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Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants

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Abstract

The organic extracts of leaves and roots of five plants used for treating malaria in Central, Nairobi and Rift Valley Provinces, Kenya were tested for brine shrimp lethality and in vitro antiplasmodial activity against chloroquine sensitive and resistant strains of *Plasmodium falciparum*. Of the plants tested, 60% were toxic to the brine shrimp ($LC_{50} < 30$ μg/ml) and eight out of ten plant parts (80%) showed in vitro antiplasmodial activity ($IC_{50} < 50$ μg/ml). Among the extracts screened, the leaves of *Cyathula polcephala* had the highest toxicity to the brine shrimp ($LC_{50} = 2.9$ μg/ml) while the leaves of *Pentas longiflora* had the best antiplasmodial activity ($IC_{50} = 11.4$ μg/ml). The plant extracts with low IC_{50} values are potential sources for novel antiplasmodial compounds.

Keywords: Plant extracts; Cytotoxicity; Antiplasmodial; Medicinal plants; *Plasmodium falciparum*; *Albizia gummifera*; *Cyathula cylindrical*; *Cyathula polycephala*; *Pentas longiflora*; *Pittosporum lanatum*

1. Introduction

Malaria is the world's leading killer among the infectious diseases. It is caused by a protozoan parasite of the genus *Plasmodium*. *Plasmodium falciparum* is the most lethal and is distributed in tropical Africa, Asia and Latin America (WHO, 2000). Over 40% of the world population remains exposed to malaria with 120 million cases reported and about two million people dying of it annually (WHO, 1998). In Kenya, more than 90% of malaria is caused by *Plasmodium falciparum* (Khaemba et al., 1994) transmitted by *Anopheles gambiae*. Resistance of the malarial parasites to the commonly used anti-malarial drugs such as chloroquine and sulfadoxine-pyrimethamine (Basco et al., 1994; USN, 2001) has enhanced morbidity and mortality and hence triggered the search of new drugs especially from plants.

Herbal remedies have been applied for treatment of many ailments since ancient time all over the world and about 25% of current drugs are derived from plants. For example, the most potent antimalarial drugs quinine and artemisinin are from the plants *Cinchona* sp. (Garnham, 1966) and *Artemisia annua* (QACRG, 1979), respectively. However, only about 20% of the plants with claimed bioactivities have been subjected to bioassay screening (Houghton, 2001).

In Kenya, plant extracts are still widely used in the treatment of malaria and other ailments. In continuation of our efforts to verify the efficacy of folk medicines (Omolo et al., 1997; Omulokoli et al., 1997; Wanyonyi et al., 2002; Muregi et al., 2003), in this paper, five plants, *Albizia gummifera* (J.F. Gmel.) C.A. Sm. (Mimosaceae), *Cyathula cylindrica* Moq., *Cyathula polycephala* Bak. (Amaranthaceae), *Pentas longiflora* Oliv. (Rubiaceae) and *Pittosporum lanatum* Hutch. & Bruce (Pittosporaceae) used for the treatment of malaria were studied for their toxicity to the brine shrimp and antiplasmodial activity.

The root decoction of *Albizia gummifera* is taken to treat malaria, stomach pains, scabies and other skin diseases (Kokwaro, 1993; Watt and Breyer-Brandwijk, 1962), and psychiatric problems (Chhabra et al., 1990). Previous phytochemical studies of this plant led to the isolation of oleanane glycoside, triterpenoids, saponins, sapogenin, macrocyclic spermine and budmunchiamine alkaloids (Lipton, 1963; Orsini et al., 1991; Rukunga and Waterman, 1996, 2001; Debella et al., 2000).

