BIOLOGICAL SCREENING OF KENYAN MEDICINAL PLANTS USING *ARTEMIA SALINA* L. (ARTEMIIDAE).

J.M.Nguta[a]*, J.M.Mbaria[a], D.W.Gakuya[b], P.K.Gathumbi[c], J.D.Kabasa[d], S.G.Kiama[e]

[a]Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya [b]Department of Clinical Studies, University of Nairobi, Nairobi, Kenya [c]Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Nairobi, Kenya [d]Department of Physiology and pharmacology, Makerere University, Kampala, Uganda [e]Department of Veterinary Anatomy and Physiology, University of Nairobi, Nairobi, Kenya

Summary

Medicinal plants constitute important components of flora and are widely distributed in different regions of Kenya. Based on ethnopharmacological significance, we collected several medicinal plants from South Coast, Kenya used in traditional medicine to treat malaria and evaluated for their toxicity. In the present study, brine shrimp (*Artemia salina*) test was used to screen antimalarial plants for their cytotoxicity. A total of 80 crude extracts from 30 plant species distributed among 18 plant families were evaluated for their toxicity against *Artemia salina*. Cytotoxicity results showed that 23 (57.5%) of organic and 7 (17.5%) of aqueous extracts showed significant toxicity to the brine shrimp (LC_{50} < 100 µg/ml). Organic extracts obtained from the leaves of *Momordica foetida* Schumach. (Cucurbitaceae), stem bark of *Warbugia stuhlmannii* Engl. (Canallaceae) and the root bark of *Zanthoxylum chalybeum* (Eng) Engl. (Rutaceae) exhibited potent activity with LC_{50} values of 8, 8 and 11 µg/ml respectively. The toxicity data obtained suggest that some of these plants would not make good malaria treatments, suggesting a need for further *in vivo* toxicological studies. The present study could be useful in the search for new antitumor compounds from the Kenyan flora.

Keywords: cytotoxic, *Artemia salina*, natural products, antimalarial plants, Kenyan biodiversity

Corresponding Author:

Dr. Joseph Mwanzia Nguta,

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi,

Box 29053-00625, Nairobi.

Tel.: +254 735 750 278. Email: joseph.nguta@uonbi.ac.ke

Introduction

During the past decade, traditional systems of medicine have become increasingly important in view of their safety (1). Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Indeed, indigenous plants play an important role in the treatment of many diseases (2) and 80% of the people worldwide are estimated to use herbal remedies (3, 4). However, few data are available on their safety, despite the fact that validation of traditional practices could lead to innovative strategies in disease control. Although modern medicine may be available in developing countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs (5).

Kenya possesses rich floristic wealth and diversified genetic resources of medicinal plants. It has a widely ranging tropical and the agro climatic conditions, which are conducive for introducing and domesticating new and exotic plant varieties. The use of the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds. Most of these traditional preparations and formulations have been found to be a reservoir of pharmaceuticals (6).

The brine shrimp lethality assay consists of exposing larvae to test sample in saline solution and lethality is evaluated after 24 h (1). The commercial availability of inexpensive brine shrimp eggs, the low cost and ease of performing the assay make brine shrimp lethality assay, a very useful bench-top method (7). A number of studies have demonstrated the use of the brine shrimp assay to screen plant extracts (8, 9, 10). Lethality assay has been used successfully to biomonitor the isolation of cytotoxic (11), antimalarial (12), insecticidal (13) and antifeedant (14) compounds from plant extracts. It has been demonstrated that activity against *Artemia salina* Leach (Artemiidae) larva correlates well with cytotoxic activity (15), as well as other pharmacological activities (16). In the current study, results of biological screening of crude plant extracts (aqueous and organic) of some important medicinal plants used in the traditional medicine to treat malaria (collected from South Coast Kenya) for lethality towards *Artemia salina* larvae are presented.

Materials and methods

Plant materials

The plant samples used in the current study were collected in August 2009 from Msambweni district of Kenya based on ethnopharmacological use through interviews with local communities and traditional health practitioners. Permission for a sustainable plant harvesting was granted by Kenya Wildlife Service (KWS) in the forest game reserve, and the local community outside the forest areas. The information gathered included part of the plant used and the method of preparation of the herbal antimalarial remedies. The plants were identified by Mr. Kimeu Musembi, a taxonomist at the University of Nairobi Herbarium, Nairobi, where voucher specimens were deposited. The plant parts were chopped into small pieces; air dried at room temperature (25°C) under shade and pulverized using a laboratory mill (Christy & Norris Ltd., England).

Antitumour drugs

Cyclophosphamide, Mfg. Lic. No.: DD/140 and batch number KB 791001, was purchased from Biochem Pharmaceutical Industries Limited (Mumbai, India). Etoposide (Etosid^{TM}), batch number J8 05 26, a semi synthetic derivative of podophyllotoxin, was purchased from CIPLA Limited, plot No.S-103 Verna.

Preparation of extracts

Considering that people in Msambweni usually use hot water to prepare their herbal remedies as decoctions and sometimes concoctions, aqueous hot infusions of each plant part was prepared (50 g of powdered material in 500 ml of distilled water) in a water bath at 60°C for 1 h. The extracts that were obtained were filtered through muslin gauze and the filtrate kept in deep freezer for 24 h, which was then lyophilized. The lyophilized dry powder was collected in stoppered sample vials, weighed and kept at -20°C until used. Organic extracts [chloroform (CHCL3): methanol (MeOH)) (1:1) (50 g of powdered material in 500 ml of solvent)] were prepared by maceration of the dried and powdered plant material with the organic solvent for 48 h. The extract was then filtered through Whatman filter paper No.1. The filtrate was concentrated to dryness *in vacuo* by rotary evaporation and weighed. The dry solid extracts were stored at -20 °C in airtight containers until used.

Product identification and description (*Artemia salina*)

Artemia eggs, batch number DE RP 33801, were purchased from JBL GmbH & Co.KG (Neuhofen, Germany) and the product was labeled as JBL Artemio Pur Brand. The *Artemia* eggs had been harvested from Great Salt Lake, Utah, USA and were identified *as Artemia salina,* based on zoogeography (17). *Artemia salina* is endemic to North and Central America (18). It has been labeled as a super species [(a set of ecologically isolated and physiologically distinct semi species and species) (18)]; this is important as it is indicative of intraspecies variation. This species is of great economic importance, as its commercial harvest from Great Salt Lake (Utah, USA) is estimated to represent 90% of the global trade in brine shrimp eggs (19). This is a substantial volume of eggs when one considers that annually over 2000 metric tons of dry *Artemia* eggs are marketed worldwide (20). *A. salina* is the best studied of the *Artemia* species (21), estimated to represent over 90% of studies in which *Artemia* is used as an experimental test organism [(very often using material sourced from Great Salt Lake, Utah, USA) (22)].

