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Original Article

Rapid Screening using GIBEX Screens-to-nature System of Ethnomedicinal Plants from Ngong Forest, Kenya for Potency against Infectious Diseases and Antioxidant Activities: A Qualitative Study

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ABSTRACT

Introduction: Plants from Kenyan flora are traditionally used to manage a number of ailments including; chronic and infectious disease, to bolster the body immunity and for general health protections. The current investigation was designed to validate the quality with respect to the pharmacological significance of 156 fresh plant materials resulting from 27 ethno-medicinal plants, from Ngong forest, Kenya. **Materials and Methods:** Pharmacological screening was carried out using the field deployable GIBEX Screens-To-Nature (STN) validated assays. The plant extracts were screened for antifungal; general protozoal lethality; round worm lethality and antioxidant potential. **Results:** Different plant parts exhibited a range of activities; related to their traditional uses; with eleven out of twenty-seven extracts exhibiting highest activities in only one out of four categories of assays studied. All plant parts of only one plant *C. axillaris* exhibited high activities in all (4/4) the categories of assays evaluated. The other plants that exhibited high activities in three out of four (3/4) categories of assays studied included; *A. oppositifolia, B. huillensis* and *T. trichocarpa*. **Conclusion:** The current investigation provided additional data in relation to the usefulness of the studied ethno-medicinal plants, mostly of the following plants; *C. axillaris*, *A. oppositifolia, B. huillensis* and *T. trichocarpa* in the management of diseases that are infectious and to bolster the immunity. The reported data will contribute towards authenticating the claimed traditional use of these plants. The extracts that exhibited high activities should be investigated further to determine their effective concentrations.

Key words: Antifungal, Antihelmintic, Antiprotozoal, Free radical Scavenger, Kenyan plant.

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INTRODUCTION

A large majority (80%) of populations in developing countries still depend solely on traditional medicine as the main resource for primary health care needs.[1-3] More than 50,000 species of flowering plants are used for medicinal purposes across the world.[4-6] In Kenya, from a flora of approximately 10,000 plants, more than 1200 members are described to have medicinal value yet more than this number are used for medicinal purposes.[7] The ethno-traditional uses of plants from Kenyan flora for the treatment of various diseases and conditions is documented in a number of books.[7-9]

One way of tackling the emergence of resistance to single drugs is the use of the total extracts containing a cocktail of compounds. A case in point is *Artemisia annua* L. whole plant extract which is 6-17 times more effective in inhibiting the growth of *P. falciparum* than artemisinin.[2] This is because the sesquiterepenes and flavonoids cocktail in the extract have synergistic interactions. African traditional health systems prescribe to a concoction of different plant extracts with synergistic interactions rather than a single plant extract.[10] The major drawback with these mixtures is the lack of precision, validation, standardization and their safety on human cells is unknown. The extracts/blends are made without any scientific investigation on their biological activities.[11-13]

The use of herbal medicine could be increased if their consistency and efficacy can be guaranteed; this can be done by ensuring in a simple way that the claimed activity is ascertained in every batch extract and that no cytotoxic constituents are present. Bioactivities are not normally assured because of variability in secondary metabolite composition and concentration for any particular medicinal plant extract depending on the stage of development, geographical location of collection, season of the year or even time of day.

In this study the rapid, affordable and easy to operate GIBEX STN bioassay systems was used to evaluate the pharmacological properties of the plant extracts to determine whether they align with their traditional medicinal uses, thus forming the basis of future validation on other traditional medicinal plants. This will transform traditional knowledge and practices to science by adding value to traditional medicine as a valid, cheap, quick and locally available means to treat infectious and chronic diseases.

MATERIALS AND METHODS

Plant Collection, Identification and Archiving

These plants were collected at random from Ngong forest (6 km from Nairobi city centre, Kenya) after obtaining consent for field sample collection from Kenya Forest Services, Ref No. RESER/1/KFS/VOL.11/27 on 26th December, 2016. Identification was done with the help of a Mr. Patrick Mutiso, a taxonomist from the School of Biological Sciences (SBS), University of Nairobi. The voucher specimens of the collected plants are deposited at the University of Nairobi Herbarium. An herbalist from the registered National Museums of Kenya (NMK) provided guid-

ance on the traditional use and cultural implication of use these plants as traditional medicine. For each plant two samples were collected, for extraction and positive taxonomic identification and retention as herbarium specimen.

Plant Extraction

Extraction of all plant materials was achieved after mincing 2 g of the plant material and then pulverizing in 4 ml ethanol in a small vial using Dremel® rotary tool for 10 min. The vial and contents were allowed to sit for approximately 5 min and then filtered through filter paper and kept at 4 °C for different bioassays.

Pharmacological Screening

Pharmacological screening of different plant parts was carried out by simple and rapid bioassays; GIBEX Screens-To-Nature (STN) system; deployable in field experiments to explore/investigate the pharmaceutical relevancy of natural plant extracts for human health protection.

The STN system involved the following activities; plant identification and collection, study of traditional, historical plant use and ethnobotany, vouchering and archiving, extraction tactics and screening plant samples using biologically relevant validated bioassays.

The resultant extracts were screened for the biological activities listed below:

General Antifungal Assay

The local strain of *Saccharomyces cerevisiae* (Common baker's yeast), obtained from the drug analysis and research unit at the University of Nairobi (UoN) was used as a source of fungi. Briefly, each well of a 24 well plate was filled with 200 ml of yeast solution (50 mg/ml) and 50 ml (approximately 0.4 mg of plant material in 1 ml of 60% ethanol) plant extract. This were mixed thoroughly and then incubated at 30 °C for 2 h, shaking gently after every 30 min. A volume of 20 ml of copper sulphate ($CUSO_4$) and 50 ml of 60% ethanol (EtOH) were used as the positive and negative controls, respectively. A 200 ml volume of yeast solution (50 mg/ml) and 20 ml 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were each added to the wells and shaken gently, before further incubation overnight at 20-30 °C. Live yeasts were detected by colour change of yellow MTT to purple, the wells containing yeasts with strong antifungal compounds will appear yellow in a few minutes but best results should be evident after 24-48 hrs.

