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IN VITRO ANTIMICROBIAL ASSAY OF PLANTS USED IN TRADITIONAL MEDICINE IN BUKOBA RURAL DISTRICT, TANZANIA.

D.P. Kisangau*[1], K.M. Hosea[2], C.C. Joseph[3] and H.V.M. Lyaruu[1]

[1]Department of Botany, University of Dar es Salaam, P.O. Box 35060, Dar es Salaam, Tanzania. [2]Department of Molecular Biology and Biotechnology, P.O. Box 35060, University of Dar es Salaam, Dar es Salaam, Tanzania.,[3]Department of Chemistry, University of Dar es Salaam, P.O. Box 35061, Dar es Salaam, Tanzania.
***E-mail:** kisangau@yahoo.com

Abstract

Plants used in traditional medicine in Bukoba Rural district in Tanzania were evaluated for their *in vitro* antimicrobial activities. Plant materials from eight plant species (*Harungana madagascariensis* (Lam) Poir., *Jatropha curcas* L., *Lantana trifolia* L., *Plectranthus barbatus* Andr., *Pseudospondias microcarpa* Engl., *Psorospermum febrifugum* Spach, *Teclea nobilis* Del. and *Vernonia adoensis* [Warp.] SL) were collected based on ethnomedical information provided by traditional herbal practitioners. Results of the study indicate that extracts from the eight plant species were active against at least one or more of the test organisms (*Bacillus subtilis*, *Staphylococcus aureus* [gram positive], *Escherichia coli*, *Pseudomonas aeruginosa* [gram negative] and *Candida albicans* [Yeast]). A profile of secondary metabolites (alkaloids, terpenoids, triterpenes, phenolics, tannins, flavonoids, anthraquinones, flavonols/flavones and /or chalcones, sterols and saponins) was obtained for three plant species (*Jatropha curcas* L., *Plectranthus barbatus* Andr., and *Pseudospondias microcarpa* Engl.). The paper discusses the probable therapeutic basis of these traditional plants based on their secondary metabolite profiles and for the first time draws research attention to Bukoba Rural district as a source for plants with potential pharmaceutical applications.

Key words: Antimicrobial activity, Secondary metabolites, Traditional medicine, Tanzania.

Introduction

Traditional medicine continues to provide health coverage for over 80% of the world population, especially in the developing world (WHO, 2002). In Africa, traditional healers have for centuries been the main providers of primary health care (Scheinman, 2002; Kala et al., 2004). In Tanzania, the use of traditional medicine for the treatment of various diseases has been practiced for generations and a large number of the population in the country use traditional medicines for their day to day healthcare needs (Moshi *et al.*, 2006; Hamza *et al.*, - In press).

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro organisms, animals and plants (Janovka et al., 2003). However, the increasing prevalence of multidrug resistant bacteria and fungi, the recent appearance of strains with reduced susceptibility to antibiotics, the side effects associated with antibiotics, the high costs of antimicrobial drugs and the re-emergence of diseases like tuberculosis are the key factors that obstruct resonant management of bacterial and fungal infections in many developing countries including Tanzania (Janovka *et al.*, 2003; Runyoro et al., 2006; Hamza et al., -In Press). The increased prevalence of HIV/AIDS virus has also in the recent past augmented the magnitude of many bacterial and fungal opportunistic infections

with frequent episodes resulting from the immune suppression of the affected persons, (Bii, 2001; Runyoro et al., 2006). Consequently, this has raised the spectre of untreatable microbial infections and adds the urgency to the search for new infection-fighting strategies. (Janovka et al., 2003).

Plants that are traditionally used in the treatment of bacterial and fungal infections or related ailments could be a good source for new, safe and biodegradable antimicrobial drugs (Hamza *et al.*, – In press), and could offer potential lead towards development of novel compounds that are active against pathogenic microbes (Runyoro *et al*., 2006). Thus, chemical studies of Tanzanian medicinal plants could provide a valuable material base for the discovery and development of new drugs of natural origin (Moshi et al., 2006).

