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Testing Beyond Ethnomedical Claims: Brine Shrimp Lethality of Some Tanzanian Plants

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Abstract

Extracts of 34 plants that are traditionally used for the treatment of different diseases exhibited various degrees of toxicity on brine shrimp. The concentration killing 50% (LC₅₀) of the shrimps was below 50 µg/ml for 14 (41.2%), 50–100 µg/ml for 10 (29.4%), and greater than 100 µg/ml for 10 (29.4%) of the plants, respectively. *Dalbergia nitidula* (LC₅₀ 0.87 µg/ml), *Ozoroa insignis* (LC₅₀ 2.21 µg/ml), *Markhamia obtusifolia* (LC₅₀ 8.94 µg/ml), *Ximenia caffra* (LC₅₀ 11.25 µg/ml), *Croton macrostachys* (LC₅₀ 13.40 µg/ml), and *Mormodica calantha* (LC₅₀ 19.38 µg/ml) were the most toxic. The results indicate the possibility that some of the plant extracts may be toxic or contain useful cytotoxic compounds, which was not reported by the traditional healers.

Keywords: Brine shrimp lethality, cytotoxic activity, testing beyond ethnomedical claims.

Introduction

Tanzania is in a unique position because of its wide diversity of plants, which comprises more than 10,000 species, among which 1122 are endemic (Polhill, 1968; Brenan, 1978). The Institute of Traditional Medicine, Muhimbili University College of Health Sciences, has already documented nearly 2500 plant species that have claims of medicinal properties. Traditional healers and local communities have played a big role in identifying these plants, whose vouchers are now kept in the herbarium of the institute. The identification of these plants was done using information obtained voluntarily

from individuals who brought information to the institute or through carrying out ethnomedical interviews of traditional healers and communities (Cox, 1994; Cox & Balick, 1994; King & Tempesta, 1994; King & Carlson, 1995). Corroboration of collected information with that in the literature plays a big role in prioritizing plants for both phytochemical and pharmacological studies.

A few of the plants in our collection that are traditionally used for management of different diseases were selected for testing on brine shrimp. Some of these plants have yielded negative results in other tests (Moshi et al., 2000), in agreement with a suggestion that some of the traditional medicines could be mere placebos (Gessler et al., 1995). There is also a possibility that they may have useful or toxic activities that are yet to be reported. This work seeks to evaluate their safety and the possibility that some may contain anticancer properties as inferred from the brine shrimp lethality test (Harwig & Scott, 1971; Meyer et al., 1982).

Materials and Methods

Materials

Ethanol was purchased from Fisher Scientific UK Ltd (Loughborough, Leicestershire, UK). Dimethylsulfoxide (DMSO) was purchased from Sigma (Poole, Dorset, UK). Brine shrimp eggs were bought from Dohse Acuaristic (Bonn, Germany). Sea salt was prepared locally by evaporating water collected from the Indian Ocean along the Dar es Salaam coast.

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Plant collection

The plants used in this study were collected from Handeni, Tanga Region (vouchers no. MJ 10–MJ 25) and Iringa Region (vouchers no. IMPP 001/0014–0031). Mr. E.B. Mhoro and Mr. F. Mbago identified the plants (Table 1), and the vouchers are kept in the Herbarium of the Institute of Traditional Medicine.

Preparation of plant extracts

Powdered plant materials (50 g) were extracted with either distilled water or 20% aqueous ethanol. Aqueous extracts were dried by freeze-drying, whereas the other

extracts were dried on a rotary evaporator, at 37°C, followed by removal of residual water by freeze-drying. Solutions were made by dissolving the aqueous extracts in distilled water and the aqueous ethanol extracts in DMSO.

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 or distilled water at varying concentrations and were incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of

Table 1. The list of plants, parts tested, and the traditional claims for which they were collected.