The root bark decoction of *Cyathula cylindrica* is drunk as a remedy for malaria and leprosy. The leaves are used for

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Table 1
Plants collected used as tradtitional anti-malarials with percent yield of extracts

Family/botanical name	Place (province)	Percent yield of extracts	
		Roots	Leaves
Amaranthaceae			
Cyathula cylindrica (GNW-SM/2/99)	Kabsabet (Rift Valley)	13.0	6.4
Cyathula polycephala (GNW-SM/3/99)	Kiambu (Central)	17.6	9.8
Mimosaceae			
Albizia gummifera (GNW-SM/1/99)	Chiromo (Nairobi)	25.5	21.5
Pittosporaceae			
Pittosporum lanatum (GNW-SM/5/99)	Makuyu (Central)	17.9	15.5
Rubiaceae			
Pentas longiflora (GNW-SM/4/99)	Cherengani (Rift Valley)	23.2	20.3

treating ankylostomiasis (Kokwaro, 1993). *Cyathula polycephala* is used to treat fever, malaria and stomach troubles (Kokwaro, 1993).

The root decoction of *Pentas longiflora* is taken against malaria, diarrhea, tapeworm, itchy rashes, as a purgative (Kokwaro, 1993), gonorrhea and syphilis (Chhabra et al., 1991). Previous chemical studies of this plant led to the isolation of naphthoquinoid pigments (Hari et al., 1991) and isagarin (Van Puyvelde et al., 1998). The lukewarm water extract of the bark of *Pittosporum lanatum* is taken as a remedy for malaria (Kokwaro, 1993). Neither chemical nor biological studies of this plant are reported in literature.

2. Materials and methods

2.1. Plant materials

The plant samples were collected from the Central, Rift Valley and Nairobi Provinces, Kenya based on their ethnomedical use (Table 1). These were identified and voucher specimens deposited at the Herbarium, Botany Department, University of Nairobi, Nairobi. The plant parts were air-dried under shade and ground using a laboratory mill (roots) and a kitchen blender (leaves).

2.2. Extraction

A known amount of dried powered plant sample (150–400 g) was extracted by maceration in methanol (3 × 48 h). The combined extracts were then concentrated under reduced pressure below 50 °C to get the crude extract (Table 1).

2.3. Toxicity testing against the brine shrimp

2.3.1. Hatching shrimp

Brine shrimp eggs, *Artemia salina* leach were hatched in artificial seawater prepared by dissolving 38 g of sea salt (Sigma chemicals Co., UK) in 1 l of distilled water. After 48 h incubation at room temperature (22–29 °C), the larvae (nauplii) were attracted to one side of the vessel with a light source and collected with pipette. Nauplii were separated from eggs by aliquoting them three times in small beakers containing seawater.

2.3.2. Brine shrimp assay

The bioactivity of the extracts was monitored by the brine shrimp lethality test (Meyer et al., 1982). Samples were dissolved in dimethylsulphoxide (DMSO) and diluted with artificial sea salt water so that final concentration of DMSO did not exceed 0.05%. Fifty microliters of sea salt water was placed in all the wells of the 96-well microtiter plate. Fifty microliters of 4000 ppm of the plant extract was placed in row one and a two-fold dilution carried out down the column. The last row was left with sea salt water and DMSO only served as the drug free control. Hundred microliters of suspension of nauplii containing about 10 larvae was added into each well and incubated for 24 h. The plates were then examined under a microscope (12.5×) and the number of dead nauplii in each well counted. Hundred microliters of methanol was then added and after 10 min, the total numbers of shrimp in each well were counted and recorded. Lethality concentration fifties (LC_{50} values) for each assay were calculated by taking average of three experiments using a Finney Probit analysis program on an IBM computer (McLaughlin et al., 1991).

2.4. Antiplasmodial testing

2.4.1. Preparation of drugs

Stock solutions of crude extracts (250 μg/ml) were made with sterile water (deionized and autoclaved) and consecutively filtered first through 0.45 and 0.22 μm microfilters under a laminar flow hood. The water insoluble extracts were first dissolved in DMSO (solvent concentration <0.02%) (Elueze et al., 1996). A stock solution of chloroquine phosphate (1 μg/ml) was similarly prepared in sterile water. All the drug solutions were stored at −20 °C and retrieved only during use.