The present study was carried out in Bukoba Rural district which is faced by severe inadequacy of health facilities, with a population-doctor ratio of 1:95,000, the lowest of all the six districts forming Kagera region in the Northern part of Tanzania (TUROT, 2003; TACAIDS et al., 2005). Majority of the population in the district are poor and cannot afford the usually expensive conventional drugs which are hardly available in many of the local health centers. Besides, many people are forced to walk for long distances to reach a health facility (TUROT, 2003). This has forced the local population to turn to traditional plant remedies which are readily available, more affordable, less toxic, and have a wide acceptance around the world (WHO, 2002). Therefore, the current study forms a strong basis for identifying plants with potential pharmaceutical applications not only in the study area, but also in the world.

Material and Methods

Criteria for selection and collection of plant materials for analysis

Different parts of eight plant species were collected from Bukoba Rural district and prepared for bioactivity and phytochemical screening. The plant species collected for analysis were *Harungana madagascariensis* (Lam) Poir., *Jatropha curcas* L., *Lantana trifolia* L., *Plectranthus barbatus* Andr., *Pseudospondias microcarpa* (A. Rich) Engl., *Psorospermum febrifugum* Spach, *Teclea nobilis* Del. and *Vernonia adoensis* (Warp.) SL. (Table 1). With a Prior Informed Consent (PIC), ethnomedical information about the eight plant species was sourced from three authentic herbal practitioners (One man and two women), who were identified with the help of local administration officers and interviewed using an open-ended semi-structured questionnaire according to Martin (1995). Voucher specimens were collected and identified by Mr. Frank Mbago and Mr. Suleiman Haji of the University of Dar es Salaam Botany Department Herbarium, where they were coded and deposited (Table1). Collection of the plant materials from the eight species for *in vitro* bioactivity assays was pegged on informant consensus on their ethnomedical uses enumerated in Table 1. Only the parts of the plants used by the herbal practitioners were collected for analysis. Leaves were collected for seven of the eight plant species while stem/bark was collected for only one plant species. No roots were collected for analysis (Tables 1, 2 and 3).

Preparation of the plant extracts

Fresh plant materials were chopped into smaller pieces before drying and then air dried under shade for a period of ten days. The dried plant materials were then pulverized using a NATIONAL SUPER® mixer grinder [MX-119] (Emerging planet India Ltd., Coimbatore 641011, India). About 300 g of each of the pulverized material was extracted using petroleum ether, dichloromethane and water in that order as successfully done in other studies (Janovka et al., 2003; Moshi et al., 2006). These crude extracts were concentrated in *vacuo* using a rotary evaporator (HEIDOLPH®, Essex Scientific laboratory supplies Ltd. Crucible house, Endway, Hadleigh, Benfleet, essex, SS7 2AN) with the bath temperature maintained at 40^0C to prevent thermal decomposition of labile compounds. The aqueous extracts were obtained by using either cold percolation or hot water to mimic the mode of extraction by the herbal practitioners. The organic solvent extracts were dissolved in DMSO while the aqueous extracts were dissolved in water at varying concentrations as done in recently reported procedure (Motsei et al., 2003).

Test microorganisms

Five test microorganisms were used in antimicrobial sensitivity tests. They were the gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (DSM 347) and gram negative bacteria

Escherichia coli (DSM 1103) and *Pseudomonas aeruginosa* (DSM 1117) and the yeast *Candida albicans* (ATCC 90028). These were obtained from the Department of Molecular Biology and Biotechnology (MBB), University of Dar es Salaam.

Preparation of the inoculum

Nutrient Broth (NB), (OXOID®, Unipath Ltd. Basingstoke, England) and Malt Extract Broth (MEB), (PRONADISA®, Conda Ltd., Madrid, Spain) were used to prepare broth cultures of bacteria and yeast test organisms respectively. The broth media were prepared according to the manufacturers' instructions. The autoclaved media was asceptically transferred to sterile capped test tubes of about 6ml each. Pure isolates of subcultured bacteria and yeast colonies were asceptically transferred to the respective broth media. The approximate cell concentration in the broth was 10^5 CFU/ml. These were incubated in DEPEX MEMMERT® incubator (Bellevue WA 98005, US) for an overnight at 37^0C and for 48 hours at 30^0C for bacteria and *Candida* respectively.

