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Research Article

Screening of *Indigofera lupatana* Baker F. root extracts for antibacterial activities

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Background: Plants produce hosts of secondary metabolites that help them fight off infections. However these phytochemicals have also been found to inadvertently protect humans against infections and are therefore a current subject in bio-prospecting for new therapeutic leads.

Objective: The aim of this study was to investigate the antibacterial properties of organic solvent root extracts of *Indigofera lupatana* Baker F.

Methodology: Powdered sample of *I. lupatana* Baker F. roots were sequentially extracted using hexane, dichloromethane and ethyl acetate. The resultant fractions were subjected to anti-bacterial assay.

Results: The general inhibition by the hexane extract was low. Gram positive bacteria and *P. aeruginosa* were not sensitive to hexane extract. The dichloromethane and ethyl acetate fractions showed marked activity in all tested bacteria. Their activities were generally greater against the Gram positive bacteria than Gram negative bacteria They were particularly active against *B. subtilis*.

Discussion: These results provide a partial justification for traditional usage of *Indigofera lupatana* Baker F. However, further *in vivo* and cytotoxicity studies are required to establish safety.

Key words: *Indigofera lupatana* Baker F., antimicrobial activity, natural products, phytochemicals

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1. Introduction

Herbal remedies as cheap alternatives to conventional medicine have contributed significantly to rural livelihoods. Apart from the traditional healers practicing herbal medicine, many people are involved in collecting and trading medicinal plants. The World Health Organization (WHO) estimates that 80% of the world's population depends on medicinal plants for their primary health care (Mothana et al, 2008; Ngoci et al, 2011). The use of traditional medicine has been explored globally and is widely used in developing countries as an alternative or to complement conventional medicine (Rates, 2001; Gupta et al, 2010).

Natural products, either as pure compounds or as standardized plant extracts, provide exceptional opportunities for new drug leads because of the unmatched chemical diversity of naturally derived compounds (Cowan, 1999; Parekh and Chanda, 2007; Mariita et al, 2010; Ngoci et al, 2011). Scientific interest in medicinal plants has burgeoned due to the recognized efficacy of plant derived drugs and ever-present concerns about the side effects of modern medicinal substances. This has fuelled the intensive

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investigation of new molecular structures from the plant kingdom as potential medicinal compounds (Mariita et al, 2010). As a result, drugs derived from unmodified natural products or semi-synthetic drugs obtained from natural sources accounted for 78% of the new drugs approved by the United States Food and Drug Administration (FDA) between 1983 and 1994 (Suffredini et al, 2006; Ngoci et al, 2011). This underscores the importance of screening natural products.

Infectious diseases are a leading cause of human and animal mortality. This is further aggravated by the rapid development of multi-drug resistance to available anti-microbial agents (Doughart and Okafor, 2007; Ngoci et al, 2011), their limited anti-microbial spectrum, their side effects (Huie, 2002), and emergence and re-emergence of opportunistic infections. Therefore, studies aimed at identifying and characterizing of the substances that exhibit activity against infectious micro-organisms, yet showing no cross resistance with existing antibiotics, are required (Olila et al, 2001). In recent years, pharmaceutical companies have focused on developing drugs from natural products that promises to counter the limitations of conventional antibiotics (Doughart and Okafor, 2007).

The bio-activity of natural products is due to phytochemicals, a group of secondary metabolites often elaborated for the plant defense against pests and herbivores or to gain an advantage over competing agents. These phytochemicals inadvertently also protect humans against pathogens (Ngoci et al, 2011). Some phytochemicals are known to have antimicrobial properties, immune-modulative properties, provide nutrition for normal cell health and repairs, inhibit carcinogens and act as antioxidants.

Indigofera lupatana Baker F., locally called 'mugiti' by the Mbeere community in Kenya, is a woody shrub found in Acacia-Combretum ecological zones of Mbeere. It is widely used for its perceived medicinal value in treating coughs and diarrhea (Riley and Brokensha, 1988; Ngoci et al, 2011), gonorrhea and pleurisy (Kokwaro, 1993; Ngoci et al, 2011).