Culture and harvesting of *Artemia salina*

Artemia salina eggs were stored at -20°C before use. The eggs were incubated for hatching in a shallow rectangular dish (14 cm x 9 cm x 5 cm) filled with 225 ml of a 3.3% solution of artificial sea water. A plastic divider with several 2 mm holes was clamped in the dish to make two unequal compartments. The eggs (1.11 g) and yeast (0.0827 g) were sprinkled into the larger compartment which was darkened. The smaller compartment was illuminated by a tungsten filament light and gently sparged with air. After 24 h, hatched *A. salina* eggs were transferred to fresh artificial seawater and incubated for a further 24 h under artificial light with air sparging (23). The phototropic nauplii were collected by pipette from the lighted side, having been separated by the divider from the shells.

Preparation of test extracts

Stock solutions of aqueous extracts (10,000 µg/ml) were made in distilled deionized water and filter sterilized using 0.22 µm membrane filters in a laminar flow hood. The organic extracts were dissolved in dimethyl sulphoxide, $CH_3.SO.CH_3$ M.W 78.13 (DMSO); batch number PJ/25/3496/709-05/6/16, (THOMAS BAKER CHEMICALS, PVT. LIMITED, MUMBAI, INDIA) followed by subsequent dilution to lower concentration of DMSO, to <1% to avoid carry over (solvent) effect (24). Test extracts at appropriate amounts (5 µl, 50 µl and 500 µl for 10 µg/ml, 100 µg/ml, and 1000 µg/ml, respectively) were transferred into 10 ml vials (5 vials for each dose and 1 for control). Five replicates were prepared for each dose level.

Preparation of antitumour drugs

Stock solutions of cyclophosphamide and etoposide (10,000 µg/ml) were prepared in distilled deionized water and filter sterilized using 0.22 µm membrane filters in a laminar flow hood. Test solutions at appropriate amounts (5 µl, 50 µl, and 500 µl for 10 µg/ml, 100 µg/ml and 1000 µg/ml, respectively) were transferred into 10 ml vials (5 vials for each dose and 1 for control). Five replicates were prepared for each dose level.

Bioassay of *Artemia salina*

For toxicity tests, ten *A. salina* nauplii were transferred into each sample vial using 230 mm disposable glass Pasteur pipettes (Ref. D812) (Poulten & Graf Ltd, Barking, UK) and filtered brine solution was added to make 5 ml. The nauplii were counted macroscopically in the stem of the pipette against a lighted background. A drop of dry yeast suspension [(Red star) (3 mg in 5 ml artificial sea water)] was added as food to each vial. All the vials were maintained under illumination. The surviving nauplii were counted with the aid of a 3x magnifying glass, after 24 h, and the percentage of deaths at the three dose levels and control were determined. In cases where control deaths occurred, the data was corrected using Abbott's formula as follows: % deaths = [(Test-control)/control x 100. The surviving nauplii were killed by the addition of 100 µl of 5% (v/v) phenol to each vial.

LC_{50} determinations

The lethal concentration fifty (LC_{50}), 95% confidence interval and slope were determined from the 24 h counts using the probit analysis method described by Finney (25). In cases where data was insufficient for this technique, the dose response data was transformed into a straight line by means of a logit transformation (26), and the LC_{50} value was derived from the best fit line obtained by linear regression analysis. LC_{50} is indicative of toxicity level of a given plant extract or antitumour drug.

Results

A total of 80 crude extracts belonging to 30 species in 26 genera and 18 families were evaluated in the current study (Table 1). The yields of the water extracts ranged between 1.06 and 21.24% w/w, while those of organic extracts were between 0.76 and 22.4% w/w (Table 1).

Table 1*:* Plant extracts used in the study (quantity obtained from 50 g of dried plant material, % dry weight, W/w).

Family	**Plant species/ Voucher specimen number**	**Plant part**	**Solvent**	**%Yield (w/w)**
Anacardiaceae	*Heeria insignis* (Delile) Kuntze (JN024)	Stem	$CHCL_3$/MeOH Water	4.78 10.4
Annonaceae	*Uvaria scheffleri* Diels (JN041)	Leaves	$CHCL_3$/MeOH Water	6.6 8.2
Apocynaceae	*Landolphia buchananii* (Hallier f.) Stapf (JN027)	Leaves	$CHCL_3$/MeOH Water	5.4 7.8
Apocynaceae	*Rauwolfia conthen.* (JN 051)	Root bark	$CHCL_3$/MeOH Water	8.8 11.4
Asteraceae	*Vernonia amygdalina* A. Chev. (JN057)	Leaves	$CHCL_3$/MeOH Water	5.6 6.8
Asteraceae	*Launea cornuta* (Hochst.ex Oliv.& Hiern) C. Jeffrey(JN028)	Leaves	$CHCL_3$/MeOH Water	5.6 8.12
Asteraceae	*Launea cornuta* (Hochst.ex Oliv.& Hiern) C. Jeffrey(JN028)	Roots	$CHCL_3$/MeOH Water	6.72 4.84
Asteraceae	*Senecio syringifolius* O. Hoffm.(JN036)	Leaves	$CHCL_3$/MeOH Water	2.08 2.66
Asteraceae	*Tridax procumbens L.* (JN 054)	Whole plant	$CHCL_3$/MeOH Water	5.4 6.6
Canellaceae	*Warbugia stuhlmannii* Engl.(JN044)	Stem bark	$CHCL_3$/MeOH Water	6.6 7.8
Combretaceae	*Terminalia spinosa* North. (JN 052)	Stem bark	$CHCL_3$/MeOH Water	3.6 4.8
Cucurbitaceae	*Momordica foetida*	Leaves	$CHCL_3$/MeOH	3.6

