Afr. J. Trad. CAM (2007) **4 (2):** 219 - 225

Research Paper
ISSN 0189-6016©2007

Afr. J. Traditional,
Complementary and Alternative Medicines
www.africanethnomedicines.net

BRINE SHRIMP TOXICITY EVALUATION OF SOME TANZANIAN PLANTS USED TRADITIONALLY FOR THE TREATMENT OF FUNGAL INFECTIONS

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Abstract

Plants which are used by traditional healers in Tanzania have been evaluated to obtain preliminary data of their toxicity using the brine shrimps test. The results indicate that 9 out of 44 plant species whose extracts were tested exhibited high toxicity with LC_{50} values below 20µg/ml. These include *Aloe lateritia* Engl. (Aloaceae) [19.1µg/ml], *Cassia abbreviata* Oliv. (Caesalpiniaceae) [12.7µg/ml], *Croton scheffleri* Pax (Euphorbiaceae) [13.7µg/ml], *Hymenodactyon parvifolium* Brig (Rubiaceae) [13.4µg/ml], *Kigelia Africana* L. (Bignoniaceae) [7.2µg/ml], and *Ocimum suave* Oliv. (Labiatae) [16.7µg/ml]. Twelve plants gave LC_{50} values between 21 and 50µg/ml, 11 plants gave LC_{50} values between 50 and 100 µg/ml, and 18 plants gave LC_{50} values greater than 100 µg/ml.

Key words: Brine shrimp test; Toxicity evaluation; Traditional antifungal plants

Introduction

In sub Saharan Africa, where 70% of the world cases of HIV/AIDS are found, *Candida* infections are very common and cause significant morbidity among patients (UNAIDS, 2004). Among problems that hamper effective management of *Candida* infections in these countries include; limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of *Candida* to commonly used antifungals, relapse of candida infections and the high cost of antifungal agents (Debruyne, 1997; Sangeorzan et al., 1994). Reports of resistance to commonly used antifungal agents like fluconazole abound (Ruhnke et al., 1994; Redding et al., 1994), including shifts from *Candida albicans* to less sensitive species such as *Candida glabrata* and *Candida krusei* (Bastert et al., 2001; Powderly, 1992). When relapses occur, the infections tend to be increasingly refractory to treatment.

These problems are of even greater relevance to poor countries, where the choice of antifungal agents is rather limited due to limited resources. In these countries, the most practical option remains to search for cheap alternatives to manage opportunistic infections. The difficulties associated with the management of *Candida* infections necessitate the discovery of new antifungal agents, in order to widen the spectrum of activity against *Candida* and combat strains expressing resistance to the available antifungal agents.

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Plants are widely used in Tanzanian traditional medicine and constitute a potentially useful resource for new and safe drugs for the treatment of opportunistic infections. According to *Medicine du Monde*, a French non-governmental organisation, 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 (AIDS Analysis Africa, 1996). Likewise, a survey conducted in Dar es Salaam showed that 21% of the people who seek care from public facilities had first consulted a traditional healer (Kilima et al., 1993).

The purpose of the present study was to evaluate the toxicities and/or potential for other biological activities of extracts of the plants that are used by traditional healers in Tanzania for management of fungal infections.

Materials and Methods

Plant collection and identification

Plants reported to be used for the treatment of oral candidiasis and skin fungal infections by the interviewed traditional healers (Table 1) were collected in four regions of Tanzania from February-March 2004. The plants were identified by Mr. Selemani, an experienced botany technician, and voucher specimens are kept at the Herbarium of the Department of Botany, University of Dar es Salaam.

Extraction of plant materials

All plant samples were air-dried and ground. Approximately 400 grams of the plant materials were macerated with 80% methanol at room temperature and after 24 h filtered through Whatman number 1 filter paper. The procedure was repeated three times to ensure exhaustive extraction of the plant material. The extracts were pooled together, concentrated, and the solvent removed by evaporation under reduced pressure in a rotar vapor, at 40°C. The extracts were further dried by freeze-drying and kept in a freezer, at -20°C, until the time of use.

The Brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the presence, in the extracts, of cytotoxic activity (Meyer et al., 1982). Solutions of the extracts were made in DMSO, at varying concentrations, and 30 µl of each incubated in duplicate vials with the brine shrimp larvae in a total volume of 5 ml. Ten brine shrimp larvae were placed in each of the duplicate vials. Brine shrimp larvae 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 (LC_{50}) 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 the regression equations. The regression equations were used to calculate LC_{16}, LC_{50} and LC_{84} values. Confidence intervals (95% CI) were calculated according to a previously reported method (Litchfield and Wilcoxon, 1949).

