

Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, north western Tanzania

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Abstract: Dichloromethane and/or ethanol extracts of 30 plants used as traditional medicines in Bukoba district, northwestern Tanzania were evaluated for brine shrimp toxicity. Among the 50 extracts tested, 32 extracts (64%) showed very low toxicity with LC₅₀ values above 100 µg/ml. Among these 12 (24%) which had LC₅₀ >500 µg/ml can be categorized as being practically non-toxic. Among the remaining extracts 19 (38%) which showed LC₅₀ >100 < 500 µg/ml are also considered to be non-toxic. Extracts that showed LC₅₀ results between 30-100 µg/ml have been categorized as mildly toxic; these include ethanol extracts of *Lantana trifolia* (LC₅₀ 32.3 µg/ml), *Vernonia bradycalyx* (LC₅₀ 33.9 µg/ml), *Antiaris toxicaria* (LC₅₀ 38.2 µg/ml) and *Rubus rigidus* (LC₅₀ 41.7 µg/ml) and the dichloromethane extracts of *Gynura scandens* (LC₅₀ 36.5 µg/ml) and *Bridelia micrantha* (LC₅₀ 32.0 µg/ml). The dichloromethane extracts of *Picralima nitida* (LC₅₀ 18.3 µg/ml) and *Rubus rigidus* (LC₅₀ 19.8 µg/ml), were only moderately toxic. *Picralima nitida* and *Rubus rigidus* extracts are only 1.1 and 1.2 less toxic than the standard drug, cyclophosphamide (LC₅₀ 16.3 µg/ml). In conclusion, the results indicate that among the 30 plants used as traditional medicines, 28 are safe for short term use. *Picralima nitida* and *Rubus rigidus* extracts are mildly toxic, but by comparison have a remote possibility to yield active anticancer compounds.

Keywords: Traditional medicine; Brine shrimp toxicity; safety evaluation

Introduction

Traditional medicines support well over 60% of the rural Tanzanian population (Kisangau *et al.*, 2007). Available evidence suggests that even in urban areas which are well served by modern healthcare facilities a good number of patients rely on traditional healers to meet some of their healthcare needs (Kilima *et al.*, 2003). The Kagera region of northwestern Tanzania is one of places where traditional medicines are widely used and thus play a significant role in the provision of health care. According to *Medicine du Monde*, a French non-governmental organization in Kagera Region, five out of every six HIV patients receive their medical attention from a traditional healer rather than from a hospital or primary health care facility (Anonymous, 1996). Due to good rains and vegetation cover in the region, there is a rich diversity of medicinal plants present. However most of these are yet to be documented and evaluated for safety and efficacy.

Current efforts therefore aim at documenting these plants and in addition evaluate them for safety and efficacy. Two recent studies in Bukoba rural district (Kisangau *et al.*, 2007; Moshi *et al.*, 2009) have set the pace and more studies are ongoing. This study reports on brine shrimp toxicity tests of extracts from some of the plants reported from Bugabo Ward in Kagera region by Moshi *et al.* (2009). These tests are normally conducted so as to

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draw some inferences on the safety of plant extracts, and to depict trends of their biological activities (Harwig & Scott, 1971; Meyer *et al.*, 1982).

Materials and Methods

Materials

Ethanol was bought from Fisher Scientific UK Ltd. (Loughborough, Leicestershire, UK), Dimethyl sulphoxide (DMSO) from Sigma (Poole, Dorset, UK and Brine shrimp eggs from O.S.I. Marine Lab. Inc., Hayward, CA 94545, USA.. Sea salt was prepared by evaporating water collected from the Indian Ocean along the Dar es Salaam coast.

The plants used in this study are among plants recently reported as being used in traditional medicine in Bukoba rural district (Moshi *et al.*, 2009). The material for this study was collected from Bukoba district in November, 2008 by Mr. Didas Ngemera, a traditional healer participating in the study. The collected material was dried in shade until completely dry and then transported to the Institute of Traditional Medicine in Dar es Salaam where it was ground into powder using a milling machine.