General Protozoal Lethality Assay Test

The local strain of the protozoa *Bodo caudatus* spp obtained from the drug analysis and research unit at the UoN was used in this assay to evaluate the impact of the total extracts on anti-trypanosomal activites. *Bodo caudatus* is a nonparasitic, nonpathogenic member in the same order as the trypanosomes, kinetoplastida, therefore was used as a suitable model in the trypanosome lethality assay. Each well of a U-bottomed 96-well plate was filled with 100 ml of *B. caudatus* culture (Best used on the third to sixth day after culture preparation) prepared from cereal grass medium innoculated with *E. coli* and incubated in the dark at 25 °C. A volume of 10 ml of antibiotics was added to each well. Thereafter, 5 ml of $CuSO_4$ (160 mg/ml) as the positive control and 10 ml of 60% EtOH was added to the wells as the negative control. The plates were incubated at room temperature for 2 h followed by addition of 5 ml MTT to each well and further incubated overnight. The presence of a purple dot at the bottom of the well was indicative of live *B. caudatus,* a small dark purple dot partially live, very small purple dot for barely alive and yellow coloration with no purple dot at the bottom of the well for dead *B. caudatus* exhibited by extracts with the highest activities. The tests were carried out in triplicate

Round Worm Lethality Assay

A local strain of the free-living roundworm, *Pangrellus redivivus* obtained from the drug analysis and research unit at the UoN was used as the model to evaluate the lethality of the total extracts against roundworms. Each of the U-bottomed 96 well plate was filled with 100 ml of the worm suspension (7-10 days round worm culture consisting of water and oatmeal) followed by addition of 5 ml plant extract before incubating for 4 h. $CuSO_4$ solution (160 mg/ml) and EtOH (5 ml of 60%) were used as the positive and negative controls, respectively. The plate was then observed from beneath for dead worms, which collected at the bottom of the U-shaped wells, using a magnifying lens (15x). The live worms continued wriggling/swimming vigorously and were evenly distributed in the tube. Each experiment was each carried out in triplicate.

Antioxidant Activity

Into each well of 96-well plates, 200 ml of 2,2-azino-*bis* (3-ethylbenzothiazoline-6-sulfonic acid) ammonium salt (ABTS) and diluted potassium persulphate were added, followed by 10 ml of the test extract. The results were observed within 5-15 min. The negative and positive controls wells contained 10 ml of 60% ethanol and ascorbic acid, respectively. The extent of the antioxidant potencies of the plant extracts were indicated by the colour discharge in the wells from green for mild, light green for moderate to clear for high antioxidant activities. The test was also carried out in triplicate.

RESULTS AND DISCUSSION

Using STN rapid bioassay systems the antifungal, protozoal lethality and antioxidant potencies of the extracts were qualitatively categorized into inactive, mild, moderate activities and high activities depending on the observed colour changes in the wells of the 96-well plates used. However, the roundworm lethality was categorized into no activity to high activity depending on whether the worms were alive after 4 h or dead in less than 4 h. The current study, from these criteria of evaluation of bioactivities, showed that the leaf extracts of fourteen Kenyan plants including; *Albizia gummifera* C.A.Sm., *Brachylaena huillensis* O.Hoffm., *Chaetachme aristata* Planch., *Chionanthus battiscombei* (Hutch.) Stearn., *Crotalaria axillaris* W.T. Aiton., *Drypetes gerrardii* Hutch. *Elaeodendron buchananii* (Loes.) Loes., *Gnidia subcordata* Meisn., *Grewia similis* K.Schum., *Mystroxylon aethopicum* (Thunb.) Loes. *Maytenus undata* (Thunb.) Blakelock, *Tragia brevipes* Pax., *Turrea mombassana* C. DC. and *Vernonia holstii* O. Hoffm; the stem bark extracts of *Acokanthera oppositifolia* (Lam.) Codd and *E. buchananii* and the root bark extract of *G. subcordata* exhibited the highest antifungal activities, of the plants tested. From Table 1, most of these plants are used extensively for ethnomedicinal purposes to manage a number of ailments including microbial infections. Furthermore, most of these plants have proven antimicrobial, antifungal antioxidant, anticancer, antimutgenic and antiparasitic potencies, from several previous studies (Table 1). Therefore, the current study agrees with previous ones which showed that different extracts of these plants elaborates a number of activities most probably attributed to the secondary metabolites including oleanane-type triterpenoid, saponins, tannins, oleanane glycosides, macrocyclic spermine and budmunchiamine alkaloids from *A. gummifera*; essential oils, coumarins, sterols, triterpenes and tannins from *Brachylaena*; pyrrolizidine alkaloids from *C. friedelane*, hopane and lupane-type triterpenoids, steroids, bioflavonoids, diterpenoids, phenanthrenone derivative and a phenanthrenone heterodimers, flavonoids, xanthones and anthraquinones from *D. ferrardii*; steroidal glycosides, eudesmane type sesquiterpenoids, dammarane tritepenoids from *E. buchananii;* diterpenoid esters with characteristic macrocyclic ring from *G. subcordata*; saponins, catechol tannins, alkaloids, steroid glycosides, flavonoids, aglycones of anthrasenosides, triterpenes (Phy-

tosterols) from *M. aethiopicum*; oleanene triterpene acids, *M. undata*; tannins, saponins, terpenoids, flavonoids, phenols, alkaloids from *T. brevipes;* sesquiterpene lactones, glaucolides and cistifoliolides from *V. holstii* and flavonoids, proanthocyanidins, toxic cardiac glycosides from *A. oppositifolia* (Table 1).

