Screening Of Some Kenyan Medicinal Plants Using The Brine Shrimp Lethality Test

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The latex of two plants and the methanol and petroleum ether extracts of thirty four plants from twenty one families were screened for their biological activity using the brine shrimp lethality test. The plants were selected on the basis of their traditional medicinal uses in Kenya. Thirty six of the seventy eight samples showed toxicity to the brine shrimp (LC_{50} < 1000 μg/ml). The most active extracts (LC_{50} < 250 μg/ml) were from *Bridelia micrantha*, *Brucea antidysenterica*, *Croton megalocarpus*, *Engleromyces goetzi*, *Euclea schimperi*, *Mondia whitei*, *Persea americana*, *Phytolacca dodecandra*, *Salvia nilotica*, *Tagetes minuta, Teclea simplifolia* and *Toddalia asiatica.* The results could be useful in the search for new compounds with pesticidal, antitumour and other pharmacological actions.

Key Words: Brine Shrimp Lethality Test, Kenya medicinal plants, methanol extract, petroleum ether extract.

INTRODUCTION

Kenya is endowed with a diverse range of plants, many of which are used by the indigenous people to make infusions and decoctions for treatment of various ailments. Although few of these plants have been evaluated for their biological activities, many species are being destroyed as their natural habitat is continually cleared to give way to human shelter and agricultural activities. There is an urgent need to evaluate these plant species biologically before they become extinct. However, many of the specific bioassays used to screen plant extracts for biological activity are costly and require special reagents, sophisticated equipment and aseptic conditions. Therefore, they are not practical or feasible within the research funds available in most developing countries.

The brine shrimp lethality test (BST), originally proposed by Meyer *et al.* [1] and later refined by McLaughlin *et al.* [2] has been developed to alleviate these handicaps. This is a simple, rapid and an inexpensive benchtop bioassay. The eggs of the brine shrimp (*Artemia salina* Leach) are readily available at low cost in pet shops as a food for tropical fish and they remain viable for years in the dry state. Upon being placed in a brine solution, the eggs hatch within 48 hours, providing a large number of larvae (nauplii), which are then used to test lethality of various substances. It has proved to be a rapid, reliable, inexpensive and convenient tool to detect general bioactivity (cytotoxicity, various pharmacological actions and pesticidal effects) [1]. It also allows the use of small quantities of extracts. The method has been used to detect and isolate bioactive compounds through bioactivity-guided screening and fractionation of bioactive plant extracts [3, 4]. It has also been applied in screening extracts of plants used in herbal medicine in Tanzania [5], Argentina [6], Nigeria [7,8] and in Jordan [9].

The present study reports the brine shrimp bioassay of some Kenyan plants used in traditional medicine. Bioassay-guided fractionations of those extracts found to be highly potent may then be carried out in order to isolate the active components. These constituents may later serve as lead compounds for the synthetic modifications by pharmaceutical industries.

EXPERIMENTAL

PLANT MATERIALS

Samples of materials were collected from different parts of Kenya, while some were supplied by herbalists. The botanical identification was confirmed by the Department of Botany, University of Nairobi and Voucher specimens were deposited at the Department of Pharmacology and Pharmacognosy, University of Nairobi.

PREPARATION OF EXTRACTS

The plant materials were air-dried and ground into a fine powder and 10 g of each sample was extracted with petroleum ether (60-80 °C) by maceration at room temperature for 4 days. The samples were then re-extracted with methanol in the same manner. The extracts were dried *in vacuo.*

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Hatching the brine shrimp

Brine shrimp eggs (Sarit Centre Pet shop, Nairobi) were hatched in a shallow rectangular plastic double-chambered box with a dividing wall which had 2-3 mm holes. The box was filled with artificial sea water (33g of sea salt in 1 litre distilled water). About 6 mg of dry yeast per litre of sea salt solution served as food for the nauplii. The eggs (about 50 mg) were sprinkled into the dark compartment while the other compartment was illuminated through a hole in the lid of the box. After 48 hours the phototropic nauplii were collected by use of a pipette from the lighted side, having been separated by the divider from their shells.

Bioassay

Ten shrimps were transferred to each sample vial using a Pasteur pipette and artificial sea water was added to make to 5 ml. The shrimps were counted macroscopically in the stem of the pipette against a lighted background. Survivors were counted after 24 hours and the percentage of deaths at each dose level and control was calculated. Additional dilutions were prepared for the more active plant extracts. In cases where deaths occurred in the control vials, the data were corrected using the equation:

% deaths = [(test-control) / control] x 100.