There is apparently no documented scientific report on anti-microbial properties of this plant. This lack of scientific corroboration has often constituted a major constraint to the consideration of the use of herbal remedies in conjunction with or as an affordable alternative to conventional medical treatment (Okeke et al, 2001). Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be important in identifying new sources of substances of economic value such as tannins, oils, gums, and precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab et al, 2003).

This study was therefore undertaken to determine the antibacterial properties of hexane, ethyl acetate and dichloromethane root extracts of *I. lupatana* Baker F.

2. Methods

2.1 Collection and Identification of Plant Samples

The plant samples for the study were collected from Mbeere district, Embu County of Kenya. The plant was taxonomically authenticated at the Department of Biological Sciences of Egerton University. A voucher sample was assigned a reference number (NSN1) and banked in the departmental herbarium.

2.2 Preparation of *Indigofera indica* root extracts

The plant roots were separated, washed, cut into small pieces, air-dried in the dark to avoid decomposition of light sensitive bioactive compounds (Houghton and Raman, 1998), at room temperature to a constant weight and milled into a powder. The powder was extracted sequentially using three organic solvents of increasing polarity - hexane, dichloromethane and ethyl acetate, in that order - as described below.

Ground material (150 g) was firstly soaked in the less polar hexane for 24 hours at room temperature with intermittent shaking followed by decanting and filtration by gravity to separate the debris. Fresh solvent was replaced and agitated for 10 minutes, decanted and filtered. The two volumes were combined together and concentrated under reduced pressure and the residue allowed to air dry. The process was repeated with material agitated in dichloromethane and ethyl acetate in the same manner as above (Houghton and Raman, 1998; Wojcikowski et al, 2008).

2.3 Test Micro-organisms

The following microbial strains were used as test cultures: three Gram-positive bacterial species - *Bacillus subtilis* BGA (Merck, Darmstadt, Germany), *Bacillus cereus* ATCC 11778 (Difco Laboratories, Detroit, USA) and *Staphylococcus aureus* ATCC 25923 (KEMRI) - and five Gram-negative bacteria - *Escherichia coli* ATCC 25922 (KEMRI), *Pseudomonas aeruginosa* ATCC 27853 (KEMRI), *Salmonella typhimurium* ATCC 13311 (KEMRI), *Klebsiella pneumoniae* and *Proteus mirabilis* (clinical isolates from KEMRI). These were maintained on nutrient agar slants at 4 °C.

2.4 Anti-microbial tests

These were used to evaluate *in vitro* activity of the various extracts.

Media preparation

The media was reconstituted using distilled water according to the manufacturer's instructions, sterilized by autoclaving at 121 °C for 15 min, and dispensed aseptically into Petri dishes (9 cm diameter). A volume of between 18-25 ml molten agar was dispensed to achieve a depth of between 3-4 mm, and left to solidify and then stored in the refrigerator at 4 °C. The inoculation plates were air dried with the lids ajar until there were no moisture droplets on the petri dish surfaces (Collins et al, 1995).

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Preparation of discs

Stock solutions of each extract (hexane extract 1.5 g/ml, ethyl acetate extract 1.4 g/ml and dichloromethane extract 1.0 mg/ml) were prepared in 1% aqueous dimethylsulfoxide (DMSO). Working extract solutions were prepared by two-fold serial dilutions of each stock solution in 1% aqueous DMSO. The aqueous 1% DMSO was used as solvent for the extracted samples because it is amphipathic, able to diffuse well in the agar and at this concentration it is non toxic (Moshi et al, 2006; Mbaveng et al., 2008).

Whatmann filter paper (No. 1) discs of 6 mm diameter were made by punching the paper, and the blank discs were sterilized in the hot air oven at 160 °C for 1 hr. They were then impregnated with 10 µl of the various working extract solutions. The discs were dried by heating at 50 °C (Ayo et al, 2007).