The extracts of the roots and stem bark of the following plants showed high antiprotozoal potencies; *C. aristata*, *C. axillaris*, *Calodendrum capense, Hibiscus hilophilus*; the root bark of *B. huillensis*, *D. gerraidii*, *T. mombassana T. trichocarpa* and *Markhamia lutea*; the stem bark of *Erythrococca bongensis* and *A. oppositifolia* and the leaves of *T. trichocarpa*. It is interesting to note that the antiprotozoal activities were exhibited mainly by the root and stem barks and rarely by the leaves of these plants. Literature review on the uses of ethnomedicinal plants has shown that protozoal diseses are mostly managed with the root and stem barks of useful plants. The leaves are rarely used to for the management of ailments related to protozoal infections. This is consistent with results observed in this study which showed that the root and stem barks are the most active plant parts for the treatment of protozoal infections. These parts of the plant could be accumulating the secondary metabolites responsible for antiprotozoal activities.

Most of the plants that exhibited high antiprotozoal activities including the roots and stem bark *C. aristata*, *C. axillaris*, *C. capense, H. hilophilus*; the root bark of *B. huillensis* and *M. lutea* and the stem bark of *Erythrococca bongensis* and *A. oppositifolia* had no report on related ethnomedicinal uses. Only one plant, *D. gerraidii* was traditionally used to manage protozoal diseases such as malaria (Table 1). Furthermore, this plant exhibited good antiprotozoal activities in previous studies, thus validating its traditional uses and also the results obtained in the current study.

It is not clear why most of the plants exhibiting interesting antiprotozoal activities using the rapid STN assays are not used in African traditional medicine practices to manage these diseases. However, the most probable explanation could be attributed to the loss of activities due to the

Table 1: Ethno-medicinal information of plants used in this study.

Botanical Name/Family / Voucher Number	Plant part	Medicinal Use	Pharmacological use	Major classes of compounds
Acokanthera oppositifolia (Lam.) Codd (Apocynaceae) (LKO2016/12/01)	Roots	Used, with caution as it a toxic plant, against syphilis and for poisoning arrows[7] normal pain and snake-bite and against tapeworm and anthrax.[22-24]	Has antioxidant,[27] anti-cancer,[33] antibacterial and antimutagenic,[34] cardiac stimulant,[35-36] good Cyclooxygenase (COX) inhibitory activities.[37]	Flavonoids, proanthocyanidins, toxic cardiac glycosides.[23,35-36,38-39]
	Leaves/ Roots	Taken for the treatment of snake bite and headache,[7] anthrax and tapeworm,[22-24] pains of the abdominal, septicaemia and convulsions[25] and heart water disease.[22]		
	Root bark	Used for the management of menorrhagia and menstruation.[26-27]		
	Stem	Used as analgesic for toothache, in the treatment of intestinal worms, as antipyretic and for the treatment from congestive heart failure.[28-29]		
	Leaves or roots and wood	Used as anthelmintics, for treating syphilis, snakebite, stomach ache, spiderbite, blood poisoning and septic spots[30-32]		
Albizia gummifera C.A.Sm. (Leguminosae) (LKO2016/12/02)	Bark	Taken to treat malaria,[7-8,58] to hasten parturition, as a snuff to treat headache, applied externally to treat scabies.[59]	Has shown anti-microbial, anti-parasitic,[66-69] anti-trypanosomal,[70-72] anti-malarial,[73] anti-cancer and cytotoxic,[74] anti-bacterial,[75] anti-plasmodial,[76-78] brine shrimp toxicity, anti-helmintic activities.[79-81]	Oleanane-type triterpenoid saponins,[74] tannins,[75] sapogenin, oleanane glycoside, triterpenoids, saponins, budmunchiamine alkaloids and macrocyclic spermine.[82-85]
	Roots and leaves	Used as a purgative, for treatment of diarrhoea and eye troubles, drunk to relieve the pain caused by sprains, to treat skin diseases, stomach-ache, sleeping sickness,[58,60] and leprosy.[60]		
	Stem bark and leaves	Used to manage throat, skin cancer,[61] against diarrhoea, cough, nervous disease, syphilis,[62] for bacterial infection, skin disease, malaria and stomach pain.[63]		
	Roots	Used for treatment diseases of the skin,[7] scabies and malaria, stomach pains[7,60] and psychiatric problems.[64]		
	Stem bark	Used against scabies[65] and malaria.[7]		