Results

Brine shrimp lethality

Among the 65 plant parts collected and belonging to 56 plant species, 50 (76.9%) plant parts of 44 plant species were tested for brine shrimp lethality. Nine plants showed high toxicity to the shrimps with LC_{50} values below 20µg/ml (Table 2). These include *Aloe lateritia* (19.1µg/ml), *Cassia abbreviata* (12.7µg/ml), *Croton scheffleri* (13.7µg/ml), *Hymenodactyon parvifolium* (13.4µg/ml), *Kigelia Africana* (7.2µg/ml), and *Ocimum suave* (16.7µg/ml). Twelve plants gave LC_{50} values between 21 and 50µg/ml, 11 plants gave LC_{50} values between 50 and 100 µg/ml, and 18 plants gave LC_{50} values greater than 100 µg/ml.

Table 1. Herbal plants reported to be used by traditional healers for treatment of fungal infections in Tanzania.

Species (Voucher Specimen No.)	**Family**	**Local name**	**Part used[a]**	**Life form**	**Preparation**
Acacia nilotica (L.) Willd ex Del (OH 58)	Mimosaceae	Kloriti	S	Shrub	Topical
Acacia robusta subsp Usambarensis (Taub) Brenan (OH 38)	Mimosaceae	Mkame	L	Tree	Topical
Acalypha fruticosa Forsk. (OH 56)	Euphorbiaceae	Siaiti	L, R	Shrub	Topical (L),
Agauria salicifolia Oliv. (OH 45)	Ericaceae	Mwomboa	L	Tree	Topical
*Albizia anthelmintica (*A. Rich) Brogn (OH 3)	Mimosaceae	Mfuleta	R	Tree	Oral
Aloe lateritia Engl. (OH 10*)*	Aloaceae	Mapunisinyamviri	WP	Shrub	Topical
Annona senegalensis Purs. (OH 11)	Annonaceae	Mnene kanda	L, R	Shrub	Topical (L),
Balanites aegyptiaca (L.) Del (OH 17)	Balanitaceae	Mudughuyu	RB	Tree	Topical
Cassia abbreviata Oliv. (OH 20)	Caesalpinaceae	Mufafati	R, SB	Tree	Oral
Cassia singuena Del (OH 12)	Caesalpinaceae	Muhufia	R	Shrub	Topical / Oral
Chrysophyllum bangweolense RE Fris (OH 15)	Sapotaceae	Mseweye	RB	Tree	Topical
Cissus petiolata Hook. F. (OH 48)	Vitaceae	Mswilaswila	R	Climber	Topical
Clausena anisata Oliv (OH 6)	Rutaceae	Mjavikali	L,SB,R	Shrub	Oral
Commiphora pteleifolia Engl. (OH 34)	Bursaraceae	Twini ndedemu	R	Shrub	Topical
Cordia africana Lam (OH 9)	Boraginaceae	Mgwengweni	R	Shrub	Topical
Coronopus didymus (L) (OH 47)	Cruciferae	Kissango	WP	Herb	Oral
Croton Scheffleri Pax (OH 24)	Euphorbiaceae	Muhalange	R	Shrub	Oral
Cucumis aculeatus Cogn. (OH 32)	Cucurbitaceae	Ingángáa	F	Climber	Topical
Cyphostemma hildebrandtii (Gilg) Desc. (OH 14)	Vitaceae	Damanyamwili	L	Herb	Topical
Diospyros usambarensis F. (OH 26)	Ebenaceae	Muriorio	R	Shrub	Topical
Drymaria cordata (L) A.Schult (OH 46)	Caryophyllaceae	Ugurashishi	WP	Herb	Topical
Elaeodendron buchananii (Loes)(OH 19)	Celastraceae	Muhorachwi	SB	Tree	Oral
Elaeodendron schlechteranum (Loes) (OH 50)	Celastraceae	Mkandekande	SB	Tree	Oral
Erythrina abyssinica Lam (OH 18)	Papilionaceae	Mkalalwanhuwa	R	Tree	Topical
Euphorbia heterophylla L. (OH 31)	Euphorbiaceae	Loo	WP	Herb	Oral
Euphorbia tirucali L. (OH 57)	Euphorbiaceae	Injokii	L	Tree	Topical
Ficus sur. Benth (OH 51)	Moraceae	Mkuyu	SB	Tree	Oral/Topical
Gonatopus boivinii Hook.f. (OH 1)	Araceae	Kunzulu	T	Herb	Topical