Chloramphenicol 30 µg/disc was used as a standard drug for positive control (STDb) against bacteria; a disc loaded with 10 µl of 1% aqueous DMSO used as a negative control (STDa) (Mbwambo et al, 2007; Mbaveng et al, 2008). The choice of chloramphenicol was based on its properties as a broad spectrum antibiotic, a very stable drug under a variety of conditions of temperature and humidity, and its low toxicity threshold when ingested (Drew et al, 1972).

Disc diffusion test

The anti-bacterial activity was assayed by disc diffusion method according to Ayo et al, (2007), CLSI (2007) and Mbaveng et al, (2008). Nutrient agar was used for sub-culturing of the test micro-organisms and Mueller Hinton agar was used for sensitivity assay (Nguemeving et al, 2006).

The bacterial strains were activated by growing them on nutrient agar at 37 °C for 18 - 24 hr. A fresh inoculum was developed by suspending activated colonies in physiological saline solution (0.85% NaCl). An inoculum of bacterial cell suspension of about 1.5×10^6 CFU/ml was determined and standardized using a McFarland turbidity standard No. 0.5. The suspension was authenticated by adjusting the optical density to 0.1 at 600 nm.

This suspension was used to aseptically inoculate Mueller Hinton agar plates by swabbing the surface. Excess liquid was air-dried under a sterile hood. The impregnated discs were then planted at equidistant points on top of the inoculated agar medium using sterile forceps. The inoculated plates were incubated at 4 °C for 2 hr to allow the pre-diffusion of extracts into the media. The plates were then incubated at 37 °C for 24 hr. Anti-bacterial activity was evaluated by measuring the diameter of the zone of inhibition. The lowest concentration of the extract that inhibited bacterial growth was recorded as the MIC of the extract fraction (Mothana et al, 2008).

2.5 Phytochemical tests

Phytochemical tests were done to determine the classes of compounds present in the various extracts. These were identified by characteristic colour changes using standard procedures according to Houghton and Raman, (1998), Edeoga et al, (2005), Shanmugavalli et al, (2009), and Ngoci et al (2011). The results were reported as (+ve) for presence, and (-ve) for absence.

2.6 Statistical analysis

The test values were expressed as mean ± standard error of the mean (SEM).

3. Results

The anti-bacterial activity test revealed extracts activity against both Gram positive and Gram negative bacteria. The activity varied depending with the extract tested, extract concentration and the test bacteria.

The general inhibition by the hexane extract was low, with the maximum inhibition (observed against *E. coli*) giving a zone of inhibition of 6.5±0.3 mm at the highest concentration tested, and MIC of 750 mg/ml. Gram positive bacteria and *P. aeruginosa* were not sensitive to hexane extract. The activity of the hexane extract is summarized in **Table 1**.

The activity of the dichloromethane extract is summarized in **Table 2**. It showed activity against all the strains of bacteria tested, and was most active against the Gram positive *B. subtilis* bacteria, showing a zone of inhibition of 28.5 ± 0.3 at the highest concentration tested, and an MIC of 31.3 mg/ml. Among the Gram negative bacteria, this extract was most active against *E. coli* (zone of inhibition of 21.7 ± 0.7 mm at the highest concentration tested, and an MIC of 125).

The ethyl acetate extract also showed activity against all the strains of bacteria tested, and was also most active against the Gram positive *B. subtilis* bacteria, showing a zone of inhibition of 26.0 ± 0.3 mm at the highest concentration tested, and an MIC of 21.9 mg/ml. Among the Gram negative bacteria, this extract was most active against *P. aeruginosa* (zone of inhibition of 21.5 ± 0.9 mm at the highest concentration tested, and MIC of 175) (**Table 3**).

The results of the phytochemical testing are shown in **Table 4**.