	Whole plant	Used against tooth aches and gingivitis.[60,64]		
	Not specified	Used for treatment of malaria, bacterial infections, skin diseases and stomach pains.[7]		
Brachylaena huillensis O.Ho ffm (Compositae) (LKO2016/12/03)	Leaves	Anticandida.[60]	Has antifungal,[41] antimicrobial,[45,47] potential dietary adjunct or therapeutic for diabetes therapy.[86]	Essential oils, main components hydrocarbons,[46-47] coumarins, sterols, triterpenes and tannins.[48]
Calodendrum capense L.f. Thunb (Rutaceae) (LKO2016/12/04)	Roots	Treatment of hypertension.[86]	Has antimicrobial/ antiinfective,[94-95] antifungal,[96] insecticidal,[97] larvicidal,[98] insecticidal and antifeedant activities.[98]	Has oil rich in oleic acid (1) and linoleic acid (2), lupeol (3),[95] limonoides (calodendrolide (4), harrisonin (5), pedonin (6) and pyroangolensolide (7),99 limonin (8), limonin diosphenol (9),[98,100] a mixture of fatty acids,[95,101] 7-O-dimethylallyl demethylenedictamnine (10), confusameline (11) and 5-methoxypsolaren (12).[94]
	Bark	Used as ingredient for skin ointment in cosmetics.[86-87]		
	Seed oil	Used for making soap.[87-88]		
	Leaves	Used to kill insects.[87]		
	Fruits	Used to soften hair and fasten its growth, clean skin irritations, teeth and rashes,[89] used to make ankle rattles worn by dancers at celebrations and feasts.[90]		
	NS	Stomach upsets, emetic.[91]		
	Bark	Used for the treatment of fever,[92] externally used to lighten skin, as a moisturizer and to treat pimples,[93] used as an ingredient of skin ointments,[86] for the treatment of fever.[92]		
	Roots	Used for the treatment of hypertension.[86]		
Canthium keniense Bullock; synonynm Afrocanthium keniense (Bullock) Lantz (Rubiaceae) (LKO2016/12/05)	Leaves	Pounded, mixed with ghee and rubbed over a newborn as poultice for skin swellings.[7]		
Chaetachme aristata Planch (Ulmaceae) (LKO2016/12/06)	Roots	Toxic.[7]	Has mutagenic and/or toxic effects,[107] bacteriostatic,[103-104,108] anti-tuberculosis,[109] genotoxic and mutagenic effects,[110] antimycobacterial activity,[111] mutagenic potential.[112]	
	Bark	Used for the treatment of haemoroids.[102-104]		
	Leaves	Used for the treatment of tuberculosis, back wounds, spinal weakness.[105-106]		
Chionanthus battiscombei (Hutch.) Stearn (Oleaceae) (LKO2016/12/07)	Bark	Used for the treatment of malaria, back pain and as anthelmintic.[7]		
Clausena anisata Hook.f., De Wild. and Staner (Rutaceae) (LKO2016/12/08)	NS	Taken for the management of convulsions or epilepsy and some mental disorders, arthritis, heart conditions, hypertension, rheumatism and other inflammatory ailments. It is also taken as anti-helmintic, to treat flatworms such as taeniasis, schistosomiasis amongst other parasitic infections. Against pains of the abdomen, constipation, gastroenteritis, hepatic diseases causing bad breath, fevers, malaria and other febrile conditions, body pains, eye complaints and headaches, against herpes Zoster and herpes simplex viral infections, influenza and other respiratory diseases, impotence, sterility, blood tonic and dysentery in cattle.[3,26,60,113-118,119-122,123-124]	Has hypoglycaemic,[125] is an inhibitor of HIV-1 and HIV-2 replication,[119] antispasmolytlc,[117] antibacterial,[121,126] antimicrobial,[127-129] behavioral and anticonvulsant,[130-131] mollucidcidal,[118] insecticidal effect,[132-135] larvicidal,[136-137] antiplasmodial and analgesic,[138] antifungal activities,[139-140] *in vivo* antimalarial and acute toxicity properties,[141] antibacterial and antioxidant,[124,142-143] antitumor,[144] antihyperglycaemic,[145] antifeedant[146] and cytotoxic activities.[126]	Monoterpenoid furanocoumarin lactones,[117] quinolone and carbazole alkaloids, γ-lactone carbazoles,[147-150] acridone alkaloids,[124] volatile constituents,[151] substituted coumarins,[152-159] carbazole alkaloids,[152-157] phytosterols,[160] essential oils.[121]

Crotalaria axillaris Aiton (Leguminasae) (LKO2016/12/09)	Leaves	Applied to eyes as a cure for ophthalmia.[7]	Applied to eyes as a cure for ophthalmia.[7]	Applied to eyes as a cure for ophthalmia.[7]
	Seeds	Applied to the back for kidney trouble.[7]		
Croton megalocarpus Hutch. (Euphorbiaceae) (LKO2016/12/10)	Bark	Taken for the management of whooping cough, severe colds, pneumonia and as anti-helmintics,[7,169-170] given to chicken with diarrhoea or swollen heads, given to livestock with anthrax of East Coast fever.[7]	Has antibacterial and anti-inflammatory activities.[169]	Clerodane diterpene, triterpene esters.[175]
	NS	Used for the treatment of wounds,[171] coryza sinusitis in livestock,[172] management of malaria.[170]		
	Leaves	Used in the management of diabetes,[173] management of respiratory diseases.[3]		
	Roots	Used for the management of tonsils,[170] induces labour,[174] for pneumonia.[7]		
Diospyros abyssinica (Hiern) F. White (Ebenaceae) (LKO2016/12/11)	Leaves, root bark	Used for the treatment of malaria, wound healing, dysentery, worms and abdominal pains.[176]	Exhibits anti-inflammatory,[178-179] lipoxygenase inhibitors, anti-oxidants,[176,179-180] anti-leishmanial and cytotoxic properties.[108]	Naphthoquinones, triterpenoids[108,178,181] and lupeol (3).[181]
	Leaves	Used for the treatment of malaria, in wound healing, against lumbago.[177]		
	Fruits	Used as a carminativum, as astringent, remedy to cure biliousness.[177]		
	Seeds	Used as sedative.[177.]		
	Bark	Used as bitter principle, astringent and febrifuge[177] and ingested by chimpanzees.[108]		
Drypetes gerrardii Hutch (Putranjivaceae) (LKO2016/12/12)	NS	Used for the treatment of malaria and other ailments.[182]	Has antiprotozoal[183] *in vitro* antiplasmodial activities,[184-185] anti-leishmanial and *in vitro* trypanocidal activities.[186]	Friedelane (13-17), hopane (18) and lupane-type triterpenoids (19), steroids, biflavonoids (20),[184-187] diterpenoids, phenanthrenone derivative, drypetenone D (21) and a phenanthrenone heterodimers, drypetenone E (22), saponin, putranoside A (24),[188] flavonoids, xanthones and anthraquinones.[184-185,187]
Elaeodendron buchananii (Loes.) Loes (Celastraceae) (LKO2016/12/13)	Roots and root bark	Used for the treatment of wounds, syphilis, coughing blood, diarrhaea.[7]	Has insect antifeedant,[190-191] antimicrobial, molluscicidal, antioxidant[190,192] and antifungal activities.[193]	Has steroidal glycosides,[194-195] eudesmane type sesquiterpenoid,[191] dammarane tritepenoids.[190.]
	Leaves	Chewed for diarrhoea.[7]		
	NS	Plant is known to be poisonous to domestic livestock in East Africa,[7] although interestingly wild animals such as giraffes are apparently unaffected.[189]		
Erythrococca bongensis Pax (Euphorbiaceae) (LKO2016/12/14)	Leaves	Used by Maasai, Luo of Kenya as food.[196-197]	Not reported	Is rich in β-carotene, lutein and α-tocopherol.[197]
Gnidia subcordata Meisn. (Thymelaeaceae) (LKO2016/12/15)	Not reported	Not reported	Has *in vivo* antileukemic activities,[198] antineoplastic activities, *in vivo* activity against the growth of P-388 lymphocytic leukemia cells.[199]	Diterpenoid esters with macrocyclic ring with one terminus at the orthoester carbon.[198]
Grewia similis K.Schum (Malvaceae) (LKO2016/12/16)	Bark	Used for the treatment of sores, wounds and snake bites.[7]	Not reported	Not reported