	Schumach. (JN060)		Water	4.8
Euphorbiaceae	*Ricinus communis* L. (JN033)	Leaves	$CHCL_3$/MeOH Water	6.1 16.66
Euphorbiaceae	*Ricinus communis* L. (JN033)	Roots	$CHCL_3$/MeOH Water	1.3 2.4
Euphorbiaceae	*Suregeda zanzibariensis* Baill. (JN045)	Root bark	$CHCL_3$/MeOH Water	13.4 16.2
Fabaceae	*Tamarindus indica* L.(JN038)	Stem bark	$CHCL_3$/MeOH Water	3.32 3.48
Lamiaceae	*Hoslundia opposita* Vahl (JN025)	Roots	$CHCL_3$/MeOH Water	2.12 1.06
Lamiaceae	*Ocimum balansae* Briq. L.(JN029)	Leaves	$CHCL_3$/MeOH Water	10.82 3.58
Lamiaceae	*Ocimum balansae* Briq. L.(JN029)	Roots	$CHCL_3$/MeOH Water	0.76 4.80
Lamiaceae	*Ocimum suave* Willd. (JN030)	Leaves	$CHCL_3$/MeOH Water	4.36 7.58
Lamiaceae	*Ocimum suave* Willd. (JN030)	Stem bark	$CHCL_3$/MeOH Water	3.28 3.75
Lamiaceae	*Plectranthus barbatus* Andr. (JN032)	Leaves	$CHCL_3$/MeOH Water	7.46 16.6
Lamiaceae	*Plectranthus barbatus* Andr. (JN032)	Stem bark	$CHCL_3$/MeOH Water	8 10
Lamiaceae	*Plectranthus barbatus* Andrews (JN032)	Roots	$CHCL_3$/MeOH Water	6.4 8.8
Lamiaceae	*Ocimum gratissimum L.*(JN058)	Leaves	$CHCL_3$/MeOH	5.6

			Water	6.8
Poaceae	*Rottboelia* Dumort (JN034)	Leaves	$CHCL_3$/MeOH Water	4.2 8.01
Polygalaceae	*Securidaca longifolia* Poepp. (JN035)	Leaves	$CHCL_3$/MeOH Water	22.4 3.95
Polygalaceae	*Securidaca longifolia* Poepp. (JN035)	Roots	$CHCL_3$/MeOH Water	22 21.24
Rubiaceae	*Pentanisia ouranogyne* S.Moore (JN031)	Roots	$CHCL_3$/MeOH Water	12.24 4.56
Rubiaceae	*Pentas bussei* K.Krause (JN048)	Root bark	$CHCL_3$/MeOH Water	8.8 9.6
Rubiaceae	*Pentas longiflora* Oliv. (JN 056)	Root bark	$CHCL_3$/MeOH Water	6.2 9.6
Rutaceae	*Teclea simplicifolia* (Engl.) L. Verd. (JN039)	Leaves	$CHCL_3$/MeOH Water	10.96 6.06
Rutaceae	*Teclea simplicifolia* (Engl.) L. Verd. (JN039)	Roots	$CHCL_3$/MeOH Water	8.08 4.62
Rutaceae	*Zanthoxylum chalybeum* Engl.(JN040)	Leaves	$CHCL_3$/MeOH Water	6.48 16.02
Rutaceae	*Zanthoxylum chalybeum* Engl.(JN040)	Stem bark	$CHCL_3$/MeOH Water	13.6 3.14
Rutaceae	*Zanthoxylum chalybeum* Engl.(JN040)	Root bark	$CHCL_3$/MeOH Water	12.64 6.38
Rutaceae	*Toddalia asiatica* (L.) Lam. (JN 055)	Root bark	$CHCL_3$/MeOH Water	9.2 3.4
Solanaceae	*Solanum incanum* L.(JN037)	Leaves	$CHCL_3$/MeOH	5.26

			Water	10.86
Solanaceae	*Solanum incanum* L.(JN037)	Roots	$CHCL_3$/MeOH Water	1.96 2.32
Verbenaceae	*Lantana camara* L.(JN026)	Leaves	$CHCL_3$/MeOH Water	9.28 19.72

The LC_{50} values of the brine shrimp obtained for extracts of these medicinal plants and that of the positive and negative controls, have been presented in Tables 2 and 3.

Table 2. Toxicity of organic ($CHCL_3$/MeOH, 1:1) crude plant extracts against brine shrimp *Artemia salina*

		Percent deaths at 24 hours					
Plant species	**Plant part**	**10 µg/ml**	**100 µg/ml**	**1000 µg/ml**	**LC_{50} value (µg/ml)*[a]** **(Organic)*[b]**	**Limits 95 % Confidence (µg/ml)**	**Slope**
Heeria insignis (Delile) Kuntze	Stem bark	**10**	**26**	**74**	**283**	**75-3275**	**0.5058**
Hoslundia opposita Vahl	Roots	**12**	**38**	**90**	**123**	**36-452**	**0.3695**
Lantana camara L.	Leaves	**8**	**68**	**100**	**56**	**20-152**	**0.3845**
Landolphia buchananii (Hallier f.) Stapf	Leaves	**20**	**36**	**92**	**101**	**25-397**	**0.3891**
Launea cornuta (Hochst.ex Oliv.& Hiern) C. Jeffrey	Leaves	**26**	**34**	**100**	**74**	**16-258**	**0.3910**
Launea cornuta (Hochst.ex Oliv.& Hiern) C. Jeffrey	Roots	**12**	**34**	**84**	**161**	**44-793**	**0.4162**
Momordica foetida Schumach.	Leaves	**54**	**86**	**100**	**8**	**0-30**	**0.7793**
Ocimum balansae Briq. L.	Leaves	**16**	**26**	**92**	**140**	**41-537**	**0.3600**

Ocimum balansae Briq. L.	Roots	**14**	**40**	**94**	**101**	**30-326**	**0.3423**
Ocimum gratissimum L.	Leaves	**26**	**34**	**100**	**74**	**16-258**	**0.3910**
Ocimum suave Willd.	Leaves	**14**	**36**	**100**	**99**	**33-284**	**0.3254**
Ocimum suave Willd.	Stem bark	**22**	**28**	**64**	**382**	**ND**	**1.0661**
Pentanisia ouranogyne S.Moore	Roots	**20**	**44**	**80**	**118**	**17-1000**	**0.5555**
Pentas bussei K.Krause	Root bark	**28**	**44**	**94**	**63**	**10-249**	**0.4451**
Pentas longiflora Oliv.	Root bark	**8**	**68**	**98**	**58**	**20-161**	**0.3742**
Plectranthus barbatus Andr.	Leaves	**14**	**36**	**94**	**110**	**33-358**	**0.3385**
Plectranthus barbatus Andr.	Stem bark	**6**	**60**	**96**	**77**	**27-219**	**0.3520**
Plectranthus barbatus Andr.	Roots	**18**	**36**	**98**	**88**	**25-276**	**0.3456**
Rauwolfia conthen.	Root bark	**36**	**62**	**94**	**31**	**1-118**	**0.5411**
Ricinus communis L.	Leaves	**10**	**28**	**88**	**171**	**52-671**	**0.3807**
	Roots	**6**	**54**	**86**	**114**	**35-394**	**0.3492**
Rottboelia Dumort	Leaves	**14**	**34**	**74**	**217**	**48-3373**	**0.5540**
Securidaca longifolia Poepp.	Leaves	**12**	**26**	**74**	**275**	**69-4067**	**0.5284**
Securidaca longifolia Poepp.	Roots	**14**	**36**	**90**	**123**	**34-472**	**0.3759**
Senecio syringifolius O. Hoffm.	Leaves	**14**	**70**	**100**	**141**	**42-527**	**0.3686**
Solanum incanum L.	Leaves	**36**	**62**	**94**	**31**	**1-118**	**0.5411**
	Roots	**24**	**38**	**90**	**91**	**17-433**	**0.4531**
Suregeda zanzibarensis Baill.	Root bark	**14**	**42**	**100**	**83**	**26-234**	**0.3212**
Tamarindus indica L.	Stem bark	**16**	**26**	**65**	**398**	**ND**	**0.7849**
Teclea simplicifolia (Engl.)	Leaves	**20**	**92**	**100**	**25**	**8-65**	**0.4270**

