolia* and commonly as African peach is a shrub known locally as *Egbesi* in Yoruba, *Ubuluinu* in Igbo, *Tafashiya* in Hausa languages in Nigeria.

In Nigeria and most parts of Africa, this plant has been described to have many ethnomedicinal uses such as in the control of diabetes and malaria (Orwa *et al*., 2009), anti-hepatotoxic and trypanocidal (Madubunyi, 1995) using the leaves and bark respectively. Other ethnomedicinal uses of the plant include treatment of diarrhea, hypertension, dysentery, pain and epilepsy (Ngo Bum *et al*., 2009). The extracts of the plant have previously been reported to be antidiabetics (Gidado *et al*., 2005), anticonvulsant, anxiolytic (Ngo Bum *et al*, 2009), antihypertensive and laxatives activities (Akpanabiantu *et al*., 2005).

One of the major hindrances encountered in farming is weed, which struggles with the crop for accessible nutrients, water, and soil space, which affect harvest yield. As a means of preventing these challenges, farmers resort to the use of synthetic herbicides which are sometimes toxic to man and causes soil and water pollution. Their long term use has also increase weed resistance (Schütte, 2003). Hence, there is need to focus on research to detect phytotoxic substances that could be used as new herbicidal templates.

The increasing rate of application of bioassay models in modern day research for the evaluation of samples including natural products could be due to its simplicity and rapidity (Krishnaraju *et al*., 2006). Bioassay lethality assay involving the use of Brine shrimps developed by Michael *et al.* (1956) is a valuable technique for evaluating preliminary toxicity. Plant metabolites are

sometimes toxic to the larvae of *Artemia salina* hence this experiment is used to ascertain the level of cytotoxic effects of metabolites present in plant and other natural products. This work is therefore aimed at determining the phytotoxic and cytotoxic characteristic of the methanol extracts of leaves, stem and root barks of *S. latifolius*.

MATERIALS AND METHODS

Collection of plant materials

The plant *Sacrocephalus latifolia* (leaves, stem and root barks) were collected between February and March 2015 in Benin City, i.e. University of Benin and Sabongida-Ora both in Edo State, Nigeria. The identity of the plant was validated at Forest Research Institute of Nigeria (F.R.I.N.) Ibadan where herbarium specimen number FHI 108340 was deposited.

Processing of Plant Materials

The plant parts were spread in the laboratory to dry for 3 days and were further dried in an oven sustained at 45°C (for the leaves) and 60°C (for the root and stem barks). Thereafter, each material was powdered using a laboratory electric milling machine (Chris Norris, England) and subsequently stored in an airtight jar.

Extraction of Plant Materials

Each plant material was collected (2.5 kg) was exhaustively extracted using a Soxhlet apparatus, and aqueous methanol (95%) as the extracting solvent. The extracts were decreased to dryness with aid of rotary evaporator at 40°C.

Preliminary Phytochemical Screening of Extracts

Phytochemical screenings of the crude extracts were performed using conventional procedures by Sofowora (2008).

Determination of Cytotoxic Effects Using Brine Shrimp

The hatching of shrimp eggs was carried out using the methods of Saima *et al.*, (2017) and the cytotoxic investigation was carried out by applying 20 mg of individual extracts previously dissolved in 2 mL distilled water to give concentrations of 10, 100 and 1000 µg/mL. The solutions were allowed to concentrate overnight. Eventually, 5 mL seawater water solution (38 g/L) was added to each vial.

Following 36 h of hatching and maturation of larvae as nauplii, 10 larvae were assigned to each vial applying a Pasteur pipette. The vials were then stored at room temperature (25-27°C) under lighting. Other vials supplemented with brine solution served as positive controls (McLaughlin, 1991). LC50 was calculated using the following regression equation.

$Y = ax + b$ ($a= 2.72$, $b= -2.28$, $Y= 5$) with $x = \text{Antilog}_{10} 2.67$.