Hibiscus calyphyllus Cav. (Malvaceae) (LKO2016/12/17)	Leaves	Used for the treatment of sores.[7]	Has antioxidant, cardioprotective, antihypertensive[201-204] and antiproliferative, antidiabetic, anticancer antibacterial, antiviral, antiulcer, antiaging, antifibrotic, antiinflammatory, analgesic, neurological, hepatoprotective, antiatherosclerotic, cardiac and nephroprotective activities.[205]	Has phenolic compounds, triterpene derivatives phytosteroids and anthocyanins, flavonoid aglycones.[206]
	Root	Used for the treatment of pneumonia.[7,200]		
	Stem	Used for the treatment of pre-hepatic jaundice and to reduce fever.[200]		
Manilkara discolor (Sond.) J.H.Hemsl (Sapotaceae) (LKO2016/12/18)	Bark	Used for stomachache and as astringent.[7]	Has antiplasmodial, cytotoxic,[207,208] *in vitro* antihelmintic,[209] antioxidant and antifungal activities.[210]	
	Root	Stomach disorders.[207]		
	Leaves and stem bark			
Markhamia lutea K.Schum. (Bignoniaceae) (LKO2016/12/19)	Young shoots or leaves	Throat disease, eye problems (conjunctivitis), snake bite wounds.[7]	Antiplasmodial and cytotoxicity,[7,213] antiviral,[220-221] *in vitro* activity against respiratory syncytial virus,[221] *in vitro* anti-parasitic activity and low cytotoxicity against MRC5 and KB cells,[222] antiplasmodial,[223] antiviral and antioxidant.[224]	Phenylpropanoid glycosides,[221,225] cycloartane triterpenoids and their xylose glycosides,[222] phaeophorbides, β-sitosterol (25) and pentacyclic triterpenes.[226-229]
	Leaves	Used as an antiparasitic agent[212] for cleaning snake bite wounds,[7] malaria, asthma, syphilis. [213]		
	Roots	Used to alleviate symptoms of watery and bloody diarrhea.[212]		
	NS	Eaten by primates such as chimpanzees and black-and-white colobus,[214] yellow fever,[215] diarrhea, asthenia and infections,[216-217] malaria,[213, 218] anaemia, various microbial and parasitic infections,[217] used as a wood preservative.[219]		
Mystroxylon aethiopicum (Thunb.) Loes (Celastraceae) (LKO2016/12/20)	Roots	Used for the treatment of headache.[230]	Androgenic effects.[231]	Has saponins, catechol tannins, alkaloids, steroid glycosides, flavonoids, aglycones of anthrasenosides, triterpenes (phytosterols).[231]
	Leaves	Used against antihelminthesis, black water in sheep and as a magic potion to keep the community together.[231]		
	Bark	Used for the treatment of haemorrhagic diarrhea.[232]		
Maytenus undata (Thunb.) Blakelock (Celastraceae) (LKO2016/12/21)	Leaves	Taken for against snakebites, chest pains, diarrhoea, rheumatism, eye and wound infection and dyspepsia.[169, 233]	Has antifungal antimicrobial and cytotoxicity,[235-237] antileishmanial,[238] antiphlogistic effect,[239] antimicrobial, anti-inflammatory, antioxidant and antimalarial,[234-240] antiplasmodial.[241-243]	Has oleanene triterpene acids, friedelane taraxerol triterpenoids.[236-237, 240]
	Roots and bark	Used in the treatment of malaria.[234]		
	Bark	Decoction of the bark used as tonic.[7]		
	Roots	The root decoction is used against syphilis, diseases of urethra.[7]		
Teclea simplicifolia (Engl.) Verdoorn (Rutaceae) (LKO2016/12/22)	Stem bark	Used for the management of malaria.[78, 244-245]	Not reported	Furoquinoline alkaloids,[249-250] triterpenoids[251-252] and an amine.[251]