Antimicrobial activity tests

Antimicrobial activity of the crude extracts was determined by Agar well and Disc diffusion assay methods as previously described by Rojas et al. (2006) and Moshi et al. (2006).

Agar well method

Nutrient Agar (LAB M™, Lancashire, UK) and Malt Extract Agar (PRONADISA®, Conda Ltd. Madrid, Spain) were prepared for bacteria and *Candida* respectively according to the manufacturers' instructions. Immediately after autoclaving, the media was allowed to cool in a 45 to 50^0C water bath. The freshly prepared and cooled media was poured into glass, flat-bottomed petri dishes (90mm in diameter) placed on a level, horizontal surface (FASTER® Laminar flow, Cornaredo via Merendi, 22 20010, Italy) to give a uniform depth of approximately 4 mm. The agar media was allowed to cool and solidify at room temperature and the plates were incubated at 35^0C for 18-20 hours before use to ensure sterility. About 0.2ml of the test inoculum was evenly spread on the surface of the solidified agar media using a sterile grigalsky spatula. Four equidistant wells of 5mm in diameter and 4mm in depth were then made on the agar using a sterile cork borer. Two more wells for positive and negative controls were made at the middle of the agar. About 25 µl of the plant extracts and controls were filled into the wells (Rojas *et al.,* 2006). Concentrations for the crude extracts ranged from 25-200mg/ml. The positive controls were Ampicillin (SIGMA®, Sigma chemical Co., st. Louis, USA.), (0.5mg/ml) for gram positive bacteria, Gentamicin (IVEE Aqua EPZ Ltd. Nairobi, Kenya), (0.5mg/ml) for gram negative bacteria and Fluconazole (ZOCON®, FDC Ltd. India), (1mg/ml) for *Candida*. The negative controls were DMSO for organic solvent extracts and distilled water for aqueous extracts. The wells were then labeled to correspond with the code numbers of the test crude extracts and controls. The treated plates were stored in a refrigerator (DAEWOO®, Daewoo Electronics, Europe GmbH, Germany) at 4 ^{0}C for atleast six hours to allow diffusion of the extracts into the agar while arresting the growth of the test microbes. The plates were then incubated for 24 hours at 37 ^{0} C for bacteria and for 48 hours at 30^0C for *Candida*. The test was carried out in duplicates. Antimicrobial activity was determined by measuring the diameters of zones of inhibition in mm. The means and standard deviations (±SD) of the diameters of zones of growth inhibitions for the treatments are shown in Table 2.

Disc diffusion method

Preparation of the media and inoculation of the test microbes were performed as described in the agar well method. However, instead of punching out wells on the agar, sterile 5 mm-Whatman No. 1 filter paper discs (WHATMAN®, Springfield Mill, Maidstone, Kent, England) were used in the disc diffusion method (Moshi *et al*., 2006). The discs were soaked into the dissolved crude extracts for a minimum of two hours. Blank discs were impregnated with Ampicillin (10µg/disc) for gram positive bacteria, Gentamicin (15µg/disc) for gram negative bacteria and Fluconazole (0.4mg/disc) for *Candida* and used as positive controls. Negative control discs were soaked in DMSO and distilled water for organic solvent and water extracts respectively. By use of a sterile forcep, four seeded discs of the plant extracts were placed

equidistantly onto each of the inoculated plates. Two extra discs for positive and negative controls were placed at the middle of plate. The treated plates were stored in a refregerator (DAEWOO®, Daewoo Electronics, Europe GmbH, Germany) at 4^0C for atleast six hours then transferred to DEPEX MEMMERT® incubator (Bellevue WA 98005, US) for 24 hours at 37^0 C for bacteria and for 48 hours at 30^0C for *Candida*. The test was carried out in duplicates. Antimicrobial activities were determined by measuring the diameters of zones of inhibition in mm. The means and standard deviations (±SD) of the diameters of zones of growth inhibitions for the treatments are shown in Table 3.