L. Verd.							
Teclea simplicifolia (Engl.) L. Verd.	Roots	**20**	**46**	**98**	**68**	**18-209**	**0.3516**
Terminalia spinosa North.	Stem bark	**36**	**62**	**94**	**31**	**1-118**	**0.5411**
Toddalia asiatica (L.) Lam.	Root bark	**30**	**34**	**88**	**91**	**10-667**	**0.5729**
Tridax procumbens L.	Whole plant	**30**	**36**	**94**	**72**	**7-327**	**0.4872**
Uvaria scheffleri Diels	Leaves	**26**	**34**	**100**	**74**	**16-258**	**0.3910**
Vernonia amygdalina A.Chev.	Leaves	**20**	**40**	**80**	**131**	**21-1233**	**0.5557**
Warbugia stuhlmannii Engl.	Stem bark	**54**	**86**	**100**	**8**	**0-30**	**0.7793**
Zanthoxylum chalybeum Engl.	Leaves	**20**	**50**	**98**	**62**	**16-185**	**0.3508**
Zanthoxylum chalybeum Engl.	Stem bark	**32**	**90**	**100**	**19**	**3-52**	**0.5212**
Zanthoxylum chalybeum Engl.	Root bark	**44**	**100**	**100**	**11**	**0-28**	**0.6782**
[b]Cyclophosphamide		**20**	**52**	**80**	**95**	**12-672**	**0.5554**
[b]Etoposide		**60**	**90**	**100**	**6**	**0-22**	**0.9269**

[*a]$CHCL_3$: MeOH (1:1)

[*b]Cytotoxic drugs

ND: Not detectable

Negative control, DMSO (LC_{50} value > 1000 µg/ml)

Table 3. Toxicity of aqueous crude plant extracts against *Artemia salina*

		Percent deaths at 24 hours					
Plant species	**Plant part**	**10 µg/ml**	**100 µg/ml**	**1000 µg/ml**	**LC_{50} value (µg/ml)[a]**	**Limits 95 % Confidence (µg/ml)**	**Slope**
Heeria insignis (Delile) Kuntze	Stem bark	10	20	70	383	ND	0.5610
Hoslundia opposita Vahl.	Roots	24	34	50	>1000	ND	2.6410
Lantana camara L.	Leaves	4	24	58	594	ND	0.6269
Landolphia buchananii (Hallier f.) Stapf	Leaves	8	24	80	249	76-1360	0.4179
Launea cornuta (Hochst.ex Oliv.& Hiern) C. Jeffrey	Leaves	22	24	56	842	ND	1.5520
Launea cornuta (Hochst.ex Oliv.& Hiern) C. Jeffrey	Roots	24	60	100	44	10-126	0.3588
Momordica foetida Schumach.	Leaves	18	32	98	96	28-316	0.3508
Ocimum balansae Briq. L.	Leaves	26	36	96	76	15-294	0.4201
Ocimum balansae Briq. L.	Roots	2	28	100	152	59-382	0.4255
Ocimum gratissimum L.	Leaves	22	46	50	572	ND	2.3098
Ocimum suave Willd.	Leaves	28	74	94	31	4-105	0.4692
Ocimum suave Willd.	Stem bark	22	28	62	437	ND	1.1650
Pentanisia ouranogyne S.moore	Roots	8	12	62	664	ND	0.6567
Pentas bussei K.Krause	Root bark	8	20	76	311	91-2561	0.4778
Pentas longiflora Oliv.	Root bark	10	18	52	>1000	ND	0.9649
Plectranthus barbatus Andr.	Leaves	22	30	64	356	ND	1.0686
Plectranthus barbatus Andr.	Stem bark	8	22	40	>1000	ND	1.4205

Plectranthus barbatus Andr.	Roots	**16**	**22**	**88**	**173**	**49-862**	**0.4003**
Rauwolfia conthen.	Root bark	**32**	**40**	**44**	**>1000**	**ND**	**12.6112**
Ricinus communis L.	Leaves	**18**	**26**	**50**	**>1000**	**ND**	**1.6704**
	Roots	**24**	**30**	**52**	**>1000**	**ND**	**2.2833**
Rottboelia Dumort	Leaves	**10**	**24**	**54**	**796**	**ND**	**0.8962**
Securidaca longifolia Poepp.	Leaves	**10**	**24**	**72**	**321**	**84-5240**	**0.5321**
Securidaca longifolia Poepp.	Roots	**8**	**18**	**42**	**>1000**	**ND**	**1.3047**
Senecio syringifolius O. Hoffman.	Leaves	**20**	**28**	**80**	**181**	**36-2410**	**0.5544**
Solanum incanum L.	Leaves	**12**	**14**	**82**	**273**	**85-1854**	**0.4258**
	Roots	**4**	**24**	**62**	**499**	**ND**	**0.5767**
Suregeda zanzibarensis Baill.	Root bark	**8**	**22**	**50**	**>1000**	**ND**	**0.9445**
Tamarindus indica L.	Stem bark	**16**	**78**	**94**	**42**	**10-126**	**0.3873**
Teclea simplicifolia (Engl.) L. Verd.	Leaves	**16**	**36**	**84**	**140**	**33-790**	**0.4483**
Teclea simplicifolia (Engl.) L. Verd.	Roots	**10**	**28**	**70**	**315**	**79-6706**	**0.5541**
Terminalia spinosa Nothr.	Stem bark	**18**	**22**	**40**	**>1000**	**ND**	**3.2053**
Toddalia asiatica (L.) Lam.	Root bark	**6**	**10**	**38**	**>1000**	**ND**	**1.3023**
Tridax procumbens L.	Whole plant	**14**	**30**	**78**	**208**	**50-1984**	**0.4996**
Uvaria scheffleri Diels	Leaves	**12**	**26**	**42**	**>1000**	**ND**	**1.7097**
Vernonia amygdalina A. Chev.	Leaves	**22**	**28**	**58**	**596**	**ND**	**1.4108**
Warbugia stuhlmannii Engl.	Stem bark	**24**	**30**	**52**	**>1000**	**ND**	**2.2833**