Phytotoxicity Assay using Lemna Minor

The effects of the various extracts on fronds of *Lemna minor* were carried out at various concentrations. The preparation of the media was done by dissolving E-medium in 100 mL of distilled water and pH was maintained at 6.0-7.0 by the addition of KOH solution. The media were autoclaved at 121°C, 15 psi for 15 min in an autoclave. A stock solution was prepared by dissolving 10 mg of the extract in 1 mL of ethanol. Three concentration replicates (10,100 and 1000 µg/mL) were made in flasks from the stock solution. The solvent was evaporated from the flask in the aseptic environment. On each container, 20 mL of the autoclaved medium together with ten plants each possessing a rosette of three fronds, were added. The percent (%) growth inhibition was analyzed with a recommendation to the negative and positive control (Atta-ur-Rahman, 1991).

$$\text{Growth inhibition (\%)} = \left(100 - \frac{\text{No of fronds in sample}}{\text{No of fronds in control}}\right) \times 100$$

Statistical Analysis

All data were expressed as Mean ± SEM and one-way Analysis of Variance (ANOVA) statistical test. The percentage cytotoxicity for brine shrimp and growth inhibition on *Lemna minor* fronds

were calculated from the mean survival larvae and fronds respectively for the three extracts as well as the control groups. The LC_{50} values of the extracts against brine shrimp were obtained from the best-fit line by regression analysis. One-way ANOVA was carried out using Graph pad Instat R version 2.05 (UK) was used to test for significance. $P< 0.05$ was considered significant.

RESULTS

From this work, the overall yield of crude methanol extracts from 2.5 kg of the powdered plant samples varied among the different morphological parts of the various plants. The root bark of *S. Latifolia* produced the highest yield of 252.75g (10.09%) while the leaf as well as the stem bark gave 189.44g (7.44%) 186.11 (7.58%) respectively. Although, the same weights (2.5kg) of the powdered samples were extracted, the barks (stem or root) were observed to produce higher extracts than the respective leaves of the same plants (Table 1).

Table 1: Yield of the extracts and their respective percentages

Extracts	Weight (g)	Yield (%)
Leaf	189.48	7.58
Root	252.27	10.09
Stem	186.11	7.44

Results of the Preliminary Phytochemical Screening of Extracts

The results of the preliminary phytochemical screening of the three morphological parts of *S. latifolius* showed variations in secondary metabolites among the plant parts (Table 2).

Results of Cytotoxicity of the Methanol Extracts on Brine Shrimp (*Artemiasalina*)

The extracts were observed to exhibit a concentration dependent activity. The larvae showed variable response towards various concentrations of the extracts. All of these three parts showed different growth inhibition at different concentrations. Using the criteria of Ali *et al* (2014) which suggested an extract could show low (30-40%), moderate (50% lethality), good (60-70%) or significant activity (>70% lethality), the extracts of *S. latifolius* particularly the leaf extract at the maximum concentration (1000 µg/mL) gave 66.7% (good activity) with LC_{50} of 671 µg/mL, while the stem and root bark extract recorded little or no activities. However, etoposide used as standard drug produce LC_{50} of 7.46 µg/mL (Table 3).

Table 2: Summary of results of the phytochemical screening of the methanol extracts

Phytochemical Groups	Extracts of *S.latifolius*		
	Leaf	**Stem bark**	**Root bark**
Anthraquinone	-	-	-
Alkaloids	+++	+	++
Cardiac glycosides	+	+	+
Flavonoids	++	-	+
Tannins	++	+	+
Terpenes	+	+	+
Saponins	++	+	+
Steroids	+	-	-

+ = present, - = absent, + += moderate amount, +++= abundant amount.

Result of the Growth Inhibitory Effects of the Methanol Extracts on *Lemna minor*

The phytotoxicity was noted to be dose dependent and the results were recorded as low (% inhibition (≤40 %), moderate (% inhibition = 40) and significant (50-100%) as prescribed by Ullah *et al.,* (2012). At the maximum concentration of 1000 µg/mL, the extract of the leaf gave a significant growth inhibition of 52.96% while the root bark extract recorded low inhibition (23.33%) respectively. However, the extract of the stem gave little or no inhibition (Table 4)..