Phytochemical screening

Screening for secondary metabolites was done for three plants, *Jatropha curcas*, *Plectranthus barbatus* and *Pseudospondias microcarpa* whose information on their bioactivity and phytochemistry is scanty with regard to their medicinal uses. Chromatograms were developed on commercial Thin Layer Chromatography (TLC) plates using different solvent systems of varying polarities (Houghton and Raman, 1998; Kinge, 2002; Orech *et al.*, 2005, Edeoga *et al.*, 2005). The chromatograms were observed under normal light and UV (254nm, 365nm) before being sprayed with the respective test reagents. Secondary metabolites were identified by color establishment using standard spray reagents on the TLC Chromatograms as exemplified in figures 2, 3 and 4. The secondary metabolites tested in all the extracts of the three plant species were alkaloids, terpenoids, triterpenes, phenolics, tannins, flavonoids, anthraquinones, flavonols/flavones and/or chalcones, sterols and saponins.

To test for alkaloids, chromatograms were sprayed with dragedorff's reagent and over sprayed with aqueous iron (III) chloride. Presence of terpenoids was detected by spraying the chromatograms with anisaldehyde and then heating them with a hot air stream from a heating gun (BLACK & DECKER®, Black & Decker, Hunt valley, MD 21031, USA). Triterpenes were detected by treating the chromatograms with 10% ammonium hydroxide. Phenolic compounds were detected by spraying the plates with 5% aqueous iron (III) chloride, then heating them with a hot air stream. Test for presence of flavonoids was done by passing the chromatograms on Ammonium hydroxide vapour. Anthraquinones were tested by spraying the chromatograms with 10% ammonium hydroxide. Presence of flavonols, flavones and/or chalcones was detected by treating the chromatograms with aluminium chloride followed by heating with hot air stream from the heating gun. Detection of tannins was done by treating the chromatograms with ferric chloride. Sterols were detected by treating chromatograms with 50% acetic anhydride in sulphuric acid followed by heating from a hot air stream. Presence of saponins was tested by shaking about 2ml of the plant extracts in distilled water.

Results

All plant species exhibited activity against at least one or more of the microorganisms tested (Tables 2 and 3). Amongst the eight plants studied, *J. curcas* and *P. barbatus* had the broadest range of activity inhibiting the growth of the gram positive, gram negative and the yeast fungus tested. *L. trifolia* had the least range of antimicrobial activity inhibiting the growth of only one of the five microbes tested. *E. coli* was the most susceptible of all the microbes tested followed by *B. subtilis.* The least susceptible microbes were *P. aeruginosa* and *C. albicans.* Generally, bacteria were more susceptible to the plant extracts than the yeast *Candida*, with Activity Index (AI) ranging between 0.22-2.96 and 0.23-0.32 for bacteria and *Candida* respectively. Moreover, activity against *C. albicans* was observed only in the disc diffusion assays but none in the agar well assays.

The highest activity was observed in the crude extracts of *V. adoensis* against *E. coli* with an Activity index (AI) of 1.76 (Table 3). The lowest activity was observed in the crude extracts of *P. microcarpa* with an Activity index of 0.22 (Table 3). However, the highest Activity index was realized in the *J. curcas* extracts at 2.96 against *S. aureus* (Table 3). The Activity Index (AI) is an expression of the degree of potency of the plant extract in relation to the standard drug (Moshi *et al.* 2006). Based on the Activity index, water extracts portrayed the highest potency of the herbal drugs screened compared to Petroleum ether or Dichloromethane extracts. There were 37 treatments which showed activity against the test organisms in the disc diffusion assays as opposed to 24 in the agar well assays (Tables 2 and 3).