	Roots	This plant part are poisonous[246] and are used against gonorrhea[247] and pneumonia.[24]		
	Leaves	Taken to manage lung infections.[24,248]		
Teclea trichocarpa (Engl.) Engl. (Rutaceae) (LKO2016/12/23	Stem bark and Root	Used to control maize weevil.[77]	Exhibits anti-feedant, antifungal and antibacterial,[78] *in vitro* anti-protozoal Activities.[79,80]	Elaborates acridone alkaloids, furoquinoline alkaloid and triterpenoids.[80,253]
	Leaves	Used as analgesic.[43]		
	NS	Used for malaria treatment, as anthelmintic and vapour inhailed as a cure for fever.[43,253]		
Tragia brevipes Pax (Euphorbiaceae) (LKO2016/12/24)	Root	Used for the treatment of labour pains and to increase the rate of contaction of the uterus,[7] tuberculosis and allied diseases.[254]	Has antibacterial,[254, 257-258] antipyretic, antiasthmatics, antiplasmodic, diuretic and analgesic[259] and antimicrobial activities.[260-261]	Tannins, saponins, terpenoids, flavonoids, phenols, alkaloids.[262-263]
	Leaves	Taken for the management of rheumatism[7] and stomach complaint.[254]		
	NS	Used for the treatment of tuberculosis,[254] helminthosis.[255-256]		
Turraea mombassana C. DC. (Meliaceae) (LKO2016/12/25)			Antiplasmodial activities,[264] anti-trypanosomal[265] and mosquito larvicidal,[266] mosquitocides activities.[267]	
Vernonia holstii O.Hoffm. (Compositae) (LKO2016/12/26)	Roots	Boiled or pounded and soaked in water used for abdominal pains.[7]	Has molluscicidal,[268] antibacterial, antifungal activities.[269-270]	Has sesquiterpene lactones, glaucolides and cistifoliolides.[271]
Zanthoxylum usambarense (Engl.) Kokwaro (Rutaceae) (LKO2016/12/27)	Bark	Used to treat rheumatism.[169]	Antibacterial and anti-inflammatory activities,[169] *in vitro* anti-plasmodial and cytoxic,[277] *in vitro* and as a curative and prophylactic agent,[278] cytotoxicity effects,[279] cryptococcal meningitis.[280]	Alkaloids of tetrahydroprotoberberine type,[281] canthin-6-one (26) oxychelerythrine (27), norchelerythrine (28), pellitorine (29), (+)-sesamin (30) and (+)-piperitol-3,3-dimethylallyl ether (31).[282]
	Stems	Used for the treatment of pneumonia and rheumatism.[273]		
	Seeds	Used in the treatment of respiratory tract infections, malaria and catarrhal fevers.[272,274]		
	Roots	Used for the management of malaria, rheumatism, coughs and as analgesic and antipyretic.[275-276]		
	Fruits	Chewed as a cough remedy,[275-276] malaria, malignant catarrhal fever.[272]		

sample/drug preparation methods used in ethno-medicine.

In African traditional medicine, the leaves are usually used as infusions where fresh leaves are crushed and kept in water ready to use. The roots and stem barks are usually processed through, cutting the barks into small pieces, drying, grinding into powder and finally making infusions of different dosages using mainly water and ethanol as the solvents.

The tedious preparation procedure of this plant parts would lead to loss of important ingredients in the extract, therefore rendering the root and stem bark extracts inactive and therefore not useful to the traditional medical practitioners. However, in the current study the plant parts were collected and extraction was effected on the same day, therefore avoiding loss of substantial amounts of important ingredients encountered during the drying process.

The root bark of *A. gummifera*, *C. axillaris*, *T. trichocarpa* and *Zanthoxyllum usambarense*; the stem barks of *C. axillaris, M. discolor* and *T. trichocarpa* and the leaves *of T. trichocarpa* showed high antihelmintic activities against round worms. The highest antioxidant activities were exhibited by the root barks of *A. oppositifolia*, *C. axillaris*, *E. buchananii*, *M. discolor, M. aethiopicum* and *T. trichocarpa;* stem bark of *A. oppositifolia, B. huillensis*, *Diospyros abyssinica*, *E. buchananii*; the leaves of *E. buchananii*, *G. similis*, *M. discolor* and *M. aethiopicum* and the roots of *V. holstii*. The high antioxidant potencies of these extracts could be attributed to due either the existence of phenolic secondary metabolites or other radical scavenging compounds in the extracts. The extracts that exhibited high activities should be investigated further to determine their effective concentrations and comprehensive phytochemistry studied fur-

Table 2: Bioactivities of the Studied Plant Extracts for General Protozoal Lethality, Round Worm Lethality and Antioxidant Potential.

		Antifungal			Antiprotozoal			Roundworm	Antioxidant		
		1	2	3	1	2	3	3	1	2	3
1	*Acokanthera oppositifolia* (Lam.) Codd (Apocynaceae) (LKO2016/12/01)			E6		E7	E6			E7	E2,E6
2	*Albizia gummifera* C.A.Sm. (Leguminosae) (LKO2016/12/02)	E6		E7		E6		E2	E2,E7		
3	*Brachylaena huillensis* O. Hoffm (Compositae) (LKO2016/12/03)	E6, E2	E2	E7	E7		E2		E7		E6
4	*Calodendrum capense* L.f. Thunb (Rutaceae) (LKO2016/12/04)	E7	E2,E6			E7	E2,E6		E6		
5	*Canthium keniense* Bullock; synonynm *Afrocanthium keniense* (Bullock) Lantz (Rubiaceae) (LKO2016/12/05)	E7	E6		E2	E7			E6,E7		
6	*Chaetachme aristata* Planch (Ulmaceae) (LKO2016/12/06)	E6	E2	E7		E7	E2,E6				
7	*Chionanthus battiscombei* (Hutch.) Stearn (Oleaceae) (LKO2016/12/07)	E2		E7						E2,E6,E7	
8	*Clausena anisata* (Willd.) Hook.f. ex Benth. (Rutaceae) (LKO2016/12/08)	E7			E6, E7				E2,E7		
9	*Crotalaria axillaris* Aiton (Leguminasae) (LKO2016/12/09)	E6		E7		E7	E2, E6	E2,E6	E6		E2
10	*Croton megalocarpus* Hutch. (Euphorbiaceae) (LKO2016/12/10)									E2, E6	
11	*Diospyros abyssinica* (Hiern) F. White (Ebenaceae) (LKO2016/12/11)									E2	E6
12	*Drypetes gerrardii* Hutch (Putranjivaceae) (LKO2016/12/12)		E6	E7		E6,E7	E2		E7		
13	*Elaeodendron buchananii* (Loes.) Loes (Celastraceae) (LKO2016/12/13)		E2	E6,E7	E7						E2,E6,E7
14	*Erythrococca bongensis* Pax (Euphorbiaceae) (LKO2016/12/14)				E2	E7	E6				
15	*Gnidia subcordata* Meisn. (Thymelaeaceae) (LKO2016/12/15)			E2,E7	E2	E6,E7			E7	E2	
16	*Grewia similis* K. Schum (Malvaceae) (LKO2016/12/16)	E6	E2	E7	E7				E7		E6