4. Discussion

The plant extracts had broad spectrum activity. They inhibited growth of both Gram positive and Gram negative bacteria. The inhibition zones increased on increasing the concentration of the extract in the discs showing a concentration dependent activity and also varied with the species of bacteria tested. Although the concentrations of the extract fractions were > 100 times more than the standard antibiotic used (chloramphenicol), they showed notable anti-bacterial activity as evidenced by their zones of inhibition. This could be due to the fact that the active components in the extract comprise only a fraction of the extract used. Therefore, the actual concentration of the active components in the extract could be much lower. It is possible that the active components could show higher antibacterial activity than those observed if they were isolated and purified.

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Table 1: Anti-bacterial activity result for the hexane root extract fraction

	Inhibition zones diameter in mm								
	Extract concentration (µg x 10^2/disc)							MIC(mg/ml)	
Micro organism	**150**	**75**	**37.5**	**18.8**	**9.4**	**STDa**	**(STDb 30µg/disc)**	**Extract**	**STDb**
Gram negative bacteria									
E. coli	6.5 ± 0.3	0	0	0	0	0	48.3±1.7	750	25
K. pneumoniae	6.0±0	0	0	0	0	0	37.6 ± 0.8	750	22.5
P. aeruginosa	0	0	0	0	0	0	24.3 ± 1.3	>750	NT
P. mirabilis	6.3±0.3	0	0	0	0	0	34.3 ± 1.3	750	NT
S. typhimurium	6.3±0.3	0	0	0	0	0	29.0 ± 1.5	750	NT
Gram positive bacteria									
S. aureus	0	0	0	0	0	0	37.3 ± 0.9	>750	31.3
B. cereus	NT	NT	NT	NT	NT	NT	NT	NT	NT
B. subtilis	NT	NT	NT	NT	NT	NT	NT	NT	NT

STDa –Represent 1% DMSO as negative control; STDb – Represents Chloramphenicol as positive control; E – Represents extract fraction and NT – Represent not tested. Values are inhibition zones in mm (mean ± SEM; n = 3).

Table 2: Anti-bacterial activity result for the dichloromethane root extract fraction

	Inhibition zones diameter in mm								
	Extract concentration (µg x 10^2/disc)							MIC(mg/ml)	
Micro organism	**100**	**50**	**25**	**12.5**	**6.3**	**STDa**	**(STDb 30µg/disc)**	**Extract**	**STDb**
Gram negative bacteria									
E. coli	21.7 ± 0.7	14.0±0.6	11.0±1	0	0	0	48.3±1.0	125	25
K. pneumoniae	11.4 ± 0.8	7.7±0.3	0	0	0	0	37.6 ± 0.8	250	22.5
P. aeruginosa	10.0 ± 0.7	8.3±0.3	7.0±0	0	0	0	24.3 ± 1.3	125	NT
P. mirabilis	10.7 ± 0.7	9.2±0.7	7.0±0.5	0	0	0	34.3 ± 1.3	125	NT
S. typhimurium	8.3 ± 0.6	0	0	0	0	0	29.0 ± 1.5	500	NT
Gram positive bacteria									
S. aureus	22.6 ± 1.0	12.4±2.8	0	0	0	0	37.3 ± 0.9	250	31.3
B. cereus	22.0 ± 0.1	20.5±0.5	17.0±2.0	12.0±0	0	0	22.3 ± 0.8	62.5	NT
B. subtilis	28.5 ± 0.3	27.5±0.5	21.5±0.5	19.0±0.6	18±0	0	32.6 ± 0.9	31.3	26.3

STDa –Represent 1% DMSO as negative control; STDb – Represents Chloramphenicol as positive control; E – Represents extract fraction and NT – Represent not tested. Values are inhibition zones in mm (mean ± SEM; n = 3).

