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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 LC₅₀ 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], *Hymenodactylon parvifolium* Brig (Rubiaceae) [13.4µg/ml], *Kigelia Africana* L. (Bignoniaceae) [7.2µg/ml], and *Ocimum suave* Oliv. (Labiatae) [16.7µg/ml]. Twelve plants gave LC₅₀ values between 21 and 50µg/ml, 11 plants gave LC₅₀ values between 50 and 100 µg/ml, and 18 plants gave LC₅₀ 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.

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 μ l 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 μ l) 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 20 μ g/ml (Table 2). These include *Aloe lateritia* (19.1 μ g/ml), *Cassia abbreviata* (12.7 μ g/ml), *Croton scheffleri* (13.7 μ g/ml), *Hymenodactylon parvifolium* (13.4 μ g/ml), *Kigelia Africana* (7.2 μ g/ml), and *Ocimum suave* (16.7 μ g/ml). Twelve plants gave LC_{50} values between 21 and 50 μ g/ml, 11 plants gave LC_{50} values between 50 and 100 μ g/ml, and 18 plants gave LC_{50} values greater than 100 μ g/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
<i>Acacia nilotica</i> (L.) Willd ex Del (OH 58)	Mimosaceae	Kloriti	S	Shrub	Topical
<i>Acacia robusta</i> subsp <i>Usambarensis</i> (Taub) Brenan (OH 38)	Mimosaceae	Mkame	L	Tree	Topical
<i>Acalypha fruticosa</i> Forsk. (OH 56)	Euphorbiaceae	Siaiti	L, R	Shrub	Topical (L),
<i>Agauria salicifolia</i> Oliv. (OH 45)	Ericaceae	Mwombo	L	Tree	Topical
<i>Albizia anthelmintica</i> (A. Rich) Brogn (OH 3)	Mimosaceae	Mfuleta	R	Tree	Oral
<i>Aloe lateritia</i> Engl. (OH 10)	Aloaceae	Mapunisinyamviri	WP	Shrub	Topical
<i>Annona senegalensis</i> Purs. (OH 11)	Annonaceae	Mnene kanda	L, R	Shrub	Topical (L),
<i>Balanites aegyptiaca</i> (L.) Del (OH 17)	Balanitaceae	Mudughuyu	RB	Tree	Topical
<i>Cassia abbreviata</i> Oliv. (OH 20)	Caesalpinaceae	Mufafati	R, SB	Tree	Oral
<i>Cassia singuena</i> Del (OH 12)	Caesalpinaceae	Muhufia	R	Shrub	Topical / Oral
<i>Chrysophyllum bangweolense</i> RE Fris (OH 15)	Sapotaceae	Mseweye	RB	Tree	Topical
<i>Cissus petiolata</i> Hook. F. (OH 48)	Vitaceae	Mswilaswila	R	Climber	Topical
<i>Clausena anisata</i> Oliv (OH 6)	Rutaceae	Mjavikali	L,SB,R	Shrub	Oral
<i>Commiphora pteleifolia</i> Engl. (OH 34)	Bursaraceae	Twini ndedemu	R	Shrub	Topical
<i>Cordia africana</i> Lam (OH 9)	Boraginaceae	Mgwengweni	R	Shrub	Topical
<i>Coronopus didymus</i> (L) (OH 47)	Cruciferae	Kissango	WP	Herb	Oral
<i>Croton Scheffleri</i> Pax (OH 24)	Euphorbiaceae	Muhalange	R	Shrub	Oral
<i>Cucumis aculeatus</i> Cogn. (OH 32)	Cucurbitaceae	Ingángáa	F	Climber	Topical
<i>Cyphostemma hildebrandtii</i> (Gilg) Desc. (OH 14)	Vitaceae	Damanyamwili	L	Herb	Topical
<i>Diospyros usambarensis</i> F. (OH 26)	Ebenaceae	Muriorio	R	Shrub	Topical
<i>Drymaria cordata</i> (L) A.Schult (OH 46)	Caryophyllaceae	Ugurashishi	WP	Herb	Topical
<i>Elaeodendron buchananii</i> (Loes)(OH 19)	Celastraceae	Muhorachwi	SB	Tree	Oral
<i>Elaeodendron schlechteranum</i> (Loes) (OH 50)	Celastraceae	Mkandekande	SB	Tree	Oral
<i>Erythrina abyssinica</i> Lam (OH 18)	Papilionaceae	Mkalalwanhuwa	R	Tree	Topical
<i>Euphorbia heterophylla</i> L. (OH 31)	Euphorbiaceae	Loo	WP	Herb	Oral
<i>Euphorbia tirucali</i> L. (OH 57)	Euphorbiaceae	Injokii	L	Tree	Topical
<i>Ficus sur.</i> Benth (OH 51)	Moraceae	Mkuyu	SB	Tree	Oral/Topical
<i>Gonatopus boivinii</i> Hook.f. (OH 1)	Araceae	Kunzulu	T	Herb	Topical

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

Key: ^aF, 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 LC₅₀ in µg/ml and 95% confidence intervals (CI).