Zanthoxylum chalybeum Engl.	Leaves	**28**	**74**	**94**	**31**	**4-105**	**0.4692**
Zanthoxylum chalybeum Engl.	Stem bark	**14**	**20**	**76**	**288**	**74-4538**	**0.5238**
Zanthoxylum chalybeum Engl.	Root bark	**16**	**60**	**98**	**56**	**17-157**	**0.3381**
[b]Cyclophosphamide		**20**	**52**	**80**	**95**	**12-672**	**0.5554**
[b]Etoposide		**60**	**90**	**100**	**6**	**0-22**	**0.9269**

>1000 (non toxic); ND: Not detectable; *[a]Acqueous extracts; *[b]Cytotoxic drugs
Negative control, distilled water (LC_{50} >1000 µg/ml)

Table 4 compares the LC_{50} values of crude plant extracts to those of positive and negative controls.

Table 4. Comparative lethality of crude plant extracts against *Artemia salina*

Family	**Plant species/ Voucher specimen number**	**Plant part**	**Solvent**	**%Yield (w/w)**	**LC_{50}** (µg/ml)*[b] **Organic*[a]**	**LC_{50}** (µg/ml)*[b] **Aqueous**
Anacardiaceae	*Heeria insignis* (Delile) Kuntze (JN024)	Stem	$CHCL_3$/MeOH Water	4.78 10.4	**283**	**383**
Annonaceae	*Uvaria scheffleri* Diels (JN041)	Leaves	$CHCL_3$/MeOH Water	4.4 5.6	**74**	**>1000**
Apocynaceae	*Landolphia buchananii* (Hallier f.) Stapf (JN027)	Leaves	$CHCL_3$/MeOH Water	5.4 7.8	**101**	**249**
Apocynaceae	*Rauwolfia conthen.* (JN 051)	Root bark	$CHCL_3$/MeOH Water	8.8 11.4	**31**	**>1000**
Asteraceae	*Vernonia amygdalina* A. Chev. (JN057)	Leaves	$CHCL_3$/MeOH Water	5.6 6.8	**131**	**596**
Asteraceae	*Launea cornuta* (Hochst.ex	Stem bark	$CHCL_3$/MeOH Water	3.32 3.48	**398**	**42**

	Oliv.& Hiern) C. Jeffrey(JN028)					
Asteraceae	*Launea cornuta* (Hochst.ex Oliv.& Hiern) C. Jeffrey(JN028)	Stem bark	$CHCL_3$/MeOH Water	6.6 7.8	**8**	**>1000**
Asteraceae	*Senecio syringifolius* O. Hoffm.(JN036)	Stem bark	$CHCL_3$/MeOH Water	3.6 4.8	**31**	**>1000**
Asteraceae	*Tridax procumbens L.* (JN 054)	Leaves	$CHCL_3$/MeOH Water	5.6 8.12	**74**	**842**
Canellaceae	*Warbugia stuhlmannii* Engl.(JN044)	Roots	$CHCL_3$/MeOH Water	6.72 4.84	**161**	**44**
Combretaceae	*Terminalia spinosa* North. (JN 052)	Leaves	$CHCL_3$/MeOH Water	2.08 2.66	**141**	**181**
Cucurbitaceae	*Momordica foetida* Schumach. (JN060)	Whole plant	$CHCL_3$/MeOH Water	5.4 6.6	**72**	**208**
Euphorbiaceae	*Ricinus communis* L. (JN033)	Leaves	$CHCL_3$/MeOH Water	3.6 4.8	**8**	**96**
Euphorbiaceae	*Ricinus communis* L. (JN033)	Leaves	$CHCL_3$/MeOH Water	6.1 16.66	**171**	**>1000**
Euphorbiaceae	*Suregeda zanzibariensis* Baill. (JN045)	Roots	$CHCL_3$/MeOH Water	1.3 2.4	**114**	**>1000**
Fabaceae	*Tamarindus indica* L.(JN038)	Root bark	$CHCL_3$/MeOH Water	13.4 16.2	**83**	**>1000**
Lamiaceae	*Hoslundia opposita* Vahl (JN025)	Leaves	$CHCL_3$/MeOH Water	4.2 8.01	**217**	**796**
Lamiaceae	*Ocimum balansae* Briq. L.(JN029)	Roots	$CHCL_3$/MeOH Water	2.12 1.06	**123**	**>1000**

Lamiaceae	*Ocimum balansae* Briq. L.(JN029)	Leaves	$CHCL_3$/MeOH Water	10.82 3.58	**140**	**76**
Lamiaceae	*Ocimum suave* Willd. (JN030)	Roots	$CHCL_3$/MeOH Water	0.76 4.80	**101**	**152**
Lamiaceae	*Ocimum suave* Willd. (JN030)	Leaves	$CHCL_3$/MeOH Water	4.36 7.58	**99**	**31**
Lamiaceae	*Plectranthus barbatus* Andr. (JN032)	Stem bark	$CHCL_3$/MeOH Water	3.28 3.75	**382**	**437**
Lamiaceae	*Plectranthus barbatus* Andr. (JN032)	Leaves	$CHCL_3$/MeOH Water	7.46 16.6	**110**	**356**
Lamiaceae	*Plectranthus barbatus* Andrews (JN032)	Stem bark	$CHCL_3$/MeOH Water	6 10	**77**	**>1000**
Lamiaceae	*Ocimum gratissimum L.*(JN058)	Roots	$CHCL_3$/MeOH Water	8 12	**88**	**173**
Poaceae	*Rottboelia* Dumort (JN034)	Leaves	$CHCL_3$/MeOH Water	5.6 6.8	**74**	**572**
Polygalaceae	*Securidaca longifolia* Poepp. (JN035)	Leaves	$CHCL_3$/MeOH Water	22.4 3.95	**275**	**321**
Polygalaceae	*Securidaca longifolia* Poepp. (JN035)	Roots	$CHCL_3$/MeOH Water	22 21.24	**123**	**>1000**
Rubiaceae	*Pentanisia ouranogyne* S.Moore (JN031)	Roots	$CHCL_3$/MeOH Water	12.24 4.56	**118**	**664**
Rubiaceae	*Pentas bussei* K.Krause (JN048)	Root bark	$CHCL_3$/MeOH Water	8.8 9.6	**63**	**311**
Rubiaceae	*Pentas longiflora* Oliv. (JN 056)	Root bark	$CHCL_3$/MeOH Water	6.2 9.6	**58**	**>1000**
Rutaceae	*Teclea simplicifolia* (Engl.) L. Verd.	Leaves	$CHCL_3$/MeOH Water	10.96 6.06	**25**	**315**