LC_{50} and 95 % confidence intervals were determined using Finney's program [10] for brine shrimp test developed and kindly provided by Prof. J. C. McLaughlin, University of Purdue, U.S.A.

RESULTS AND DISCUSSION

Table 1 lists the traditional uses of the medicinal plants under study, while table 2 shows the brine shrimp bioassay results of the extracts under study. The latex of *Plumeria alba* and of *Synadenium compactum* had LD_{50} values (confidence intervals) of 471(359-565) and 602 (225-2879), respectively. The results indicate that 36 of 78 samples were active (LC_{50} < 1000 µg/ml). The most active samples (LC_{50} < 250 µg/ml) were from *Bridelia micrantha* (ME), *Brucea antidysenterica* (PE), *Croton megalocarpus* (ME, PE), *Engleromyces goetzi* (ME, PE), *Euclea schimperi* (ME), *Mondia whitei* (ME), *Persea americana* (ME), *Phytolacca dodecandra* (ME, PE), *Salvia nilotica* (ME, PE), *Tagetes minuta* (PE) *Teclea simplifolia* (ME) and *Toddalia asiatica* (ME, PE).

Table 1. Ethnobotanical data of the plants under study

FAMILY Botanical name	PART USED	POPULAR USE
APOCYNACEAE ***Plumeria alba*** L.	SB, L	Laxative, fever, skin diseases [11]; warts, skin eruptions, fruit edible [12].
ASCLEPIADACEAE *Mondia whitei* (Hook F.) Skeels	R	Gonorrhea, aphrodisiac [13]; stomach disorders in infants, mouth freshener [14].
Periploca lineafolia Dill & Rich.	R	Skin sores, chest pains, fevers [14].
BIGNONACEAE *Kigelia africana* (Lam.) Benth.	F, SB, L	Dysentery, constipation, wound dressing, boils [11]; malaria, headaches, measles, causes abortions, causes sexual organs to enlarge, beer flavor [13].
CAPPARACEAE *Gynodropsis gynadra* (L.) Briq.	L, R	Vegetable, aching ears, epilepsy, stomach ache, conjunctivitis, threadworms, facilitate birth [13].
CESALPINIACEAE *Cesalpinia volkensii* Harms	L, R, S	Malaria, pains in pregnant women, aphrodisiac, [13]; gonorrhea, bilhazia, stomach ulcers, aphrodisiac, infertility [14].

FAMILY Botanical name	PART USED	POPULAR USE
COMPOSITAE *Aspilia pluriseta* Schweinf	L	Skin diseases, cuts, wounds, trauchoma [13]; eye diseases, diabetes [14].
Bidens pilosa L.	R, L	Fungal infections, insecticide [11]; constipation, antihelmintic, stomach aches, stomach ulcers, malaria, conjunctivitis [13].
Psiadia punculata (DC.) Vatke.	L	Abdominal pains, colds, burns [13].
Sphaeranthus suaveolens (Forsk.) DC.		Malaria, cough [13] , spasmodic dysmenorrhea, purgative, pain of the spleen [12].
Tarchonanthus camphoratus L.	L	Pain relief, restlessness, anxiety states [11]; slight narcosis, headaches, venereal diseases, stomach problems, asthma, toothaches, bronchitis, diaphoretic, spasmodic, tonic [12].
CONVOLVULACEAE *Cuscuta campestris* Yunker.	SJ	Scabies, pimples and warts [14].
CUCURBITACEAE *Coccinia trilobata* (Cogn.) C. Jeffrey	L	Vegetable [14].
EBENACEAE *Euclea schimperi* (A. DC.) Dandy	L, RB	Anglostomiasis, snake bite[12]; spasmodic, dysmenorrhea, splenic pain, purgative [13].
Croton macrostachyus DEL.	L, R	Malaria, venereal diseases, antihelmintic against tapeworms, purgative, promotes clotting of cuts, cough, skin rashes, seeds are poisonous [13].
Croton megalocarpus Hutch	SB	Antihelmintic, tonic [12]; intestinal worms, whooping cough [13].
Synadenium compactum N. E. Br.	SB	East Coast Fever [13, 15].
HYPOCREACEAE *Engleromyces goetzi* P. Hennings	W	Fevers, malaria, pneumonia [14].
LABIATAE *Ajuga remota* Benth	L	Fever, toothaches, dysentry, high blood pressure [13]; malaria, pneumonia, stomach aches, liver problems [14].
Ocimum basilicum L.	R, L, W	Carminative, flu, fevers, cough [11], mosquito repellant, nasal and bronchial catarrh, constipation and stomach pains in pregnant women [13].
Ocimum suave Willd.	L	Oral hygiene, fevers, colds, cough [11], blocked nose, coughs, ear troubles, abdominal pains, sore eyes, disinfectant, insecticide [13].
LAURACEAE *Persea americana* Mill	F, S, L	Wounds, toothaches, intercostal neuralgia, aphrodisiac, emmenagogue, rubifacient, pectoral, stomachic, antihelmintic, pain relief, astrigent, antidysentric, haemolytic, kidney stones [12].
PAPILIONACEAE *Crotalaria brevidens* Benth.	L	Vegetable, stomach pains and stomach swellings [13].
PHYTOLACCACEAE *Phytolacca dodecandra* L'Herit	SB,L, F	Fish poison, purge taenifuge [11], tapeworm, emetic, fever, venereal diseases, purgative, haemostatic, epilepsy, stimulant, tonic, cathartic, abortifacient, homicidal poison [12].
ROSACEAE *Prunus africana* (Hook. F.) Kalkm	B, L	Fever, stomach ache, purgative for cattle, appetizer [13], liver problems, constipation, prostate enlargement [14].