Binomial name	Part tested	LC₅₀ µg/ml	(95% CI)
<i>Acacia robusta</i>	Stem	108.5	87.8-134.0
<i>Acalypha fruticosa</i>	Roots	23.9	16.5-34.7
	Leaves	113.9	91.2-142.3
<i>Agauria salicifolia</i>	Leaves	>240	-
<i>Albizia anthelmintica</i>	Roots	24.9	14.1- 44.0
<i>Aloe lateritia</i>	Whole plant	19.1	13.2-27.8
<i>Balanites aegyptica</i>	Root bark	> 240	-
<i>Cassia abbreviata</i>	Roots	12.7	8.1-19.8
<i>Commiphora pteleifolia</i>	Roots	>240	-
<i>Cordia africana</i>	Roots	211.4	117.6-380.1
<i>Croton scheffleri</i>	Roots	13.7	21.5-8.7
<i>Chrysophyllum banguelense</i>	Root bark	96.3	65.5-141.6
<i>Cyphosterna hilderbrandtii</i>	Leaves	25.7	16.9-39.0
<i>Drymaria cordata</i>	Whole plant	>240	-
<i>Elaeodendron schlechteranum</i>	Stem bark	37.5	28.1-50.1
<i>Elaeodendron stuhlmannii</i>	Stem bark	>240	-
<i>Erythrina abyssinica</i>	Root	>240	-
<i>Euphorbia heterophylla</i>	Whole plant	80.2	57.3-112.5
<i>Euphorbia tirucali</i>	Leaves	196.2	72.7- 529.7
<i>Ficus sur</i>	Stem bark	146.1	116.1-183.9
<i>Hymenodictyon parvifolium</i>	Roots	13.4	8.3-21.5
<i>Hypericum roeperanum</i>	Leaves	46.6	34.2-63.6
<i>Indigofera rhynchocarpa</i>	Roots	28.3	20.5-39.0
<i>Jatropha multifida</i>	Leaves	21.7	16.4-28.7
	Stem	58.3	41.3-82.4
	Roots	26.1	17.3-39.2
<i>Khaya anthotheca</i>	Stem bark	38.7	28.6-52.2
<i>Kigelia africana</i>	Fruit	>240	-
	Roots	7.2	3.9-13.8
<i>Lannea stuhlmannii</i>	Leaves	25.3	16.6-38.8
<i>Lobelia giberroa</i>	Leaves	>240	-
<i>Ocimum basilicum</i>	Whole plant	85.3	68.2-106.6
<i>Ocimum suave</i>	Leaves	16.7	11.6-24.1
<i>Plumbago zeylanica</i>	Roots	>240	-
<i>Rapanea melanophloeus</i>	Stem bark	152.4	84.6-274.5
	Leaves	12.1	8.6-17.2
<i>Rhoicissus tridentate</i>	Stem	>240	-
<i>Salvadore persica</i>	Roots	>240	-
<i>Securidaca longipedunculata</i>	Roots	77.1	45.3-131.1
<i>Solanum incanum</i>	Whole plant	90.2	75.7-107.4
<i>Spirostachys africana</i>	Leaves	16.4	9.4-28.8
	Stem	45.2	24.2-84.5
<i>Sterculia africana</i>	Leaves	94.5	57.9-154.9
<i>Strophanthus eminii</i>	Root bark	38.9	27.4-55.2
<i>Strychnos pototorum</i>	Leaves	87.6	39.5-194.2
<i>Tegetes minuta</i>	Leaves	19.9	14.5-27.3
<i>Turraea holstii</i>	Leaves	96.3	42.5-218.5
<i>Zanthoxylum chalybeum</i>	Root bark	68.9	36.9-128.6
<i>Zehneria scabra</i>	Whole plant	138.1	93.7-203.4
<i>Ziziphus pubescens</i>	Leaves	68.2	50.5-92.1
Cyclophosphamide	-	16.3	10.6-25.2

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 (LC₅₀ 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.

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