	(JN039)					
Rutaceae	*Teclea simplicifolia* (Engl.) L. Verd. (JN039)	Roots	$CHCL_3$/MeOH Water	8.08 4.62	**68**	**>1000**
Rutaceae	*Zanthoxylum chalybeum* Engl.(JN040)	Leaves	$CHCL_3$/MeOH Water	6.48 16.02	**62**	**31**
Rutaceae	*Zanthoxylum chalybeum* Engl.(JN040)	Stem bark	$CHCL_3$/MeOH Water	13.6 3.14	**19**	**288**
Rutaceae	*Zanthoxylum chalybeum* Engl.(JN040)	Root bark	$CHCL_3$/MeOH Water	12.64 6.38	**11**	**56**
Rutaceae	*Toddalia asiatica* (L.) Lam. (JN 055)	Root bark	$CHCL_3$/MeOH Water	9.2 3.4	**91**	**>1000**
Solanaceae	*Solanum incanum* L.(JN037)	Leaves	$CHCL_3$/MeOH Water	5.26 10.86	**31**	**273**
Solanaceae	*Solanum incanum* L.(JN037)	Roots	$CHCL_3$/MeOH Water	1.96 2.32	**91**	**499**
Verbenaceae	*Lantana camara* L.(JN026)	Leaves	$CHCL_3$/MeOH Water	9.28 19.72	**56**	**594**

[*a] $CHCL_3$: MeOH (1:1); [b] Cytotoxic drug, Cyclophospamide (LC_{50} =95µg/ml); *[b] Cytotoxic drug, Etoposide (LC_{50} =6µg/ml); W/w, weight by weight
Negative control, distilled water (LC_{50} >1000 µg/ml);

Negative control, DMSO (LC_{50} >1000 µg/ml);

A total of 40 organic crude extracts were screened for lethality against *A.salina*, out of which 23 extracts (57.5%) exhibited strong toxicity against *A.salina* (LC_{50}<100 µg/ml), while 17 extracts (42.5%) demonstrated moderate cytotoxicity against *A. salina* [(LC_{50} value ranged between 100-500 µg/ml) (Fig. 1)].

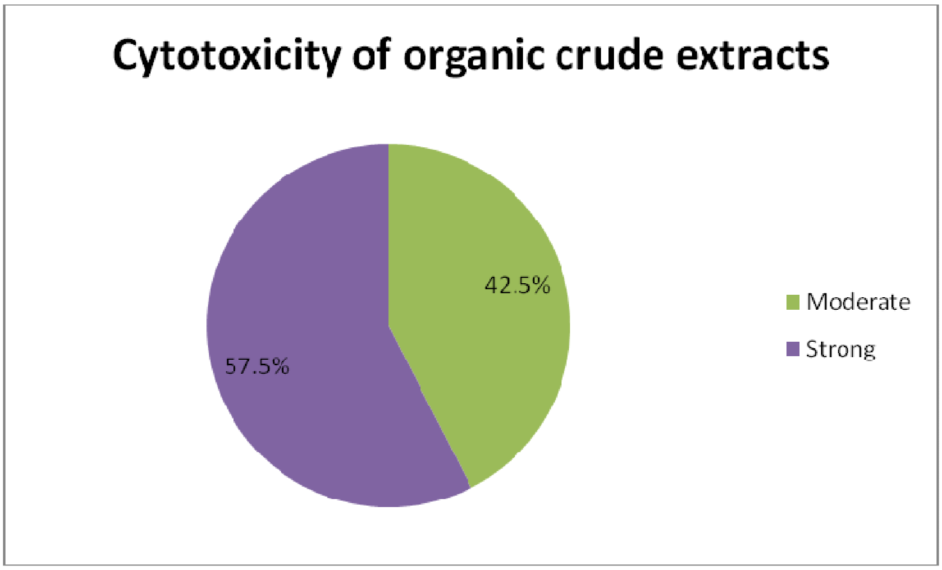

Figure 1. Lethality of organic ($CHCL_3$/MeOH, 1:1) crude extracts to *Artemia salina*

The results obtained from screening 40 aqueous crude extracts from 30 different plant species against *A. salina* larvae are shown in Table 3. Approximately 17.5% (7) of the aqueous extracts demonstrated activity at or below 100 µg/ml and were considered to have strong cytotoxic activity, 37.5% (15) of the screened crude extracts had LC_{50} values between 100 µg/ml and 500 µg/ml and were considered to be moderately toxic, 15% (6) of the crude extracts had LC_{50} values between 500 µg/ml and 1000 µg/ml and were considered to have weak cytotoxic activity while 30% (12) of the aqueous extracts had LC_{50} values greater than 1000 µg/ml and were categorized as non toxic (Fig. 2).