Hymenidictyon parvifolium Brig (OH 2)	Rubiaceae	Pekawake	R	Shrub	Topical
Hypericum roeperanum Schimp. ex A. Rich (OH 44)	Gutteferae	Mwambaziwa	L	Shrub	Topical
Indigofera rhynchocarpa Bak. Var (OH 16)	Papilionaceae	Igangula	R	Shrub	Topical
Jatropha multifida L. (OH 53)	Euphorbiaceae	Maugwamwipoli	L,S,R	Shrub	Topical
Khaya anthotheca (Welw.) C.Dc (OH 52)	Meliaceae	Mgolaminzi	SB	Tree	Topical
Kigelia africana L. (OH 49*)*	Bignoniaceae	Mungungu	RB, F	Tree	Oral
Lannea stuhlmanii Engl. (OH 7)	Anacardiaceae	Muhungilo	L	Tree	Topical
Lobelia giberroa Neumeleg (OH 35)	Campanulaceae	Gongoa	L	Herb	Topical
Ocimum basilicum L. (OH 29)	Labiatae	Irumbasi	WP	Herb	Oral
Ocimum suave Oliv. (OH 13)	Labiatae	Suameno	L	Herb	Topical
Plumbago zeylanica L. (OH 36)	Plumbaginaceae	Chambula	R	Herb	Oral
Pteridium aquilinum (L.) Kuhn (OH 41)	Densitraediaceae	Shilu	L	Herb	Topical
Rapanea melanophloeus (L.) Mez (OH 5)	Myrsinaceae	Mpaja	L, SB	Tree	Oral
Rhoicissus tridentata (Lf) Wild & Drumm (OH 27*)*	Vitaceae	Iforiyo	T	Climber	Oral
Salvadora persica L (OH 30)	Salvadoraceae	Mukunkuni	R	Tree	Topical
Sclerocarya birrea. (A.Rich.) Hochst. subsp. caffra (Sond.) (OH 8)	Anacardiaceae	Muongozi	L, R	Tree	Topical
Securidaca longipedunculata Fres (OH 28)	Polygonaceae	Musatu	R	Shrub	Oral
Senecio deltoidea Less (OH 33)	Cucurbitaceae	Ulenge	WP	Climber	Oral
Solanum incanum L (OH 23)	Solanaceae	Mtula ndulele	WP	Herb	Oral
Spirostachys africana Sonder (OH 54)	Euphorbiaceae	Ormotanga	S	Tree	Topical
Sterculia africana (Lour) Fiori (OH 39)	Sterculiaceae	Muhoza	L	Tree	Oral
Strophanthus eminii Asch & Pax (OH 25*)*	Apocynaceae	Muhunguti	RB	Shrub	Oral
Strychnos potatorum Gilg. (OH 21)	Loganiaceae	Mumpande	L	Tree	Oral
Tagetes minuta L. (OH 43)	Compositae	Mbangi	L	Climber	Topical
Turraea holstii Gurk (OH 37)	Meliaceae	Muhenga	L	Shrub	Oral
Zanthoxylum chalybeum L. (OH 22)	Rutaceae	Mulungu	RB	Tree	Topical/Oral
Zehneria scabra (L.f) Sond (OH 42)	Cucurbitaceae	Foiza	WP	Climber	Topical
Ziziphus pubercens Oliv. (OH 55)	Rhamnaceae	Indigrishi	L	Shrub	Topical

Key: [a]F, Fruit; L, Leaves; R, Roots; RB, Root bark; S, Stem; SB, Stem bark; T, Tubor; WP,whole plant. [b]* No other uses report.

Table 2: The brine shrimp lethality results represented as LC_{50} in µg/ml and 95% confidence intervals (CI).