The hexane fraction had the lowest activity against the test bacteria. This could have been due to absence of phytochemicals such as flavonoids, tannins, saponins, cardiac glycosides, terpenoids and phenolics (**Table 4**) that have been associated with antimicrobial activity or due to antagonistic actions of specific compounds in the hexane crude extract (Cowan, 1999; Ogunwenmo et al, 2007; Al-Bayati and Al-Mola, 2008).

The dichloromethane fraction showed marked activity in all tested bacteria. This activity was probably due to phytochemicals detected in the extract fraction such as tannins that act by complexing bacterial proteins, interfering with bacterial adhesion, inactivating enzymes and disrupting bacterial cell membrane (Cowan, 1999; Okuda, 2005; Biradar et al, 2007; Samy and Gopalakrishnakone, 2008; Kaur and Arora, 2009). Also, activity could be due to flavonoids, saponins, cardiac glycosides, phytosteroids and terpenoids that were detected in the extract and could have been responsible for the antibacterial activity (Ngoci et al, 2011)..

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Table 3: Anti-bacterial activity result for the ethyl acetate root extract

Micro organism	Inhibition zones diameter in mm: Extract concentration (µg x 10^2/disc) 140	70	35	17.5	8.8	STDa	(STDb 30µg/disc)	MIC(mg/ml) Extract	STDb
Gram negative bacteria									
E. coli	16.3 ± 0.7	14.0±1.0	12.0±0.5	0	0	0	48.3 ± 1.0	175	25
K. pneumoniae	10.6 ± 0.9	8.0±0.5	0	0	0	0	37.6 ± 0.8	350	22.5
P. aeruginosa	21.5 ± 0.9	10.0±1.0	7.0±0	0	0	0	24.3 ± 1.3	175	NT
P. mirabilis	10.4 ± 1.1	6.0±0	0	0	0	0	34.3 ± 1.3	350	NT
S. typhimurium	17.3 ± 0.3	0	0	0	0	0	29.0 ± 1.5	700	NT
Gram positive bacteria									
S. aureus	7.0±0.1	0	0	0	0	0	37.3 ± 0.9	700	31.3
B. cereus	21.2 ± 0.6	19.5±0.5	15.0±1.0	13.5±0.5	8.0±1.7	0	22.3 ± 0.8	43.8	NT
B. subtilis	26.0 ± 0.3	24.5±0.5	23.0±1.0	21.0±0.6	18±0.5	0	32.6 ± 0.9	21.9	26.3

STDa –Represent 1% DMSO as negative control; STDb – Represents Chloramphenicol as positive control; E – Represents extract fraction and NT – Represent not tested. Values are inhibition zones in mm (mean ± SEM; n = 3)

Table 4: Phytochemical test results

Phytochemical constituent	Hexane extract	Dichloromethane extract	Ethyl acetate extract
Alkaloids	-ve	-ve	-ve
Flavonoids	-ve	+ve	+ve
Tannins	-ve	+ve	-ve
Saponins	-ve	+ve	+ve
Cardiac glycosides	-ve	+ve	+ve
Phlobatannins	+ve	-ve	+ve
Phytosteroids	+ve	+ve	+ve
Terpenoids	-ve	+ve	+ve
Phenolics	-ve	+ve	+ve

+ve: *presence of the tested phytochemicals in the sample extract*
–ve: *absence of the tested phytochemicals in the sample extract*

The ethyl acetate fraction showed marked activity in all tested bacteria. It was particularly active against *P. aeruginosa, B. cereus and B. subtilis*, with the highest sensitivity recorded against *B. subtilis*. This observed antibacterial activity could be due to flavonoids that have been shown to act by complexing proteins and disrupting membranes (Navarro et al, 2003; Al-Bayati and Al-Mola, 2008; Samy and Gopalakrishnakone, 2008; Kaur and Arora, 2009), saponins that have been demonstrated to act by inhibiting bacterial colonization, lowering surface tension of extracellular medium or by lysing bacterial membranes (Al-Bayati and Al-Mola, 2008), phytosteroids and terpenoids that acts by disrupting bacterial membrane (Cowan, 1999; Soares et al, 2005; Ogunwenmo et al, 2007; Samy and Gopalakrishnakone, 2008). Cardiac glycosides and phlobatannins that were also detected could be responsible for antibacterial activity.