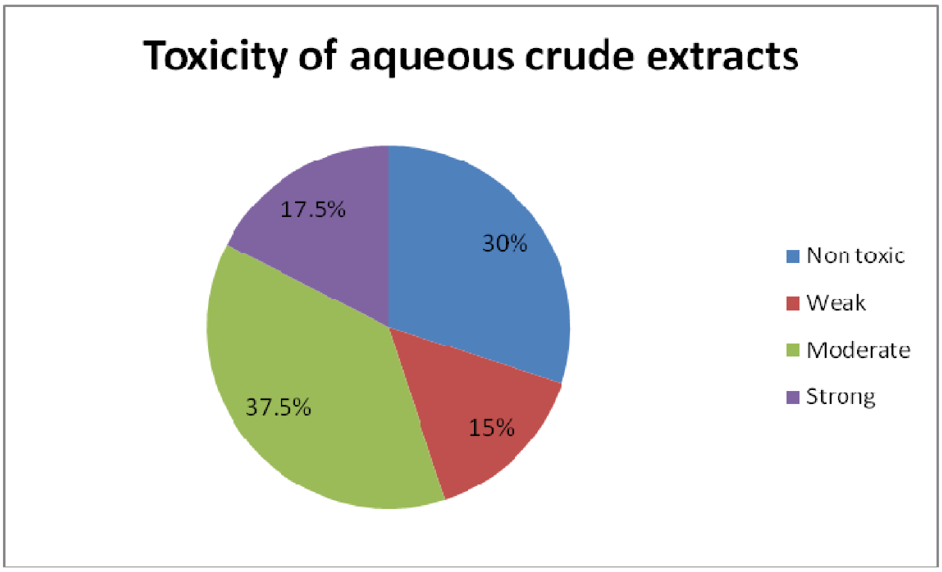

Figure 2. Cytotoxicity of aqueous crude extracts to *Artemia salina*

Discussion

Brine shrimp lethality is a simple bioassay useful for screening large number of extracts for safety in the drug discovery process from the Kenyan medicinal plants. The procedure of Meyer et al (15), was adopted to determine the lethality of crude plant extracts traditionally used as antimalarial remedies in Msambweni district, Kenya and the positive controls, cyclophosphamide and etoposide to brine shrimp (*Artemia salina*). The method allows the use of smaller quantity of the test substances and permits larger number of samples and dilutions within a shorter time than using the original test vials (27). The assay is based on the premise that bioactive compounds are often toxic in high doses and that *in vivo* lethality in a simple organism can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products (7). Literature data suggest a good correlation between the activity in the brine shrimp assay and the cytotoxicity against some tumor cell lines (28), hepatotoxic activity (29) as well as other pharmacological activities (16). Increase in mortality was observed to be proportional to increase in concentration, which provided linearity in the dose-effect relationship of every extract and determination of the LC_{50} value. Maximum mortalities took place at a concentration of 1000 µg/ml whereas least mortalities were at 10 µg/ml. The positive controls, cyclophosphamide and etoposide exhibited strong activity against *A.salina,* with LC_{50} values of 95 and 6 µg/ml respectively (Table 2). Cyclophosphamide, a standard antitumour drug has also been used in other cytotoxicity studies as a positive control (30).

The most toxic extracts were the organic extracts from the leaves of *Momordica foetida* Schumach. (Cucurbitaceae) and stem bark of *Warbugia stuhlmannii* Engl. (Canallaceae), which has been used in traditional medicine for the treatment of antitumour and anti-inflammatory diseases (31) and the lethality (LC_{50}) value, was 8 µg/ml (Table 2). The activity results of *Warbugia stuhlmannii* were found to be consistent with existing phytochemical knowledge of this plant as a source of cytotoxic and antitumour compounds (32). In addition the organic extracts from the leaves of *Momordica foetida* Schumach. (Cucurbitaceae) and the root bark of *Zanthoxylum chalybeum* (Eng) Engl. (Rutaceae) showed lethality to brine shrimp comparable to that of the positive control, etoposide. The LC_{50} values were found to be lower than 100 µg/ml. It is notable that, *Zanthoxylum chalybeum,* which has exhibited strong cytotoxicity has also shown strong antiplasmodial activity (IC_{50} value of 3.65 µg/ml) in other studies (33), suggesting a strong correlation between its cytotoxic and antiplasmodial activity. These data seem to correlate well with antitumor activity. Indeed, the toxicity data would suggest that these plants could not make safe malaria treatments.

It is notable that the aqueous extracts, which in most cases are the ones used by traditional healers were slightly less toxic on brine shrimps. The cytotoxic activity was considered weak when the LC_{50} was between 500 and 1000 µg/ml, moderate when the LC_{50} was between 100 and 500 µg/ml, as strong when the LC_{50} ranged from 0 to 100 µg/ml (34) and designated as non toxic when the LC_{50} >1000 µg/ml (15). On that basis, the most toxic aqueous extracts (LC_{50}<100 µg/ml) were the root bark of *Zanthoxylum chalybeum* (Eng) Engl. (Rutaceae); leaves of *Zanthoxylum chalybeum* (Eng) Engl. (Rutaceae); leaves of *Ocimum suave* Willd (Labiatae); leaves of *Ocimum bacilicum* L. (Labiatae); leaves of *Momordica foetida* Schumach. (Cucurbitaceae); roots of *Launea cornuta* (Oliv and Hiern) C. Jeffrey (Compositae) and the stem bark of *Tamarindus indica* L. (Caesalpiniaceae). It is interesting to note that both the aqueous and organic extracts from *Zanthoxylum chalybeum* exhibited strong cytotoxic activity. The generated data suggest that these plants are not safe for use as antimalarial remedies. The observed effect calls for further bioactivity guided fractionation to isolate the cytotoxic compounds.

The current study evaluated the cytotoxicity of crude plant extracts and antitumour drugs as positive controls against *A. salina*. The standard *A.salina* bioassay is a useful screen for the toxicity based detection of plant extracts and could replace the more ethically challenged mouse bioassay for this purpose. It is also a useful screen for bioactive compounds in natural products (35, 36). *Artemia* can be maintained indefinitely in the laboratory in their cyst form, and are easily induced to hatch. As such *Artemia* provides a constantly available bioassay species to screen for phytotoxins and evaluation of cytotoxic status of antitumor drugs. Furthermore, the *A.salina* bioassay is more sensitive than the mouse bioassay and the unit costs much lower compared to *in vitro* protein synthesis assays. Finally, while the *A. salina* bioassay provides a simple method for toxicity assessment of crude plant extracts, this should continue to be complemented by appropriate phytochemical analytical methods (37). From the cytotoxicity screening, we have identified numerous extracts of Kenyan medicinal plants used for malaria treatment with strong cytotoxic activity against brine shrimp. The fact that twenty three (23) organic crude extracts (57.5%) and seven (7) aqueous crude extracts (17.5%) out of the 80 crude extracts screened for toxicity against brine shrimp had LC_{50} values less than 100 μg/ml is interesting and correlates with antitumour activity, suggesting a need for further *in vivo* toxicological studies and isolation of cytotoxic compounds. Based on the possible relationship between brine shrimp lethality and plant bioactivity, this work could serve for further pharmacological and phytochemical research.

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References

1. Krishnaraju AV, Rao TVN, Sundaraju D, Vanisree M, Tsay H-S, Subbaraju G. Biological screening of medicinal plants collected from Eastern Ghats of India using Artemia salina (brine shrimp test). Int J Appl Sci Eng 2006; *4*: 115-125.
2. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kiama SG. Antimalarial herbal remedies of Msambweni, Kenya. J Ethnopharmacol 2010; 128: 424-432
3. Phillipson J.D. Natural products as drugs. Trans R Soc Trop Med Hyg 1994; 88: 17-19.
4. Geoffrey C, Kirby GC. Medicinal plant and the control of protozoa disease with particular reference to malaria. Trans R Soc TropMed Hyg 1996; 90: 605-609.
5. Farnsworth NR, Soejarto D D. Global importance of medicinal plants. In: Akerele, O., Heywood, V., Synge, H. (Eds.) "*The Conservation of Medicinal Plants*", Cambridge University Press, Cambridge 1991; 25-51.

6. Arora S, Kaur K, Kaur S. Indian medicinal plants as a reservoir of protective phytochemicals. Teratogensis Carcinogenesis Mutagensis 2003; 1: 295-300.