Preparation of extracts brine shrimp lethality test

Powdered plant material was soaked sequentially in dichloromethane (99 %) and then ethanol (96 %), each for 48 h. However, extracts were obtained from some of the plants using ethanol only. The extracts were filtered and solvents removed using a rotary evaporator at a temperature of 40° C. The extracts were further dried in a freeze dryer to remove any residual water and then stored in a freezer at -20° C until the day of use.

Solutions of the extracts were made in DMSO, at varying concentrations, and incubated in duplicate vials with the brine shrimp larvae in a total volume of 5 ml. Ten brine shrimp larvae were then placed in each of the duplicate vials. Others were placed in a mixture of DMSO (30 µl) and seawater to serve as a negative control. Cyclophosphamide, an anticancer drug, was used as a positive control. After 24 h the nauplii were examined against a lighted background, with a magnifying glass and the average number of survived larvae was determined. The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the larvae (LC₅₀) was determined from the graph.

Data analysis

The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the Fig P computer program (Biosoft Inc, USA), which also gives regression equations. The regression equations were used to calculate LC₁₆, LC₅₀ and LC₈₄ values. Confidence intervals (95% CI) were calculated according to a previously reported method (Litchfield and Wilcoxon, 1949).

Results

The yield of extracts from plants used ranged between 1.04 - 4.02 %. Of the fifty (50) plant extracts tested, 8 showed little to no toxicity to brine shrimps (Table 1). These included the dichloromethane extracts of *Antiaris toxicaria*, *Asystasia gangetica*, and *Bersama abyssinica* and the ethanol extracts of *Anthocleista grandiflora*, *Canna indica*, *Gynura scandens* and *Oxalis latifolia* which had LC₅₀ values above 1000 µg/ml. Dichloromethane extracts of *Cratispermum*

schweinfurthii and *Lantana trifolia* and the ethanol extracts of *Blumea auriculata* and *Pseudospondius microcarpa* were also practically non-toxic to brine shrimps with LC₅₀ values of between 500 and 800 µg/ml. Dichloromethane extracts of eight plants and ethanol extracts of twelve plants gave LC₅₀ values between 100 and 500 µg/ml (Table 1). The extracts which have potential for toxicity with LC₅₀ values below 100 µg/ml can be categorized into those with LC₅₀ values of 30–100 µg/ml and those whose LC₅₀ values were below 30 µg/ml. In the former category are dichloromethane extracts of *Blumea auriculata*, *Boerhavia diffusa*, *Clausena anisata*, *Teclea nobilis* and *Vernonia bradycalyx*, and ethanol extracts of *Clausena anisata*, *Garcinia b Buchananii*, *Hibiscus cannabinus*, *Hugonia castenifolia*, *Lantana trifolia*, *Vernonia bradycalyx*, *Antiaris toxicaria*, *Bidens shimperi* and *Bridelia micrantha*, ethanol extract of *Rubus rigidus* and dichloromethane extract of *Gynura scandens*. In the second category are dichloromethane extracts of *Picralima nitida* (LC₅₀ 18.3 µg/ml) and *Rubus rigidus* (LC₅₀ 19.8 µg/ml) which were almost as toxic as cyclophosphamide (LC₅₀ 16.3 µg/ml).