Table 3: Cytotoxic effects of the crude extracts against Brine shrimps nauplii

Extracts of *S. latifolius*	% Mortality			
	10µg/mL	**100µg/mL**	**1000µg/mL**	**LC_{50} (µg/mL)**
Leaf	0.00 ± 0.00	16.99 ± 0.30	66.67 ± 1.44	467.74
Stem bark	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	>1000
Root bark	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 0.30	>1000
***Ectoposide*(positive control)**	70.00 ± 0.67	100.00 ± 0.00	100 .00± 0.00	6.76
Distilled water (Negative control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-

Values are expressed as the mean ± SEM of three independent observations. LC_{50} of the leaf extract x from regression equation

Y= ax +b, x =antilog$_{10}$ 2.67= 467.74, Standard drug (Ectoposide), x= 69.83, LC_{50} = 6.76µg/mL

Table 4: Phytotoxic activity of the crude extracts against the *Lemna minor*

Extracts	**% Growth Inhibition**			
	10µg/mL	**100**µg/mL	**1000**µg/mL	Standard Drug(µg/mL)
Leaf	3.33 ± 0.8	16.66± 1.00	52.96 ± 1.11	
Stem bark	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100 % Inhibition
Root bark	0.00 ± 0.00	6.66 ± 0.33	23.33 ± 2.99	
Distilled water (-ve control)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	

Values are expressed as the mean ± SEM of three independent observations. Conc. Standard drug (Paraquat) 0.015 µg/mL.Incubation conditions = 28 ± 1 °C.

Discussion

The capacity of plants to manifest certain therapeutic activities is a representation of the active constituents they contain (Kinghorn *et al*., 2011). The outcome of the phytochemical screening of extracts of the three morphological parts of *S. latifolius* confirmed the presence of alkaloids, cardia glycosides, flavonoids, tannings, saponins in varying intensities. The preliminary biological screening for cytotoxicity and phytotoxicity of the extracts of the leaves, stem and root barks of *S. latifolius* was carried out at 10-1000 µg/mL respectively.

These variations in phytochemical groups could be as a result of the time of collection of the individual plant parts. The constituents were observed more in the leaves than stem and barks, which could due to the fact that they were produced in the leaves and were later translocated to other parts of the plant.

The activity of the extract increased with increased concentrations implying a concentration-dependent activity. For example, the highest mortalities (100%) were observed at a 1000 µg/mL. Following this, the mortality rate of the extracts on the shrimp could be said to be concentration-dependent which could be as a result of increased concentrations of potent cytotoxic and probably antitumor components of the extracts.

As recommended by Meyer *et al*. (1982), a substance is said to be toxic (active) if it has an LC_{50} value of less than 1000 g/mL and non-toxic (inactive) if it is greater than 1000 mg/mL. Hence, the leaf extract could be said to be active unlike the other plant parts. Baravalia *et al* (2012) recorded significant mortality (73 %) of the

methanol extract of *Woodfordia fruticosa* flowers at 1000 µg /mL and LC_{50} of 763.34 µg /mL which is in line with this work.

Apart from the determination of allelopathic potential of a plant, phytotoxicity also plays an essential role in the developing and design of natural plant growth regulators or biological herbicides (Tranel and Wright, 2002). Weed control using synthetic herbicides has led to the development of weed resistance towards herbicides and also pollution of aquatic environment (Binkley and Brown, 1993).

Previous work by Khurm *et al*, (2016) on the dichloromethane extract of *Heliotropium strigosum*, recorded significant growth inhibition of 50% at 1000 µg/mL and low (35%) and moderate inhibitions (40%) at 10 and 100 µg/mL respectively. Similarly, Ullah *et al*. (2012) in their phytotoxic study of the methanol extract of *Calendula arvensis* recorded moderate (46.03%) and low (31.74%) inhibitions at 1000 and 100 µg/mL. These findings are in line with results of our phytotoxic studies.

CONCLUSION

The extracts of the leaves of *S. latifolius* exerted cytotoxic and phytotoxic effects on the brine shrimp and *Lemna minor* more effectively than the other extracts. Thus, the leave of *S. latifolius* will constitute a promising source in search of cytotoxic and phytotoxic compounds which could be utilized in the formulation of biological weedicides with less risk to human well-being and also the surroundings.

ACKNOWLEDGEMENTS

This work is supported by NAM S&T (2014) and was carried out at the International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan.

REFERENCES

Akpanabiantu, M.I., Umoh, I.B., Udosen, E.O., Udo, A.E., Edet, E.E. (2005). Rat serum electrolytes, lipid profile and cardiovascular activity of *Nauclea latifolia* leaf extract administration. *Indian Journal* of *Clinical Biochemistry*, **20:** 29–34.