2.4.2. Cultures of Plasmodium falciparum

Laboratory-adapted *Plasmodium falciparum* cultures of M24, K39 (chloroquine-sensitive isolates, originally obtained from patients in Mombasa and Kisumu, Kenya, respectively) and the international reference isolate VI/S (chloroquine-resistant) were used in this study. The strains have been cultured and maintained at the Malaria Laboratories of Kenya Medical Research Institute (KEMRI), Nairobi. The culture medium was a variation of that

described by Trager and Jensen (1976) and consisted of RPMI 1640 supplemented with 10% human serum, 25 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) and 25 mM $NaHCO_3$. Human type O+ erythrocytes (<28 days old) served as host cells and the cultures were incubated at 37 °C in an atmosphere of 3% CO_2, 5% O_2 and 92% N_2.

2.4.3. Bioassays

The in vitro semi-automated micro-dilution assay technique that measured the ability of the extracts to inhibit the incorporation of [*G*-^{3}H]hypoxanthine into the malaria parasite was used. Precise details of the protocol were described earlier (Muregi et al., 2003). The extracts were tested in duplicate at 11 concentrations in two-fold dilutions and the experiment was repeated twice for each extract.

Computation of the concentration of drug causing 50% inhibition of [*G*-^{3}H]hypoxanthine uptake (IC_{50}) was carried out by interpolation after logarithmic transformation of both concentration and cpm values using the formula,

$$IC_{50} = \text{antilog}\left[\frac{(\log X_1 + [(\log Y_{50} - \log Y_1) \times (\log X_2 - \log X_1)]}{\log Y_2 - \log Y_1}\right]$$

where Y_{50} is the cpm value midway between parasitisized and non-parasitisized control cultures and X_1, Y_1, X_2 and Y_2 are the concentrations and cpm values for the data points above and below the cpm midpoints (Sixsmith et al., 1984).

3. Results and discussion

Results of the toxicity against brine shrimp of the extracts are shown in Table 2.

A total of 10 methanol extracts were tested for their toxicity against brine shrimp using the brine shrimp lethality assay. The extracts of the leaves and roots of *Cyathula polycephala* (Amaranthaceae), *Pentas longiflora* (Rubiaceae) and *Pittosporum lanatum* (Pittosporaceae) showed significant toxicity against brine shrimp with LC_{50} values 2.9, 8.4; 12.3, 6.4; 27.4 and 17.8 μg/ml, respectively (Table 2). The leaves and the roots extracts of *Cyathula cylindrica* (Amaranthaceae) and *Albizia gummifera* (Mimosaceae) did not show any significant cytotoxicity (Table 2). Since in most cases toxicity is associated with pharmacological properties, it was deduced that the extracts from *Cyathula polycephala* and *Pentas longiflora* had the best bioactivity.

Table 2
The mean LC_{50} values ± S.D for plant extracts screened against brine shrimp larvae (*Artemia salina* leach)

Plant species	LC_{50} ± S.D. (μg/ml)	
	Leaves	Roots
Pentas longiflora	12.3 ± 0.3	6.4 ± 1.1
Cyathula cylindrica	153.3 ± 3.8	137.2 ± 2.7
Cyathula polycephala	2.9 ± 0.3	8.4 ± 0.2
Albizia gummifera	274.4 ± 2.6	86.9 ± 2.3
Pittosporum lanatum	27.4 ± 0.3	17.8 ± 0.5
Emetine hydrochloride[a]	20.1 ± 0.2	

[a] Included as a positive control.

All the 10 extracts were further screened for in vitro antiplasmodial activity against the chloroquine sensitive (M24 and K39) and resistant (V1/S) *Plasmodium falciparum* strains and the results are summarized in Table 3.