Table 1: Brine shrimp toxicity of plant extracts

Botanical name	Part tested	LC ₅₀ (95% CI) µg/ml			
		Dichloromethane	LC ₅₀ DCMex LC ₅₀ CPMD	Ethanol	LC ₅₀ EToHex LC ₅₀ CPMD
<i>Acanthus pubescens</i>	L	133.5(80.9-220.3)	8.2	286.4(207.5-395.2)	17.6
<i>Anthocleista grandiflora</i>	SB	355.6(192.2-657.9)	21.8	>1000	>61.3
<i>Antiaris toxicaria</i>	WP	>1000	>61.3	38.2(27.9-52.2)	2.3
<i>Asystasia gangetica</i>	L	>1000	>61.3	306.8(189.4-497.0)	18.8
<i>Bersama abyssinica</i>	SB	>1000	>61.3	127.7(95.9-164.7)	7.8
<i>Bidens shimperi</i>	L	-	-	46.9(25.5-86.3)	2.9
<i>Blumea auriculata</i>	L	57.7(38.2-87.1)	3.5	682.0(296.5-1568.6)	41.8
<i>Boerhavia diffusa</i>	AP	71.5(45.5-112.2)	4.4	232.4(139.2-388.1)	14.2
<i>Bridelia micrantha</i>	R	298.0(181.7-488.7)	18.3	32.0(20.3-50.3)	2.0
<i>Canna indica</i>	L	273.9(167.8-447.0)	16.8	>1000	>61.3
<i>Clausena anisata</i>	R	71.9(49.6-104.2)	4.4	60.5(43.3-84.5)	
<i>Crassocephalum vitellinum</i>	R	-	-	>1000	>61.3
<i>Craterispermum schweinfurthii</i>	AP	513.0(278.8-943.9)	31.5	-	-
<i>Ficus asperifolia</i>	SB	332.4 (211.2-523.2)	20.4	250.4 (145.7-430.40)	15.4
<i>Ficus exasperate</i>	L	-	-	126.9(96.1-167.5)	7.8
<i>Garcinia b Buchananii</i>	L	207.0(122.5-349.8)	12.7	60.6(45.6-80.6)	3.7
<i>Gynura scandens</i>	L	36.5(15.3-85.8)	2.2	>1000	>61.3
<i>Hibiscus cannabinus</i>	AP	-	-	97.8(73.0-131.0)	6.0
<i>Hugonia castenifolia</i>	L	217.1(109.6-429.8)	13.3	66.7(50.9-87.4)	4.1
<i>Jasminum dichotomum</i>	L	-	-	190.7(157.6-230.7)	11.7
<i>Lantana trifolia</i>	AP	756.0(315.0-1014.4)	46.4	32.3(20.2-51.7)	2.0
<i>Maesopsis eminii</i>	L	133.4(83.9-212.1)	8.2	218.1(138.9-342.4)	13.4
<i>Oxalis latifolia</i>	L	-	-	>1000	>61.3
<i>Picralima nitida</i>	SB	18.3(11.9-28.2)	1.1	104.4(73.5-148.2)	6.4
<i>Plumbago zeylanica</i>	L	-	-	232.3(177.3-304.3)	14.2
<i>Pseudospondius microcarpa</i>	L	-	-	541.2(300.7-974.2)	33.2
<i>Rubus rigidus</i>	SB	19.8(11.4-34.4)	1.2	41.7(30.0-58.0)	2.5
<i>Teclea nobilis</i>	AP	75.5(56.8-100.4)	4.6	156.6(101.7-241.2)	9.6
<i>Vangueria infausta</i>	L	-	-	144.7(115.8-180.9)	8.9
<i>Vernonia bradycalyx</i>	L	90.8(61.3-134.4)	5.6	33.9(24.2-47.5)	2.1
<i>Cyclophosphamide</i>	-	16.3 (10.6-25.2)	Data from Moshi et al, 2004.		

Key: - = not done; L = leaves; AP = aerial parts; SB = stem bark; R = roots; WP = whole plant; DCMex = dichloromethane extract, EToHex = ethanol extract, CPMD = Cyclophosphamide

Discussion

The brine shrimp results in this study are interpreted as follows: $LC_{50} < 1.0 \mu\text{g/ml}$ – highly toxic; $LC_{50} 1.0\text{-}10.0 \mu\text{g/ml}$ – toxic; $LC_{50} 10.0\text{-}30.0 \mu\text{g/ml}$ – moderately toxic; $LC_{50} > 30 < 100 \mu\text{g/ml}$ – mildly toxic, and $> 100 \mu\text{g/ml}$ as non-toxic. Cyclophosphamide ($LC_{50} 16.3 \mu\text{g/ml}$) was used as a standard so that it can allow some inference to be made for potential to yield anticancer compounds.