Ali, N., Vishwakarma, P. K., Khan, I. and Sohaib, M. (2014). In Vitro Antibacterial, Antifungal and Phytotoxic Activities of FicusCaricaMethanolic Leaves Extracts, *International Journal of Current Biotechnology,* **2**(2): 11-15.

Atta-ur-Rahman, Choudary MI, Thomsen JW (1991). In: Manual of bioassay techniques for natural product Research, Harward Academic Press, Amsterdam. 82-84.

Baravalia Y., Vaghasiya Y., Chanda S., 2012 Brine shrimp cytotoxicity, anti-inflammatory and analgesic properties of Woodfordia fruticosa Kurz flowers. *Iranian Journal of Pharmaceutical Research*, **11**(3):851-861.

Binkley, D. and Brown, T.C. (1993). Forest Practices as Nonpoint Sources of Pollution in North America, *Water Resources Bulletin*, **29**, 729 - 740.

Gidado, A., Ameh, D.A., Atawodi, S.E. (2005). Effect of Nauclea latifolia leaves aqueous extracts on bloods glucose levels of normal and alloxan-induced diabetic rats. *African Journal of Biotechnology*, **4:** 91–93.

Khurm, M., Chaudhry, B.A., Uzair, M. and Janbaz, K.H. (2016). Antimicrobial, cytotoxic, phytotoxic and antioxidant potential of *Heliotropium strigosum* Willd. *Medicines*,**3**(3): 20.

Kinghorn, A.D., Pan, L., Fletcher, J.N., Chai, H. (2011). The relevance of higher plants in lead compound discovery programs, Journal of *Natural Products*, **74**:1539–1555.

Krishnaraju AV, Tayi Rao VN, Sundararaju D, Vanisree M, Tsay HS, and Subbaraju GV (2006). Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). *International Journal of Applied Science and Engineering*, **2:** 115-125.

Madubunyi, I. I. (1995). Anti-Hepatotoxic and Trypanocidal Activities of the Ethanolic Extract of *Naulea latifolia* Root Bark. *Journal of Herbs, Spices* & *Medicinal Plants*, **3**(2): 23-25

McLaughlin, J.L., Chang, C.J. and Smith, D.L. (1991). Bench Top Bioassays for the Discovery of Bioactive Natural Products: An Update. In: Studies in Natural Products Chemistry, Atta-Ur-Rahaman, (Ed.). Vol. 9, Elsevier, Amsterdam, pp: 383-409.

Meyer,B.N.,Ferrigini,R.N., Putnam,J.E.,Jacobsen,L.B.,Nichols, D.E.,McLauglin,J.L.(1982)Brineshrimp:A convenient general bioassay for active plant constituents. *Planta Medica*, 45, 31-35.

Michael, AS, Thompson CG, Abramovitz M (1956). Artemiasalina as a test organism for bioassay. **Science**, **123**:(3194)464.

Ngo Bum, E., Taiwe, G.S., Moto, F.C.O., Ngoupaye, G.T., Nkantchoua, G.C.N., Pelanken, M.M., Rakotonirina, S.V., Rakotonirina, A. (2009). Anticonvulsant, anxiolytic and sedative properties of the roots of *Nauclea latifolia* Smith in mice. *Epilepsy* and *Behavior,* **15**:434–440.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simona, A. (2009). Agroforestree Database: a tree reference and selection guide version 4.0 (http/www.worlddagroforestry.org/af/treedbi). Accessed on 10/6/13.

Saima, S., Muhammad, A. K., Barkatullah, I. (2017): Cytotoxic and Phytotoxic Activities of *Reinwardtiatrigyna*(ROXB.). *International Journal of Applied Agricultural Sciences*, **3**(2): 32-36

Schütte, G. (2003). "Herbicide Resistance: Promises and Prospects of Biodiversity for European Agriculture." *Agriculture and Human Values,* **20**(3): 217–30.

Sofowora, L. A., (2008). Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., Spectrum Books Ltd., Ibadan, Nigeria, ISBN: 9789780298814, Pages: 289.

Tranel PJ and Wright TR (2002). Resistance of weeds to ALS-inhibiting herbicides: What have we learned? *Weed Science*, **50**: 700–712

Ullah, F., A. Bano and A. Nosheen. 2012. Effects of plant growth regulators on growth and oil quality of canola (Brassica napus L.) under drought stress. *Pakistan* Journal of *Botany*, **44**(6): 1873-188.