The most active extracts were of *Pentas longiflora*, the root extract and the leaves extracts having IC_{50} values 20.4, 14.1, 25.8 and 24.3, 11.4, 29.0 μg/ml against chloroquine sensitive (K39, M24) and resistant (V1/S) strains, respectively. The next active plant was *Albizia gummifera*, the root and leaves extracts having IC_{50} values 37.6, 27.4, 34.2 and 30.5, 21.3, 27.6 μg/ml against chloroquine sensitive (K39, M24) and resistant (V1/S) strains, respectively. The order of activity was followed by the extracts of the roots and leaves of *Pittosporum lanatum*. The extract of the roots of *Cyathula cylindrica* was the least active among all the extracts while the extract of the leaves of *Cyathula polycephala* was the least active among the leaves of all the plant studied.

From the results obtained for toxicity against the brine shrimp (Table 2), it was obvious that the extracts of

Table 3
In vitro antimalarial activities for methanol roots and leaves extracts from five different Kenyan plants used in traditional medicine against different strains of *Plasmodium falciparum*

Plant species	IC_{50} ± S.D. (μg/ml) of the isolates					
	Roots			Leaves		
	K39	V1/S	M24	K39	V1/S	M24
Pentas longiflora	20.4 ± 0.1	25.8 ± 2.3	14.1 ± 1.0	24.3 ± 1.1	29.0 ± 0.2	11.4 ± 0.9
Cyathula cylindrical	255.0 ± 2.5	269.0 ± 1.5	187.6 ± 0.7	54.0 ± 0.8	50.1 ± 1.2	49.0 ± 0.2
Cyathula polycephala	47.2 ± 0.6	60.3 ± 5.4	42.8 ± 1.1	86.7 ± 0.9	87.3 ± 2.8	66.4 ± 1.1
Albizia gummifera	37.6 ± 2.1	34.2 ± 1.9	27.4 ± 0.4	30.5 ± 2.8	27.6 ± 0.3	21.3 ± 2.7
Pittosporum lanatum	34.0 ± 0.3	41.5 ± 0.7	31.2 ± 0.2	38.4 ± 1.1	39.3 ± 0.3	24.2 ± 0.3
Chloroquine (μg/ml)[a]	0.021 ± 0.02	0.105 ± 0.01	0.054 ± 0.01			

[a] Included as a positive control.

Cyathula polcephala were most toxic followed by that of *Pentas longiflora* while the in vitro antiplasmodial studies (Table 3) indicated that extracts of *Pentas longiflora* and *Albizia gummifera* had comparable good activities. On comparing the toxicity against the brine shrimp and the in vitro antiplasmodial activities of the extracts of *Pentas longiflora* and *Albizia gummifera*, it was evident that the extracts of *Albizia gummifera* were selectively toxic to malaria parasites.

This study showed that the five plants used by traditional healers to treat malaria had some antiplasmodial properties albeit the roots extract of *Cyathula cylindrica* had very low activity (187–269 μg/ml). The methanol extracts of *Pentas longiflora* commonly used in Rift Valley Province had the highest in vitro antiplasmodial activity (about 20 μg/ml). Both the leaves and the root extracts were found to be biologically active against the chloroquine sensitive and resistant strains.

The low antiplasmodial activity in some plants could partly be explained by the circumstances that many plants are used in the treatment of malaria, not for their anti-parasitic effect (that is, curing the disease) but because of other activities with therapeutic value for a patient with malaria. These activities would include reducing fever, easing convulsions and headache, and possibly even immunostimulatory effects (Rasoanaivo et al., 1992). Another reason is that traditional healers give a mixture of some plants for the treatment of diseases. The mixture could be active due to synergistic effects (Gessler et al., 1994).

4. Conclusion

The fact that three out of the five plants (60%) screened for toxicity against the brine shrimp had LC_{50} values less than 30 μg/ml and eight out of ten plant parts (80%) screened for in vitro antiplasmodial activity had IC_{50} values less than 50 μg/ml is interesting and lends support to the traditional use of these plants for malaria treatment, but in vivo test are required to support this. After detailed in vivo antimalarial evaluation and thorough toxicological studies, some of these plants may find use as antimalarials in known dosages especially in rural communities where the conventional drugs are unaffordable or unavailable and the health facilities inaccessible.

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