Binomial name	Vernacular name	Traditional uses
<i>Acacia kirkii</i> Oliv. (Mimosaceae)	Mgunga	Diabetes/malaria
<i>Acacia mellifera</i> (Vahl) Benth (Mimosaceae)	Msasa	Stimulant
<i>Albizia petersiana</i> (Bolle) Chiv. (Mimosaceae)	Mchala	Headaches
<i>Albizia versicolor</i> Wew. ex Oliv (Mimosaceae)	Mkingu	Impotence/aphrodisiac
<i>Asparagus africanus</i> Lam. (Asparagaceae)	Mkalakanga	Hypertension
<i>Bridelia cathartica</i> Bertol (Euphorbiaceae)	Kikwindile kwima	Aphrodisiac, wounds
<i>Caesalpinia bonducella</i> (L.) Flem. (Caesalpiniaceae)	Msoro/mkomve	Diabetes
<i>Catunaregam spinosa</i> (Thunb.) Tirvengadam subsp. Taylori (S.Moore) Verdc (Rubiaceae)	Mpongolo	Bilharzia/diarrhea
<i>Cissus quadrangularis</i> (L.) (Vitaceae)	Kihindihindi	Backache/body pain
<i>Croton macrostachys</i> Del. (Euphorbiaceae)	Mhulugu, mbiha	Diabetes
<i>Croton sylvaticus</i> Krauss (Euphorbiaceae)	—	No claimed use
<i>Dalbergia nitidula</i> Kabk. (Papillionaceae)	Mlengwe	Kifafa
<i>Diospyros mespiliformis</i> Hochst. ex DC. (Ebenaceae)	Mlengwe	Skin eruptions
<i>Deinbolia borbonica</i> Schweinff (Sapindaceae)	Mkwambekwambe	Impotence/cancer
<i>Ehretia amoena</i> Klotzsch (Boraginaceae)	Mvungaliza	Skin diseases/wounds
<i>Euphorbia candelabrum</i> Kotschy var. <i>bilocularis</i> (N.E.Br.) S. Carter (Euphorbiaceae)	Mlangali	HIV
<i>Garcinia semseei</i> Verdc. (Guttiferaceae)	—	Skin eruptions/HIV
<i>Gardenia ternifolia</i> Schumach. & Thonn. subsp. <i>jovis-tonantis</i> (Welw.) Verdc. (Rubiaceae)	Kilemandembwe	Epilepsy
<i>Lannea stuhlmannii</i> (Engl.) (Anacardiaceae)	Mumbu	Malaria, sores
<i>Leptactina bengulensis</i> (Benth. & Hook.f) R.Good (Rubiaceae)	Kimakasa	Epilepsy
<i>Markhamia obtusifolia</i> Bak Spragne (Bignoniaceae)	Myuyu	Diabetes
<i>Maytenus putterlickioides</i> (Loes) Excell & Mendonca (Celastraceae)	Mtulavuha	Antibacterial
<i>Milletia ariocalyx</i> Dunn (Papillionaceae)	—	Skin eruptions
<i>Milletia puguensis</i> Gillett (Papillionaceae)	—	Skin eruptions
<i>Mormodica calantha</i> Gilg (Cucurbitaceae)	Mtundwa	Diabetes
<i>Ozoroa insignis</i> Del. var. <i>reticulata</i> (Baker.f) Gillett (Anacardiaceae)	Motaponzi	Bilharzia
<i>Piliostigma thorningii</i> (Schumach.) Milne-Redh (Caesalpiniaceae)	—	Diabetes/pruritus
<i>Rauvolfia caffra</i> Sond. (Apocynaceae)	Mvelevele	Epilepsy
<i>Ricinodendron heudelotii</i> (Baill) (Euphorbiaceae)	—	—
<i>Syzygium guineense</i> (Willd.) DC (Myrtaceae)	Muvengi	Diabetes
<i>Terminalia sericea</i> Burch. ex DC (Combretaceae)	Mpululu	Gonorrhea
<i>Vangueria tomentosa</i> Hochst (Rubiaceae)	Mvilu	Diabetes
<i>Ximenia americana</i> L. var. <i>caffra</i> Sond. (Olaceae)	Mtundwi	Gonorrhea
<i>Ximenia caffra</i> Sond. (Olaceae)	Mdunula/mnyaudende	Fungal infection

Table 2. The brine shrimp lethality results, represented as LC₅₀ values and 95% confidence intervals (CI).

Name of plant/test item	Part tested	Type of extract	LC ₅₀ (µg/ml)	95% CI
<i>Acacia kirkii</i>	Whole stem	AE	>240	—
<i>Acacia mellifera</i>	Stem bark	AE	>240	—
<i>Albizzia petersiana</i>	Whole stem	AE	48.51	35.15–63.55
<i>Albizzia versicolor</i>	Whole stem	AE	93.65	52.90–165.80
<i>Asparagus africanus</i>	Whole stem	AE	91.50	62.1–135.01
<i>Bridelia cathartica</i>	Stem bark	AE	58.45	41.4–82.4
<i>Caesalpinia bonducella</i>	Roots	AQ	65.08	43.7–96.85
<i>Catunaregum spinosa</i>	Whole stem	AE	>240	—
<i>Cissus quadrangularis</i>	Whole stem	AE	>240	—
<i>Croton macrostachys</i>	Roots	AE	13.4	8.7–20.62
<i>Croton sylvaticus</i>	Whole stem	AE	29.73	21.1–41.92
<i>Dalbergia nitidula</i>	Roots	AE	0.87	0.35–2.16
<i>Deinbolia borbonica</i>	Leaves	AE	>240	—
<i>Diospyros mespiliformis</i>	Whole stem	AE	63.3	36.34–110.27
<i>Ehretia amoena</i>	Stem bark	AE	65.0	43.04–98.15
<i>Euphorbia candelabrum</i>	Whole stem	AE	>240	—
<i>Garcinia semseii</i>	Whole stem	AE	>240	—
<i>Gardenia ternifolia</i>	Roots	AE	54.48	38.92–76.27
<i>Lannea stuhlmannii</i>	Whole stem	AE	67.87	47.5–97.05
<i>Leptactina bengulensis</i>	Whole stem	AE	20.95	12.81–34.25
<i>Markhamia obtusifolia</i>	Whole stem	AE	8.94	4.9–16.36
<i>Maytenus putterlickioides</i>	Stem bark	AE	>240	—
<i>Milletia aeriocalyx</i>	Stem bark	AE	38.32	33.8–43.5
<i>Milletia puguensis</i>	Stem bark	AE	29.43	18.9–45.90
<i>Momordica calantha</i>	Roots	AE	19.38	14.6–25.68
<i>Ozoroa insignis</i>	Roots	AE	2.21	1.38–3.53
<i>Piliostigama thornningii</i>	Whole stem	AE	>240	—
<i>Rauvolfia caffra</i>	Whole stem	AE	42.95	27.5–67.1
<i>Ricinodendron heudelotii</i>	Whole stem	AE	23.72	15.30–36.78
<i>Syzygium guineensis</i>	Roots	A	>240	—
<i>Terminalia sericea</i>	Roots	AE	79.91	53.43–99.32
<i>Vangueria tomentosa</i>	Whole stem	AE	28.60	24.7–33.20
<i>Ximenia americana</i>	Whole stem	AE	87.45	50.26–152.2
<i>Ximenia caffra</i> Sond. (Olacaceae)	Whole stem	AE	11.25	8.09–15.64