The bioactivities demonstrated by the different extracts may be attributed to the diversity of structures and/or the uneven distributions of chemical constituents between these extracts. Each extract had a different degree of inhibitory activity and specificity against bacteria and this could be related to the polarity of the extracting solvent (Przybylski et al., 1998). The polar solvents extracted phenolics, tannins, flavonoids, terpenoids that are responsible for the bioactivity of these extracts and could either have exhibited synergistic or additive effects when used in their crude form (Mohamed et al, 2010).

Gram positive strains were generally more susceptible to the extracts than Gram negative strains. This is in agreement with previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Parekh and Chanda, 2006; Mohamed

et al, 2010). The higher sensitivity of Gram-positive bacteria could be attributed to their outer peptidoglycan layer which is not an effective permeability barrier as compared to the outer phospholipid membranes of Gram-negative bacteria (Trombetta et al, 2005; Tomczykowa et al, 2008; Kaur and Arora, 2009).

The present work showed the potential of the tested extracts against the causative agents of nosocomial infections and morbidity among immune-compromised and severely ill patients such as *P. aeruginosa* and *S. aureus* (Bastos et al., 2009; Kaur and Arora, 2009). Infections caused by *P. aeruginosa* and *B. cereus* are difficult to combat (Aliero and Afolayan, 2005) and therefore their susceptibility to the extracts is a pointer to extracts' potential against these bacteria.

The plant extracts also showed commendable activity toward pathogen responsible for the gastrointestinal disorders that leads to diarrhea, cholecystitis, and urinary tract infections e.g. *E. coli*, *S. typhimurium* (Moshi et al., 2006; Matasyoh et al., 2007) and this partially supports the traditional use of this plant for the treatment of diarrhea (Riley and Brokensha, 1988).

5. Conclusion

From this study it can be concluded that the *Indigofera lupatana* Baker F. extracts had broad spectrum bioactivity as they inhibited both Gram-positive and Gram-negative bacteria. This partially supports the traditional usage of this plant for therapeutic purposes. However further studies on *in vivo* activity as well as determination of cytotoxicity using cell lines needs to be undertaken.

Conflict of Interest declaration

The authors declare no conflict of interest

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References

Al-Bayati FA and Al-Mola HF (2008). Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. *J. Zhej. Uni. Sci.* **9:** 154–159.

Aliero AA and Afolayan AJ (2005). Antimicrobial activity of *Solanum tomentosum. Afr. J. Biotech*. **5:** 369-372.

Ayo RG, Amupitan JO and Zhao Y (2007). Cytotoxicity and anti-microbial studies of 1,6,8-trihydroxy-3-methyl-anthraquinone (Emodin) isolated from the leaves of *Cassia nigricans* Vahl. *Afr. J. Biotech.* **6:** 1276-1279.

Bastos MLA, Lima MRF, Conserva LM, Andrade VS, Rocha EMM and Lemos RPL (2009). Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. *J. Evid. Based Complement. Altern. Med.* **8:** 16-21.

Biradar YS, Jagatap S, Khandelwal KR and Singhania SS (2007). Exploring of Antimicrobial Activity of Triphala Mashi - an Ayurvedic Formulation. *J. Evid. Based Complement. Altern. Med*. **5:** 107–113.

Clinical and Laboratory Standards Institute (2007). Methods for determining bactericidal activity of antimicrobial agents. Tentative standards M26-T. National Committee for Clinical Laboratory standards, Wayne , PA.

Collins CH, Lyne PM and Grange JM (1995). Microbiological methods, 7th ed. Butterworth-Heinemann, Great Britain. pp 178-205.