Binomial name	**Part tested**	**LC_{50} µg/ml**	**(95% CI)**
Acacia robusta	Stem	108.5	87.8-134.0
Acalypha fruticosa	Roots	23.9	16.5-34.7
	Leaves	113.9	91.2-142.3
Agauria salicifolia	Leaves	>240	-
Albizia anthelmintica	Roots	24.9	14.1- 44.0
Aloe lateritia	Whole plant	19.1	13.2-27.8
Balanites aegyptica	Root bark	> 240	-
Cassia abbreviata	Roots	12.7	8.1-19.8
Commiphora pteleifolia	Roots	>240	-
Cordia africana	Roots	211.4	117.6-380.1
Croton scheffleri	Roots	13.7	21.5-8.7
Chrysophylum banguelense	Root bark	96.3	65.5-141.6
Cyphosterma hilderbrandtii	Leaves	25.7	16.9-39.0
Drymaria cordata	Whole plant	>240	-
Elaeodendron schlechteranum	Stem bark	37.5	28.1-50.1
Elaedendron stuhlmannii	Stem bark	>240	-
Erythrina abbysinica	Root	>240	-
Euphorbia heterophylla	Whole plant	80.2	57.3-112.5
Euphobia tirucali	Leaves	196.2	72.7- 529.7
Ficus sur	Stem bark	146.1	116.1-183.9
Hymenodictyon parvifolium	Roots	13.4	8.3-21.5
Hypericum roeperanum	Leaves	46.6	34.2-63.6
Indigofera rhynchocarpa	Roots	28.3	20.5-39.0
Jatropha multifida	Leaves	21.7	16.4-28.7
	Stem	58.3	41.3-82.4
	Roots	26.1	17.3-39.2
Khaya anthotheca	Stem bark	38.7	28.6-52.2
Kigelia africana	Fruit	>240	-
	Roots	7.2	3.9-13.8
Lannea stuhlmannii	Leaves	25.3	16.6-38.8
Lobelia giberroa	Leaves	>240	-
Ocimum basilicum	Whole plant	85.3	68.2-106.6
Ocimum suave	Leaves	16.7	11.6-24.1
Plumbago zeylanica	Roots	>240	-
Rapanea melanophloeus	Stem bark	152.4	84.6-274.5
	Leaves	12.1	8.6-17.2
Rhoicissus tridentate	Stem	>240	-
Salvadore persica	Roots	>240	-
Securidaca longipedunculata	Roots	77.1	45.3-131.1
Solanum incanum	Whole plant	90.2	75.7-107.4
Spirostachys africana	Leaves	16.4	9.4-28.8
	Stem	45.2	24.2-84.5
Sterculia africana	Leaves	94.5	57.9-154.9
Strophanthus eminii	Root bark	38.9	27.4-55.2
Strychnos pototorum	Leaves	87.6	39.5-194.2
Tegetes minuta	Leaves	19.9	14.5-27.3
Turraea holstii	Leaves	96.3	42.5-218.5
Zanthoxylum chalybeum	Root bark	68.9	36.9-128.6
Zehneria scabra	Whole plant	138.1	93.7-203.4
Ziziphus pubescens	Leaves	68.2	50.5-92.1
Cyclophosphamide	-	16.3	10.6-25.2

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Discussion

Previous investigations of our group on the *in vitro* antifungal activity of the plants support the therapeutic claims of the traditional healers (Hamza et al., in Press). Identification of herbal medicines for the treatment of fungal infections in HIV/AIDS patients could be pivotal in supporting the needs of these patients in terms of easy availability, affordability, and possibly to cope with the problem of recurrent *Candida* infections and emergence of resistance.

Apart from efficacy, safety of herbal medicines is of paramount importance as there is not much that is known about many plants that are used in traditional medicine. We have used the brine shrimp lethality test as a preliminary tool to evaluate the toxicity of the identified plants. Unfortunately not all the plants collected were tested. However, among those tested 9 were quite toxic to the shrimps. Since the test is also used to identify potential anticancer substances, the results may mean that these plants are either outright toxic or may have potential anticancer activity. Two of the plants *Euphorbia heterophylla* L. (Rocha e Silva, 1943) and *Jatropha multifida* are reported to be toxic (Levin et al., 2000), thus supporting what was reported by the healers. The extracts of the roots and leaves of *Jatropha multifida* also exhibited relatively high toxicity on the shrimps, while for *Euphorbia heterophylla* the toxicity was low (LC_{50} 80.2 µg/ml). Toxicity results from animals will be crucial as a way to definitively judge the safety of these plants, as and when they are found to have enough potential for development. The present results only suggest possibility of other hitherto unreported biological activities, of toxic nature or even anticancer activity. Among the plants tested were seven plants that in earlier investigations of our group showed to have potent antifungal activity (Hamza et al., in Press). The toxic effect of these plants are shown in Table 2. All these plants need to be further investigated for their potential as a source of antifungal compounds.

The results of this toxicity study showed the relative toxicities of the plants. More work is needed in order to determine their usefulness as potential antifungal and anticancer agents.

Acknowledgements

We are grateful to Mr. Selemani of Botany Department, UDSM, for helping with collection and identification of the plants used in this study. We are grateful to the NAPRALERT Data base of the University of Illinois, at Chicago, for allowing us access to literature.

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