FAMILY Botanical name	PART USED	POPULAR USE
RUTACEAE		
Teclea simplifolia (Engl.) Verdoorn	R	Treats gonorrhea, root is poisonous [16].
Toddalia asiatica (L.) Lam	L, F, R	Treats fever, inflammation, insect bites [11], nasal and bronchial pains, coughs, colds, stomachache, snake bites, it's a powerful emetic [13].
SIMAROUBACEAE		
Brucea antidysenterica J. F. Mill	L, R, F, SB	Parasitic skin diseases [11], diarrhea, dysentry, fever, asthma, antihelmintic, bone carries, scrofula, leprosy, cancer, galls and pressure sore on the mule [12], stomach ache, indigestion, asthma, leprosy, skin diseases, wounds, dysentry, bloating in cattle [13].
URTICACEAE		
Urtica massaica	L	Common vegetable [14].
VERBENACEAE		
Lippia javanica (Burm. F.) Spreng	L, F, R	Headache, fever, skin diseases [11], fever, stuffy nose, malaria, cuts, wounds, tapeworm [13]; insect repellant [14]; coughs, colds, bronchial troubles, influenza, measlesprophylactic, lung inflammations, disinfection of anthrax - infected meat, gangrenous rectitis, urticaria, Blackwater fever, malaria, dysentry, ingestion by stock results in photosensitisation [12].

L: leaves, F: fruits, R: root, SB: stem bark, SJ: stem juice, W: whole plant

Table 2. Brine shrimp lethality test. LC_{50} (confidence intervals) of latex, petroleum ether and methanol extracts tested at 10, 100 and 100 µg/ml

FAMILY NAME Botanical name	PART USED	LC_{50} (µg/ml)		
		Latex	PE extract	ME extract
APOCYNACEAE				
Plumeria alba	SB	-	>1000	>1000
Plumeria alba	Latex	471(359-565)	-	-
ASCLEPLADACEAE				
Mondia whitei	R	-	>1000	30 (11-77)
Periploca linearifolia	W	-	>1000	>1000
BIGNONIACEAE				
Kigelia africana	F	-	362 (132-1259)	>1000
CAPPARACEAE				
Gynodropsis gynadra	L	-	>1000	>1000
CELASTRACEAE				
Maytenus cordata	R	-	>1000	516 (192-1717)
CESALPINIACEAE				
Cesalpinia volkensii	L	-	>1000	>1000
COMPOSITAE				
Aspilia pluriseta	L	-	>1000	>1000
Bidens pilosa	L/ F	-	388 (277-557)	>1000
Psiadia punculata	L	-	>1000	>1000
Sphaeranthus suaveolens	L	-	556 (229-1669)	627(225-5527)
Tagetes minuta	L	-	8 (2.5-26)	317 (230-411)
Tarchonanthus camphoratus	L	-	>1000	>1000
CONVOLVULACEAE				
Cuscuta campestris	W	-	>1000	>1000
CUCURBITACEAE				
Coccinia trilobata	L	-	>1000	>1000