17	*Hibiscus calyphyllus* Cav. (Malvaceae) (LKO2016/12/17)		E2,E6,E7			E7	E2,E6					
18	*Manilkara discolor* (Sond.) J.H. Hemsl (Sapotaceae) (LKO2016/12/18)	E2,E7			E7			E6				E2,E7
19	*Markhamia lutea* K. Schum. (Bignoniaceae) (LKO2016/12/19)	E2	E7			E7	E2					
20	*Mystroxylon aethiopicum* (Thunb.) Loes (Celastraceae) (LKO2016/12/20)	E6,E2		E7							E7	E2, E6
21	*Maytenus undata* (Thunb.) Blakelock (Celastraceae) (LKO2016/12/21)		E2, E6	E7		E7			E2,E6,E7			
22	*Teclea simplicifolia* (Engl.) Verdoorn (Rutaceae) (LKO2016/12/22)		E7			E2			E2			
23	*Teclea trichocarpa* (Engl.) Engl. (Rutaceae) (LKO2016/12/23)				E6		E2, E7	E2, E6, E7	E7			E2,E6
24	*Tragia brevipes* Pax (Euphorbiaceae) (LKO2016/12/24)			E7		E7						
25	*Turraea mombassana* C. DC. (Meliaceae) (LKO2016/12/25)		E2, E6	E7		E7	E2		E7			
26	*Vernonia holstii* O.Hoffm. (Compositae) (LKO2016/12/26)	E1, 6		E7		E7			E6			E1
27	*Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae) (LKO2016/12/27)	E6	E2,E7		E6	E2, E7		E2,	E2,E6,E7			

E1: Roots, E2: Root bark, E3: Shoots, E4: Stem and twigs, E5: Wood, E6: Stem bark, E7: Leaf, E8: Inflorescence, E9: Flower, E10: Fruit, E11: Seed, 1: Low activity, 2: Moderate activity, 3: High activity.

ther and the bioactivities of the constituent compounds determined. It is interesting to note from these studies that different plant parts of only one plant *C. axillaris* exhibited high activities in the four categories of assays carried out.

The traditional use of all plants studied in the current investigation is compiled in Table S1 with special stress on the management of microbial and protozoal infections, antiheminthics, antioxidant and associated symptoms. The four plants, with interesting activities in all or three of the categories tested are described in the following paragraphs in more details.

Crotalaria axillaris (Ait): Plants belonging to the genus *Crotolaria* are mainly found in Australia, Asia, South America and the tropics and subtropics of Africa.[14-15] Despite their cytotoxicity these plants are used in East African traditional medicine. For example, the leaf infusion is applied to eyes for the treatment of opthalmia and a poultice made from crushed seeds applied to the back for kidney troubles.[7] The genus *Crotalaria* elaborates important secondary metabolites, pyrrolizidine alkaloids, based on the numerous biological activities including; acute hepatotoxic,[16] carcinogenic,[17] mutagenic,[18] teratogenic,[19] anticancer properties[20] and neuroactive properties.[21] In the current study, the leaves of *C. axillaris*, exhibited good antifungal activities, the root and stem bark had good antiprotozoal and antihelmintic activities against round worms and the root bark extracts had good antioxidant activities (Table 2). These interesting bioactivities shown by various parts of this plant are most likely due to the presence of pyrrolizidine alkaloids known to have the above-mentioned activities (Table 2).

Acokanthera oppositifolia (A.D.C) Schweinf. root decoction is used for the management of microbial infections including syphilis and against anthrax,[7,22-23] and hence authenticating the observed antimicrobial activities in the current study. However, the treatment is usually administered with caution as the plant is known exhibit toxic effects is used as an arrow poison.[7] The leaves and wood of this plant are used to relieve

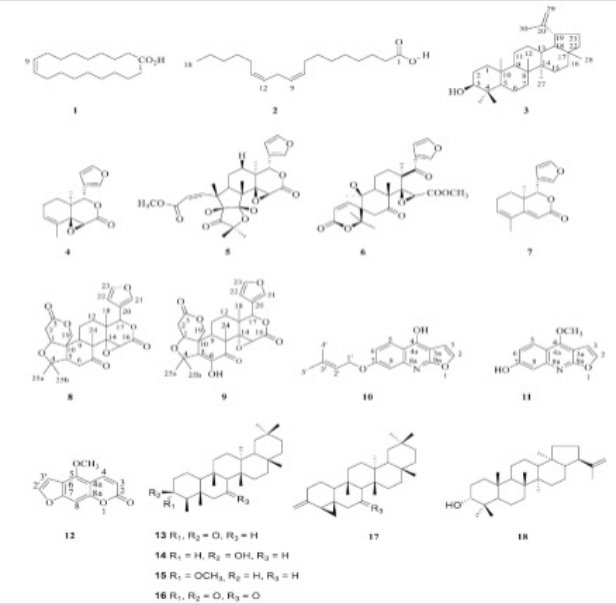

Figure 1: Chemical Structures of Compounds from some Plants in Table 1.