Cowan MM (1999) Plant products as anti-microbial agents. *Clin. Microbiol. Rev.* **12:** 564-582.

Doughart JH and Okafor B (2007). Anti-microbial activity of *Senna alata linn*. *E. C. Afr. J. Pharm. Sci.* **10:** 17-21.

Drew WL, Arry AL, O'toole R and Sherris JC (1972). Reliability of the Kirby-Bauer Disc Diffusion Method for Detecting Methicillin-Resistant Strains of *Staphylococcus aureus. J. Appl. Microbiol.* **24:** 240-247.

Edeoga HO, Okwu DE and Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants*. Afr. J. Biotech.* **4:** 685-688.

Gupta VK, Shukla C, Bisht GR S, Kumar S and Thakur RL (2010). Detection of anti-tuberculosis activity in some folklore plants by radiometric BACTEC assay. *Lett. Appl. Microbiol.* **52:** 33-40.

Houghton PJ and Raman A (1998). Laboratory Hand book for the Fractionation of Natural Extracts, 1st ed, Chapman and Hall, London, pp 1-153.

Huie CW (2002). Review: Modern sample preparation techniques for the extraction and analysis of medicinal plants. *J. Anal. Bioanal. Chem.* **373:** 23-30.

Kaur GJ and Arora DS (2009). Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi. J. BMC: Compliment. Altern. Med.* **9**: 30-41.

Kokwaro JO (1993). Medicinal plants of East Africa. 2nd ed. Kenya Literature Bureau, Nairobi, Kenya, pp 149.

Matasyoh JC, Kiplimo JJ, Karubiu NM and Hailstorks TP (2007). Chemical composition and antimicrobial activity of essential oil of *Tarchonanthus camphorates*. *J. Food Chem.* **101:** 1183-1187.

Mariita RM, Ogol CKP, Oguge NO, and Okemo PO (2010). Antitubercular and Phytochemical Investigation of Methanol Extracts of Medicinal Plants Used by the Samburu Community in Kenya. *Trop. J. Pharm. Res.* **9**: 379-385.

Mbaveng AT, Ngameni B, Kuete V, Simo IK, Ambassa P, Roy R, Bezabih M, Etoa F, Ngadjui BT, Abegaz BM, Meyer JJM, Lall N and Beng VP (2008). Anti-microbial activity of the crude extracts and five flavonoids from the twigs of *Dorstenia barteri* (Moraceae). *J. Ethnopharm.* **116:** 483-489.

Mbwambo ZH, Moshi MJ, Masimba PJ, Kapingu MC and Nondo RS (2007). Anti-microbial and brine shrimp toxicity of extracts of *Terminalia brownii* roots and stem. *BMC Complement. Altern. Med.* **7:** 1472-1477.

Mohamed LT, El Nur BS and Abdelrahman MN (2010). The Antibacterial, antiviral activities and phytochemical screening of some Sudanese medicinal plants. *EurAsian J. BioSci.* **4:** 8-16.

Mojab F, Kamalinejad M, Ghaderi N and Vahidipour HR (2003). Phytochemical Screening of Some Species of Iranian Plants. *Iran. J. Pharm. Res.* **2** :77-82.

Moshi MJ, Mbwambo ZH, Kapingu MC, Viva H, Mhozya VH and Marwa C (2006). Antimicrobial and Brine Shrimp Lethality of Extracts of *Terminalia Mollis* Laws. *Afr. J. Trad. Compliment. Altern. Med.* **3:** 59-69.

Mothana RA, Abdo SA, Hasson S and Althawab FM (2008). Antimicrobial, Antioxidant and Cytotoxic Activities and Phytochemical Screening of Some Yemeni Medicinal Plants. *J Evid Based Complement. Altern Med*. doi:10.1093/ecam/nen004:

Navarro MC, Montilla MP, Cabo MM, Galisteo M, Caceres A, Morales C and Berger I (2003). Antibaterial, antiprotozoal and antioxidant activity of five plants used in izabal for infectious diseases. *J. Phytoth. Res.* **17:** 325-329.