7. McLaughlin J L, Chang CJ, Smith D L. Bench-top bioassays for the discovery of bioactive natural products: an update. In: Rhaman, A. U. (Ed.), "*Studies in Natural Products* 1991.

8. Sleet R B, Brendel K. Improved methods for harvesting and counting synchronous populations of *Artemia nauplii* for use in developmental toxicology. Ecotoxicol Environ Saf 1983; 7: 435-446.

9. Harwing J, Scott P. Brine shrimp (*Artemia nauplii* L.) larvae as a screening system for fungal toxins. Appl Microbiol 1971; 21: 1011-1016.

10. Pelka M, Danzl C, Distler W, Petschelt A. A new screening test of dental materials. J Dent.2000.

11. Siqueira M J, Bomm D M, Pereira N FG, Gareez WS, Boaventura M A D. Estudo fitoquimico de Unonopsis lindmanii- Annonaceae, biomonitorado peloensaio de toxicidade sobre *Artemia salina* LEACH. *Quim Nova* 1998; 21: 557-559.28: 341-345.

12. Perez H, Diaz F, Medina J. D. Chemical investigation and *in vitro* antimalarial activity of *Tabebuia ochracea* ssp. neochrysantha. Int J Pharm 1997; 35: 227-231.

13. Oberlies NH, Rogers L L, Martin J M, McLaughlin J L.1998. Cytotoxic and insecticidal constituents of the unripe fruit of *Persea Americana.* J Nat Prod 1998; 61: 781-785.

14. Labbe C, Castillo M, Connoly JD. Mono and sesquiterpenoids from *Satureja gilliesii.* Phytochemistry 1993; 34: 441-444.

15. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Mc Laughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 1982; 45: 31–4.

16. Solis PN, Wright CW, Anderson MM, Gupta MP Phillipson JD. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med 1993; 59(3): 250–252

17. Van Stappen G. Zoogeography. In: Abatzopoulos, T.J., Beardmore, J.A., Clegg, J.S., Sorgeloos, P., editors. *Artemia*: Basic and Applied Biology. Dordrecht: Kluwer Academic Publishers. Pp 171-224. 2002.

18. Bowen ST, Fogarino EA, Hitchner KN, Dana GL, Chow VHS, Buoncristiani MR, Carl .R. Ecological isolation in *Artemia*: Population differences in tolerance of anion concentrations. J Crustacean Biol 1985; 5: 106-129.

19. Treece G.D. *Artemia* production for marine larval fish culture. Southern Regional Aquaculture Center Publication No. 702. 2000.

20. Van stappen G. Introduction, biology and ecology of *Artemia*. In: Lavens, P., Sorgeloos, P., editors. Manual on the Production and use of live food for Aquaculture. FAO Fisheries Technical Paper No.361. Rome: FAO. Pp 79-106. 1996.

21. Gajardo G, Abatzopoulos TJ, Kappas I, Beardmore J.A. Evolution and speciation. In: Abatzopoulos, TJ, Beardmore JA, Clegg JS, Sorgeloos P, editors. *Artemia*: Basic and applied Biology. Dordrecht: Kluwer Academic Publishers.2002; Pp 225-250.

22. Sorgeloos P, Beardmore JA. Editorial note: Correct taxonomic identification of *Artemia* species. Aqua Res 1995; 26: 147.

23. Campbell DL, Lawton LA, Beattie KA, Codd GA. Comparative assessment of the specificity of the brine shrimp and Microtox assays to hepatotoxic (microcystin-LR containing) cyanobacteria. Environ Toxicol Water Qual 1994; 9: 71-77.*Chemistry*", Elsevier, 383-409.

24. Dorin D, Le Roch K, Sallicandro P, Alano P, Parzy D, Poullet P, Meijer L, Doerig C. Pfnek-1, a NIMA-related kinase from the human malaria parasite *Plasmodium falciparum*; biochemical properties and possible involvement in MAPK regulation. Eur J Biochem.2001; 268: 2600-2608.

25. Finney D.J. Probit analysis, 3rd ed., Cambridge. 1971.
26. Hafner E, Heiner E, Noack E. Arzneim-Forsch 1977; 27: 1871.
27. Sam T. W. Toxicity testing using the brine shrimp: *Artemia salina*. In: Colegate, S. M., Molyneux, R. J. (Eds.), "*Bioactive Natural Products Detection, Isolation, and Structural Determination*", CRC Press, Boca Raton, FL, 1993; 442-456.
28. Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. Phytochem Anal 1991; 2: 107–111.
29. Kiviranta J, Sivonen K, Niemelä SI, Huovinen K. Detection of toxicity of cyanobacteria by *Artemia salina* bioassay. Environ Toxicol Wat Qual 1991; 6: 423–436.
30. Mbwambo ZH, Moshi MJ, Masimba PJ, Kapingu MC, Nondo RSO. Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownie* roots and stem. BMC Complement Altern Med 2007; 7: 9 doi: 10.1186/1472-6882-7-9.
31. Beentje J. Kenya trees, shrubs and lianas. National Museums of Kenya. National Museums of Kenya, Nairobi. 1994.
32. Manguro LOA, Ugi I, Herman R, Lemmen P. Flavonal and drimane-type sesquiterpene glycosides of *Warbugia stuhlmannii* leaves. Phytochemistry 2003; 63: 497-502.
33. Rukunga GM, Gathirwa GW, Omar SA, Muregi SW, Muthaura CN, Kirira PG, Mungai GM, Kofi-Tsekpo W.M. Antiplasmodial activity of the extracts of some Kenyan medicinal plants. J Ethnopharmacol 2009; 121: 282-285
34. Padmaja R, Arun PC, Prashanth D, Deepak M, Amit A, Anjana M. Brine shrimp lethality bioassay of selected Indian medicinal plants. Fitoterapia 2002; 73: 508–510.
35. Colombo ML, Bugatti C, Mossa A, Pescalli N, Piazzoni L, Pezzoni G, Menta E, Spinelli S, Johnson F, Gupta R.C, Dasaradhi L. Cytotoxicity evaluation of natural coptisine and synthesis of coptisine from berberine. Farmaco 2001; 56: 403-409.
36. Favilla M, Macchia L, Gallo A, Altomare C. Toxicity assessment of metabolites of fungal biocontrol agents using two different (*Artemia salina and Daphnia magna*) invertebrate bioassays. Food Chem Toxicol 2006; 44: 1922-1931.

37. Eaglesham GK, Norris RL, Shaw GR, Smith MJ, Chiswell RK, Davis BC, Neville CR, Seawright AA, Moore MR. Use of HPLC-MS/MS to monitor cylindrospermopsin, a blue-green algal toxin for public health purposes. Environ Toxicol 1999; 14: 151-154.