In some cases, the three extracts of the same plant (petroleum ether, dichloromethane and water) had antimicrobial activity against the same microorganism. For instance, the three extracts of *H.*

madascariensis were active against *B. subtilis*, the three extracts of *P. febrifugum* were active against E. coli while those of *P. barbatus* were active against *C. albicans*. Different kinds of secondary metabolites were detected in all the plant extracts tested (Table 5). Appearance of colour establishments shown in table 4 was positive indication of the secondary metabolites tested. *P. barbatus* had the highest number of secondary metabolites, with eight out the ten metabolites screened. The plant with the least number of secondary metabolites was *P. microcarpa* with only three of the ten metabolites screened. Phenolics, tannins and sterols were present in all the extracts, while alkaloids and saponins were absent in all the extracts screened.

Table 1: Traditional uses of the plants recorded in the field.

Plant name	**Local name (Kihaya)**	**Family**	**Disease treated**	**Part used**	**Preparation and administration**	**Collection No.**
Harungana madagascariensis (Lam) Poir.	Omujumbo	Clusiaceae	Chronic diarrhea, malaria, jaundice	Leaves	Boiled and decoction drunk one cup x 3/day	DK006/06
Jatropha curcas L.	Ekiyo	Euphorbiaceae	Body sores, skin infections	Leaves	Leaves soaked in water, crushed and infusion bathed with.	DK011/06
Lantana trifolia L.	Mushikira	Verbenaceae	Chronic cough	Leaves	Boiled and decoction drunk	DK009/06
Plectranthus barbatus Andr.	Kasindano	Lamiaceae	Oral thrush, skin rashes, ring worms, Diarrhoea	Leaves	Soaked in water and infusion bathed with.	DK010/06
Pseudospondias microcarpa (A. Rich) Engl.	Omuziru	Anacardiaceae	Chronic cough	Leaves	Boiled and decoction drunk	DK005/06
Psorospermum febrifugum Spach	Ekiana	Clusiaceae	Skin infections (Body sores, skin rushes-Herpes zoster)	Bark	Boiled and decoction drunk one cup x3/day. Also decoction bathed with.	DK003/06
Teclea nobilis Del.	Omuzo	Rutaceae	Weight loss, Chronic cough	Leaves	Boiled and decoction drunk one cup x 3/day	DK005/06
Vernonia adoensis (Warp.) SL.	Nyakibasi	Asteraceae	TB	Leaves	Boiled and decoction drunk	DK008/06

Discussion

The nominal susceptibility of *C. albicans* to the plant extracts could be associated with the presence of supramolecular complexes of its cell wall including chitin, which may be difficult to digest, thus imposing resistance to prospective drugs (Marcilla et al., 1991). In the cases where the three kinds of extracts of the same plant depicted activity against the same microorganism, would possibly mean that the compound responsible for the antimicrobial activity was present in each extract at a different concentration as similarly observed by Rojas et al. (2006).An antimicrobial assay on the root extracts of *J. curcas* by Atindehou *et al.* (2002) reported a negative activity against *C. albicans.* The anti *Candida* activity of the leaf extracts recorded in the present study (Table 3) may be an indication that the active principles against the fungus are present in the leaves of the plant. It is however important that further studies on toxicity potential on leaf extracts of *J. curcas* are carried out as the oil from the seeds of the plant have been reported to be rich in toxalbumins which are poisonous proteins and usually irritant in nature (Kokwaro, 1993).

An antimicrobial flavonoid umuhengerin isolated from *L. trifolia* (Rwangabo, 1988) showed activity against *S. aureus* as opposed to the present data where there was no activity against the microbe. However, it was not clear from which part of the plant the active compound was isolated. The effects of extracts from *H. madagascariensis* against *S. aureus* and *B. subtilis* (Atindehou et al., 2002; Moulari *et al.*, 2006) and the anti *Candida* activity of *P. barbatus* (Runyoro *et al.*, 2006) are confirmed in this work

Most of the medicinal applications of the secondary metabolites reported in literature are in harmony with the traditional medicinal uses of the plants recorded here (Table 6), thus confirming the rationalization for the traditional use of the herbal remedies in treating various disease conditions.