FAMILY NAME Botanical name	PART USED	LC_{50} (µg/ml)		
		Latex	PE extract	ME extract
EUPHORBIACEAE				
Bridelia micrantha	L	-	>1000	>1000
B. Micrantha	SB	-	>1000	31 (12-82)
Croton macrostachyus	L	-	>1000	>1000
Croton megalocarpus	L	-	>1000	>1000
C. Megalocarpus	SB	-	13 (7-25)	152 (61-444)
C. Megalocarpus	S	-	6 (0-14)	107 (34-315)
Synadenium compactum	SB	-	>1000	437.9 (150-2672)
Synadenium compactum	Latex	602(225-2879)	-	-
HYPOCREACEAE				
Engleromyces goetzi	W	-	248 (119-599)	116 (46-316)
LABIATAE				
Ajuga remota	L	-	>1000	>1000
Ocimum basilicum	L	-	>1000	909 (438-4302)
Ocimum suave	L		>1000	602.9 (194-12144)
Salvia nilotica	L	-	247 (105-621)	85 (31-220)
LAURACEAE				
Persea americana	L	-	49 (11-149)	14 (11-18)
Persea americana	P	-	>1000	2.7 (0.9-7.9)
PAPILIONACEAE				
Crotalaria brevidens	L	-	453 (179-1552)	>1000
PHYTOLACCACEAE				
Phytolacca dodecandra	SB	-	91 (24-311)	24.8 (17-35)
ROSACEAE				
Prunus africana	SB	-	>1000	>1000
RUTACEAE				
Teclea simplifolia	SB	-	>1000	217 (102-522)
Toddalia asiatica	S	-	120 (80-179)	145 (40-672)
SIMAROUBACEAE				
Brucea antidysenterica	SB	-	59 (22-160)	>1000
URTICACEAE				
Urtica massaica	R	-	338 (114-1619)	362 (120-2544)
VERBENACEAE				
Lippia javanica	L	-	>1000	>1000

L: leaves, F: fruits, P: peel, R: root, S: seed, SB: stem bark, W: whole plant , PE: Petroleum ether, ME: Methanol extract

There have been arguments as to whether BST activity lies in the polar or non-polar fractions [6]. The present results indicate that activity could reside in any of the portions (44 % and 56 % of the active extracts were from polar and non-polar fractions, respectively). For some plants activity was found only in the polar portion (e.g. *Persea americana* peel) while for others it was only in the non-polar fraction (e.g. *Brucea antidysenterica*). Little work has been carried out on the phytochemistry and/or biological activity of these plants.

However, there are interesting findings on related species (table 3), most of which have been studied in other countries. Although BST certainly indicates that the plant has biological activity, lack of lethality, however, does not mean absence of biological activity. This is indicated in the case of *Prunus africana*, which showed no BST lethality, but has clearly been demonstrated to counteract benign prostatic hyperplasia [57]. Some well known drugs like atropine and phentolamine also showed no BST activity in another study [64].