Ngoci SN, Mwendia CM and Mwaniki CG (2011). Phytochemical and cytotoxicity testing of *Indigofera lupatana* Baker F. *J. Anim. Plant Sci.* **11**: 1364-1373.

Nguemeving JR, Azebaze AGB, Kuete V, Carly NNE, Beng VP, Meyer M, Blond A, Bodo B and Nkengfack AE (2006). Laurentixanthones A and B, anti-microbial xanthones from *Vismia laurentii*. *J. phytochem.* **67:** 1341-1346.

Ogunwenmo KO, Idowu OA, Innocent C, Esan EB and Oyelana OA (2007). Cultivars of *Codiaeum variegatum* (L.) Blume (*Euphorbiaceae*) show variability in phytochemical and cytological characteristics. *J. Biotech.* **20:** 2400-2405.

Okeke MI, Iroegbu CU, Eze EN, Okoli AS and Esimone CO (2001). Evaluation of extracts of the root of *Landolphia owerrience* for anti-bacterial activity. *J. Ethnopharm.* **78:** 119-127.

Okuda T (2005). Systematics and health effects of chemically distinct tannins in medicinal plants. *J. Phytochem.* **66:** 2012-2031.

Olila D, Olwa-Odyek and Opuda-Asibo J (2001). Anti-bacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis,* Ugandan medicinal plants. *J. Afr. Healt. Sci.* **1:** 66-72.

Parekh J and Chanda S (2006). *In-vitro* antimicrobial activities of extractsof *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). *Afr. J. Biomed. Res.* **9:** 89-93.

Przybylski R, Lee RY and Eskin NAM (1998). Antioxidant and radical-scavenging activities of buckwheat seed components. *J. Amer. Oil Chem. Soc.* **75:** 1595–1601

Rates SMK (2001) Plants as source of drugs. *Toxicon* **39:** 603–613.

Riley BW and Brokensha D (1988). *The Mbeere in Kenya*; *Botanical identity and use*, Vol 2, University Press, USA, pp 76-77.

Samy RP and Gopalakrishnakone P (2008). Review: Therapeutic potential of plants as anti-microbials for drug discovery. *J. Evid. Based Complement. Altern. Med* doi:10.1093/ecam/nen036.

Shanmugavalli N, Umashankar V and Raheem (2009). Antimicrobial activity of *Vanilla planifolia*. *Ind. J. Sci. Tech.* **2:** 37-40.

Soares MB, Brustolim D, Santos LA, Bellintani MC, Paiva FP, Ribeiro YM, Tossami TC and Santos R (2005). Physalins B, F and G, *Seco*-steroids purified from *Physalis angulata* L., inhibit lymphocyte function and allogeneic transplant rejection. *J. Inter. Immunopharm.* **6:** 408-414.

Suffredini BI, Paciencia MLB, Nepomuceno DC, Younes RN and Varella AD (2006). Anti-bacterial and cytotoxic activity of Brazilian plant extracts - *Clusiaceae . Memórias do Instituto Oswaldo Cruz*, **101:** 1590-1598.

Tomczykowa M, Tomczyk M, Jakoniuk P and Tryniszewska B (2008). Antimicrobial and antifungal activities of the extracts and essential oils of *Bidens tripartite. J. Folia Histochem. Et Cytobiol.* **46:** 389-393.

Trombetta D, Castelli F, Sarpietro M, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G and Bisignano G (2005) Mechanisms of anti-bacterial action of three monoterpenes. *J. Antimicr. Agen. Chemother.* **49:** 2474-2478.

Wojcikowski K, Wohlmuth H, Johnson DW, Rolfe M and Gobe G (2008). An *in vitro* investigation of herbs traditionally used for kidney and urinary system disorders: Potential therapeutic and toxic effects. *J. Nephrol.* **14:** 70-79.

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