The presence of terpenoids in *J. curcas* and *P. barbatus* could be responsible for the medicinal properties associated with these plants as they were the major group of compounds expressed on the chromatograms after treatment with Anisaldehyde spray reagent (Figure 2). The presence of tannins in *P. barbatus* could also contribute to its medicinal use for treatment of diarrhea as reported by the traditional healers. Tannins are glycosides of gallic or protocatechuic acids and have the property of precipitating proteins and mucus and also constricting blood vessels. Such stringent action of tannins gives them the medicinal value of preventing diarrhea, controlling haemorrhages and treating wounds (Kokwaro, 1993; Kisangau, 1999).

Moreover, the skin healing properties of *P. barbatus* could be associated with the presence of anthraquinones detected in the plant, which could be responsible for the plant's inhibitory activities against *S. aureus* (Tables 2 and 3). Previous reports indicate that anthraquinones are useful in healing wounds and have been used as dressing for burns and other skin lesions (Kokwaro, 1993). They are also a remedy for general skin eruptions and have the ability to inhibit the growth of *S. aureus*, a major cause of many skin infections (Mona, 1997). They could also be responsible for its use as purgative, treatment of colics, diarrhea, peptic ulcers, typhoid and stomach aches as they have the ability to stimulate bile flow and promote activity of the entire digestive process (Mona, 1997).

Phenolic compounds have been recorded to have diuretic and choleretic effects decreasing the viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis (Farombi, 2003). This means that the presence of phenolics in the three traditional plants could be possible candidates for use in the management of hypertensive cases. However, it is imperative to note that the healing effects of the reported plants could be due to synergistic action of the various compounds found in the plants. For example, the traditional use of *P. barbatus* for the treatment of diarrhea, colics or as purgative could be an effect of the presence of triterpenes, tannins, anthraquinones, flavonols/flavones or chalcones detected in the plant extracts (Table 5).

Conclusion

The fact that water extracts portrayed the highest potency of the herbal extracts against the test microbes justifies the rationale for use of water as medium for extracting herbal medicines from plants. The study unveils rich resources of bioactive plants in Bukoba rural district and lays crucial needs for isolation

and development of pharmaceuticals from the extracts reported here. In this study, we are starting efforts to incorporate the traditional healers in all stages of our research by signing Material Transfer Agreement (MTA) with them. This will ensure that they stand to benefit in the event of pharmaceutical production of their knowledge. Any new studies on ethnobotany and ethnomedicine are encouraged to do the same.

Acknowledgements

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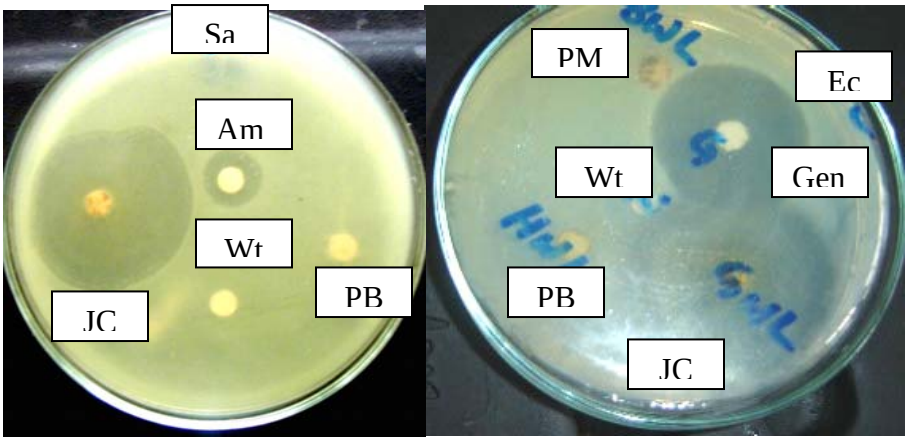


Fig 1: -Activity of *Jatropha curcas* (JC) against *S. aureus* (Sa) **(A)** and E. coli (Ec) **(B).**
-PB (*Plectranthus barbatus*), PM (*Pseudospondias microcarpa*), Am (Ampicillin), Gen (Gentamicin), Wt (water).