AQ, aqueous extract; AE, 20% aqueous ethanol extract.

the duplicate vials. Control brine shrimp larvae were placed in a mixture of seawater and DMSO only. After 24 h, the nauplii were examined against a lighted background, and the average number of survived larvae in each triplicate was determined. The mean percentage mortality was plotted against the logarithm of concentrations, and the concentration killing 50% of the larvae (LC₅₀) was determined from the graph (Meyer et al., 1982).

Data analysis

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

Wilcoxon (1949). A LC₅₀ value greater than 100 µg/ml was considered to represent an inactive compound or extract.

Results

Table 1 shows the plants that were tested, their vernacular names, and ethnomedical uses for which they were collected. Among the 34 plants, 18 were collected on claims related to treatment of symptoms manifesting in diabetes mellitus (plants used as aphrodisiacs, antifungals, used as tonics or to increase energy, polyuria, etc.), 2 for malaria, 1 for hypertension, 4 for epilepsy, and 7 for skin eruptions or HIV/AIDS-related skin conditions, excluding Kaposi sarcoma. Only one plant, *Deinbolia borbonica*, was mentioned for the treatment of cancer. Table 2 shows that, among the 34 plants, 90% were toxic to brine shrimp. The concentrations

killing 50% of the shrimps (LC₅₀) were less than 50 µg/ml for 14 (41.2%), 50–100 µg/ml for 10 (29.4%), and greater than 100 µg/ml for 10 (29.4%) of the plants, respectively. *Dalbergia nitidula* (LC₅₀ 0.87 µg/ml), *Ozoroa insignis* (LC₅₀ 2.21 µg/ml), *Markhamia obtusifolia* (LC₅₀ 8.94 µg/ml), *Ximenia caffra* (LC₅₀ 11.25 µg/ml), *Croton macrostachys* (LC₅₀ 13.4a µg/ml) and *Mormodica calantha* (LC₅₀ 19.38 µg/ml) were the most active.

Discussion

The brine shrimp lethality test is used to predict compounds or extracts that may have anticancer activity (Meyer et al., 1982). According to literature, *Dalbergia nitidula* (Van Heerden et al., 1978; Nyanzema, 1984), *Lannea stuhlmannii* (Chhabra et al., 1984), *Mormodica calantha* (Hedberg et al., 1983), *Rauvolfia caffra* (Chhabra & Uiso, 1991), and *Gardenia ternifolia* (Chhabra et al., 1991) are traditionally used for the treatment of swellings or wounds, and *Ximenia americana* (Adoum & Takeda, 2000) and *Lannea stuhlmannii* (Chhabra et al., 1987) for the treatment of cancer. Previous work on *Ximenia americana* using cancer cell lines (Asres et al., 2001) and the current results suggest the possibility that its traditional uses could be related to the treatment of cancer. Previous results on cancer cell lines for *Bridelia cathartica* (Suffness et al., 1988), *Croton macrostachys* (Kupchan et al., 1969), *Maytenus putterlickioides* (Shibuta, 1984), *Ozoroa insignis* (Abreu et al., 1999), and *Ximenia americana* (Asres et al., 2001) show the same pattern of cytotoxic activity as the brine shrimp lethality test. Some differences were noted: whereas *Cissus quadrangularis*, *Deinbollia borbonica*, *Ehretia amoena*, *Lannea stuhlmannii*, *Markhamia obtusifolia*, *Vangueria tomentosa* (Massele & Nshimo, 1995) and *Ximenia americana* (Asres et al., 2001) were previously reported to be inactive, they have now shown toxicity in brine shrimp. One cytotoxic compound, a cyclohexane crotopoxide, has been reported from the leaves of *Croton macrostachys* (Kupchan et al., 1969). A number of other compounds have been isolated by our group from this plant (Kapingu et al., 2000) that are toxic to brine shrimp. Further work is in progress to determine the activity of the isolated compounds on cancer cell lines.

This work suggests that some of the extracts may be toxic or contain useful cytotoxic compounds, which was not reported by the local communities or traditional healers.

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