Figure 2: Images of some Representative Plants.

various kinds of pain, as an antidote for snake bites, against abdominal pains, convulsions, septicaemia, for heart water disease in ruminants and as antihelmintics to expel tapeworms which, is consistent with the present findings of the roots, which exhibited high antihelmintic activities.[22-30] The dried leaves and roots are taken for the management of headaches and snake bites, the root bark for the treatment of irregular menstruation, while the stem is used to relieve toothache, in the treatment of intestinal worms, for aches and colds and for the treatment of patients suffering from congestive heart failure.[31-32] Previous studies have shown that different parts of this plant exhibit the following biological activities; antioxidant,[27] anti-cancer,[33] antibacterial and antimutagenic,[34] cardiac stimulant[35-36] and good Cyclooxygenase (COX) inhibitory activities.[37] The good antioxidant potencies shown by the root and stem bark of *A. oppositifolia* in the present studies could be supported with the occurrence of flavonoids previously characterized from this plant.[23,35-36,38-39] The other families of secondary metabolites that could be attributable for the traditional uses and biological activities of this plant include; proanthocyanidins and toxic cardiac glycosides.[23,35-36,38-39] In South African traditional medicine, *Brachylaena huillensis* O. Hoffm leaves are administered for treating oral candidiasis, prevalent in persons infected with HIV.[40-41] If oral candidiasis is left untreated, it results to difficulty in chewing and swallowing in most cases and sometimes leading to severe diarrhoea[42-43] and associated weight loss.[44] Furthermore, in the Maputo area in southern Africa, where diarrhoel diseases are one of the highest causes of mortality, this plant is used in combination with *Psidium guajava* to treat this microbial infection.[45] The fractional inhibitory concentration index for the combination of these plants ranged between 0.09 (Synergestic) to 2.25 (Non-interactive) when tested against pathogens associated with diarrhea.[45] The antifungal potencies of this plant would most probably be attributed to the presence of essential oils containing mainly; α-copaene (9.0%), *cis*-calamenene (10.5%), β-cubebene (15.5%) and caryophyllene (19.1%),[46-47] coumarins, sterols, triterpenes and tannins.[48] *Teclea trichocarpa* (Engl.) is used extensively by Kenyan traditional healers, for the treatment of variety of parasitic infections including malaria, trysonomiasis and leishmaniasis. As a cure for fevers, the steam prepared from the leaves of this palnt is usually inhaled,[7] while the leaves and stem barks are used to control maize weevils in some counties in Kenya.[49] Previous bioassay studies of this plant have reported several potencies including anti-feedant, pesticidal, antifungal and antibacterial,[50] *in vitro* anti-protozoal activities.[49,51-52] In the current study, different plant parts mainly the root bark of *T. trichocarpa* had high activities in three out of four (3/4) categories of assays tested including; antiprotozoal, antihelmintic and antioxidant activities thus authenticating their use in traditional medicine. The leaves only showed good antiprotozoal and antihelmintic activity, while the stem bark exhibited high antihelmintic and antioxidant activities. The results from the current study clearly show that the three plant parts (Leaves, root and stem bark) can be used effectively as antihelmintics to manage infections caused by worms. The bioactivities of the extracts of *T. trichocarpa* may be attributed mainly to acridone and furoquinoline alkaloids previously characterized from this plant with proven potencies.[51,53-54] Previous investigations reported the interesting *in vitro* and *in vivo* biological activities of several acridone alkaloids against *P. yoelli*[55-56] as well as the *in vitro* bioactivities against *P. falciparum* of acridones and furoquinolines.[57]

CONCLUSION

The current investigation provided additional data in relation to the usefulness of the studied ethno-medicinal plants, mostly of the following plants; *C. axillaris, A. oppositifolia, B. huillensis* and *T. trichocarpa* in the management of diseases that are infectious and to bolster the immunity. The reported data will contribute towards evaluating the pharmacological properties of the plant extracts to determine whether they align with their traditional medicinal uses. The extracts that exhibited high activities should be investigated further to determine their effective concentrations.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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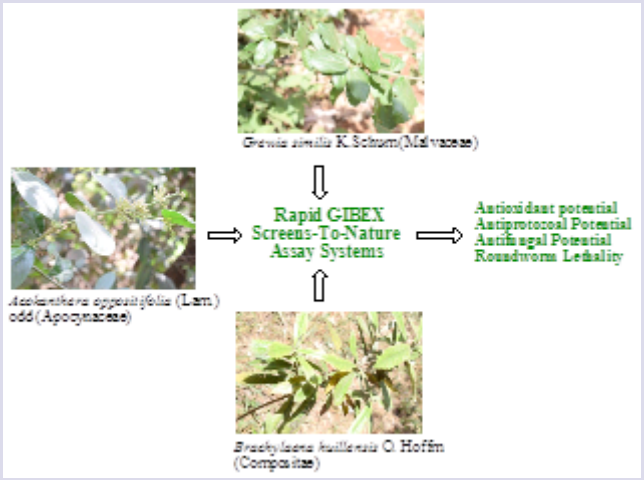
PICTORIAL ABSTRACT



SUMMARY

- The Rapid Screening using GIBEX Screens-To-Nature Systems are reliable assays for screening ethnomedicinal plants for potency against infectious diseases and antioxidant activities.
- Different plant parts exhibited a range of activities; related to their traditional uses; with eleven out of twenty-seven extracts exhibiting highest activities in only one out of four categories of assays studied.
- All plant parts of only one plant *C. axillaris* exhibited high activities in all (4/4) the categories of assays evaluated.
- The other plants that exhibited high activities in three out of four (3/4) categories of assays studied included; *A. oppositifolia, B. huillensis* and *T. trichocarpa*.

ABOUT AUTHORS

Dr. Leonidah Kerubo Omosa: is a Senior Lecturer in Organic Chemistry, in the Department of Chemistry, University of Nairobi, Kenya. Her research interest includes: Drug discovery from Kenyan ethnomedicinal flora with anti-plasmodial, anti-microbial anti-oxidant and anti-cancer potencies. Her interest also includes modifications of compounds with modest bioactivities in order to improve on their activities. Recently, she has ventured into ex¬ploring the possibility of discovering bioactive compounds and enzymes with potential application in textile and leather industry from microbes inhabiting Kenyan Soda lakes. She is also interested in promoting smallholder access to fungal biopesticides formulations. To date her research work has resulted in 35 publications in different peer reviewed journals.