Table 3. Previous work done on plants under study and/or related species

FAMILY Botanical name	RESEARCH FINDINGS
APOCYNACEAE	
Plumeria rubra	Rubrinol, a new antibacterial triterpenoid was isolated [17]. Fulvoplumierin, an anti-HIV compound was isolated [18]. Some cytotoxic compounds were isolated from the stem bark [19].
Plumeria acutifolia	Plumeride, plumericin, fulvoplumierin, lupeol and other compounds were isolated [20].
ASCLEPIADACEAE	
Periploca nigrescens	The cardenolides cymarin, strophanthidin and a strophanthidin glycoside were isolated [21].
BIGNONACEAE	
Kigelia punculata	After extracting the fruit with petroleum ether, ethanol and methanol, the subsequent aqueous extract showed neoplasm inhibiting effect in mice [22].
CESALPINIACEAE	
Caesalpinia bonducella	Caesalpin F, a new furanoditerpene was isolated from the seeds [23].
C. crista	Isolation and characterisation of (+)-ononitol was carried out [24].
C. japonica/sappan	Homoflavonoids and related known compounds were isolated from the wood [25-27].
COMPOSITAE	
Aspilia mossambicensis	The dithiopolyenes thiarubicin A, B and C were isolated from the roots [28].
A. africana	The anticoagulant properties of the leaf extract were studied [29].
Bidens pilosa	Phenylheptatriyne, extracted from leaves, showed photoxicity to *Escherichia coli* [30] and cercaricidal effect in snails [31].
Psiadia punculata	Flavonoids and diterpenes isolated [32, 33], extracts have hypotensive, neuromuscular blockade and bronchodilatory effects [34].
Tagetes minuta	Thiophenes, which have antiviral activity, were isolated [35].
CONVOLVULACEAE	
Cuscuta chinensis	Seed extracts have been used in combination with inflammation inhibitors for treatment of acne and dandruff [36].
Brucea antidysenterica	Extracts found to be active against chloroquine resistant strains of *Plasmodium falciparum* and *Entamoeba histolytica* [37].
EUPHORBIACEAE	
Bridelia ferrugina	Quercetin-3-hesperidoside (rutin) and other flavonoids were isolated as the active hypoglycemic agents [38].
B. tomentosa	A new triterpenoid was isolated from the roots [39].
Croton macrostachyus	Extracts showed antineoplastic activity on Ehrlich and sarcoma 180 carcinomas [40]. Lupeol, betulin and a number of fatty acids were isolated from the stem bark [41].
Croton megalocarpus	Chiromodine, a novel clerodane, was isolated as a major constituent [42]. Epoxychiromodine, a new diterpeneand other known compounds were isolated [43-44].
Synadenium compactum	2-Methylbutanoate tetraacetate and 2-methylbutanoate pentaacetate derivatives of synadenol were isolated and characterized [45].

FAMILY Botanical name	RESEARCH FINDINGS
LABIATAE *Ocimum suave*	The chemical constituents, as well as the mosquito repellant and antimicrobial activity of the essential of was reported [46].
Ocimum basilicum	Essential oil showed insecticidal activity against stored grains insects [47]. The essential oil and combined alcohol and aqueous extracts showed antibiotic activity against seven microorganisms [48]. The compounded powder of the plant showed inhibition of production of toxin by three species of toxigenic *Aspergillus* [49].
LAURACEAE *Persea americana*	Brine shrimp lethality test (BST) was done on unripe fruit. Three major compounds were isolated and they were found to be cytotoxic on six human cell lines and have insecticidal activity against yellow fever mosquito larvae [50].
Persea major	The chloroform partition of ethanol extract of the bark was insecticidal and significantly active in (BST) (LC_{50} = 2.60 μg/ml). Two bioactive compounds, majorynolide and majorenolide, were isolated by activity-directed fractionation using BST[51].
PAPILIONACEAE *Crotalaria assamaica*	Monocrotaline, an alkaloid from the plant, inhibited growth of transplanted tumours in mice [52].
PHYTOLACCACEAE *Phytolacca dodecandra*	The molluscicidal activity of the plant was reported [53]. Isolation of saponins from berries [54-55]. Dodecandrin, a new ribosome-inhibiting protein was reported [56].
ROSACEAE *Prunus africana*	Different compunds from the plant (fatty acids, sterols, pentacyclic triterpenes, two linear alcohols and their esters) exert synergistic action, counteracting some of the biochemical functional changes that characterise benign prostatic hyperplasia [57].
SIMAROUBACEAE *Brucea antidysenterica*	Bruceantin, a new potent antileukemic simaroubalide was isolated [61] and characterised [62].
URTICACEAE *Urtica dioica* L.	Clinical trial have been carried out on the use of the root extract for therapy of benign prostatic carcinoma. Patients have shown significant improvement and very good tolerability [63].

About 54 % of the active extracts are from plants that are traditionally used for various gastrointestinal problems or as anthelmintic agents (table 1). Some of the plants which were active, such as *Plumeria alba*, *Kigelia africana*, *Maytenus cordata*, *Tagetes minuta*, *Croton* species, *Crotaralia* species and *Toddalia asiatica,* have also been reported to contain compounds with anticancer or anti-HIV activity (table 3). The results obtained should serve as a guide to further research on the chemical constituents and biological activities of the active plant extracts.

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