The brine shrimp test results indicate that 64% of the plant extracts tested had LC_{50} values above $100 \mu\text{g/ml}$ which suggests that they are practically non-toxic. As traditional medicines, most of the extracts are prepared as decoctions, which, in a way is mirrored on the ethanol extracts, the results of which suggest that the way they are used poses no threat of acute toxicity. Some of the extracts, including *Antiaris toxicaria* ($LC_{50} 38.2 \mu\text{g/ml}$), *Bidens shimperi* ($LC_{50} 46.9 \mu\text{g/ml}$), *Bridelia micrantha* ($LC_{50} 32.0 \mu\text{g/ml}$), *Lantana trifolia* ($LC_{50} 32.3 \mu\text{g/ml}$), *Rubus rigidus* ($LC_{50} 41.7 \mu\text{g/ml}$) and *Vernonia bradycalyx* ($LC_{50} 33.9 \mu\text{g/ml}$), are mildly toxic and probably have no obvious danger of outright toxicity during acute exposure.

Dichloromethane extracts for *Picralima nitida* ($LC_{50} 18.3 \mu\text{g/ml}$) and *Rubus rigidus* ($LC_{50} 19.8 \mu\text{g/ml}$) were the most toxic. However extracts from these two plants used as traditional medicines are unlikely to have any ill effects on patients as they are not on the highly toxic category.

Some brine shrimp results that are already available (Moshi *et al.*, 2006; 2004) provide a circumstantial evidence that plant extracts with LC_{50} values below $20 \mu\text{g/ml}$ have a likelihood of yielding anticancer compounds. This corroboration is demonstrated by *Bridelia cathartica* (Moshi *et al.*, 2004; Suffness *et al.*, 1988), *Croton macrostachys* (Moshi *et al.*, 2004; Kupchan *et al.*, 1969), *Maytenus putterlickioides* (Moshi *et al.*, 2004; Shibuta, 1984), *Ozoroa insignis* (Moshi *et al.*, 2004; Abreu *et al.*, 1999), *Psorospermum febrifugum* (Moshi *et al.*, 2006; Abou-Shoer *et al.*, 1988; Kupchan *et al.*, 1980), *Phyllanthus engleri* (Moshi *et al.*, 2006; Ratnayake *et al.*, 2009) and *Ximenia americana* (Moshi *et al.*, 2004; Asres *et al.*, 2001). This cut-off point has also been suggested elsewhere (Geran *et al.*, 1972). In the 2004 study using brine shrimps, *Phyllanthus engleri* gave an LC_{50} of $0.47 \mu\text{g/ml}$ (Moshi *et al.*, 2004), and recently the plant yielded englerin A, a selective anti-cancer compound against kidney cancer cells (Ratnayake *et al.*, 2009), which provides further corroborative evidence on the potential of the brine shrimp test to predict the presence of anti-cancer compounds in plant extracts. It is therefore possible that in this study, dichloromethane extracts of *Picralima nitida* ($LC_{50} 18.3 \mu\text{g/ml}$) and *Rubus rigidus* ($LC_{50} 19.8 \mu\text{g/ml}$) may have potential to yield compounds active against cancer cell lines, and is consistent with the results of cyclophosphamide ($LC_{50} 16.3 \mu\text{g/ml}$) and the criterion set by Geran *et al.* (1972). As compared to cyclophosphamide the two LC_{50} values are only 1.1 and 1.2 times higher, and probably not too far fetched to speculate of their possibility to yield cancer cell line active compounds.

In conclusion most of the extracts of the plants tested seem to be innocuous on short term use. Dichloromethane extracts of *Picralima nitida* and *Rubus rigidus* which were the most toxic among the tested extracts have LC_{50} values that suggest a remote possibility that they may yield cancer cell line active compounds.

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