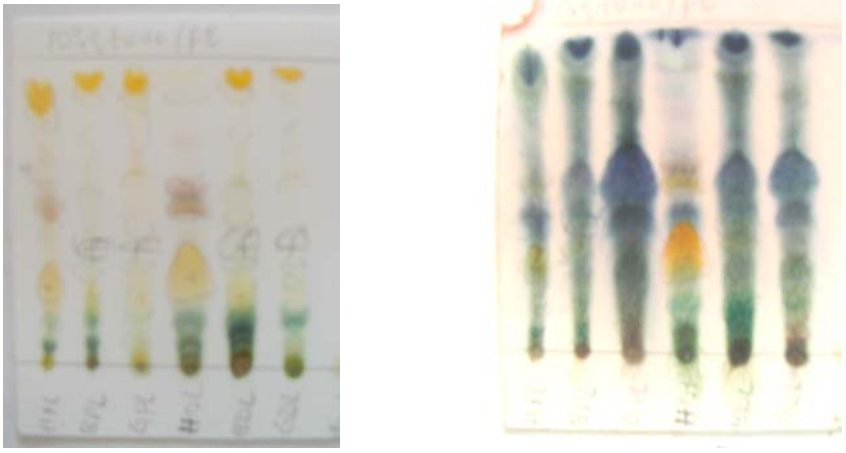


Figure 2: Test for Terpenoids using Anisaldehyde reagent: **(A)-**Reference plate **(B)-**Treated plate. (Solvent system: EtOAc: Pet ether/10:90)

Table 2: Growth inhibition zone means ±SD and AI of plant crude extracts observed in Agar well assays.

Plant name	**Part extracted**	**Extract**	**Conc (mg/ml)**	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***
Harungana madagascariensis	Leaves	Pet ether	150	3.50±0.70 AI: 0.23	24.00±1.41 AI: 1.33	-	-	-
		CH_2CL_2	130	-	9.00±0.00 AI: 0.5		-	-
		H_2O	80	-	-	8.50±0.70 AI: 0.32	-	-
Jatropha curcas	Leaves	Pet ether	160	-	-	-	-	-
		CH_2CL_2	130	-	10.50±0.70 AI: 0.58	-	-	-
		H_2O	85	-	-	-	22.00±0.00 AI: 0.74	-
Lantana trifolia	Leaves	Pet ether	25	-	-	8.00±1.41 AI: 0.31	-	-
		CH_2CL_2	40	-	-	-	-	-
		H_2O	80	-	-	-	-	-
Plectranthus barbatus)	Leaves	Pet ether	130	7.50±0.70 AI : 0.5	16.50±2.12 AI : 0.92	-	-	-
		CH_2CL_2	70	-	-	23.5±2.12 AI : 0.90	-	-
		H_2O	60	33.00±0.00 AI : 2.4	10±2.62 AI : 0.55		-	-
Pseudospondias microcarpa	Leaves	Pet ether	90	-	-	7.5±2.12 AI: 0.29	-	-
		CH_2CL_2	160	-	-	-	8.00±00 AI: 0.27	-
		H_2O	100	-	8.5±0.70 AI: 0.47	-	-	-

Table 2: Growth inhibition zone means ±SD and AI of plant crude extracts observed in Agar well assays ontinued

Psorospermum febrifugum	Bark	Pet ether	100	-	-	8.00±1.41 AI: 0.31	-	-
		CH_2CL_2	170	-	7.50±2.12 AI: 0.42	9.00±1.41 AI: 0.34	-	-
		H_2O	100	-	9.00±0.00 AI: 0.50	11.00±00 AI: 0.42	-	-
Teclea nobilis	Leaves	Pet ether	170	-	10.00±0.00 AI: 0.55	4.50±0.70 AI: 0.17	-	-
		CH_2CL_2	170	-	-	-	-	-
		H_2O	170	-	-	-	-	-
Vernonia adoensis	Leaves	Pet ether	70	-	-	4.00±0.00 AI: 0.15	-	-
		CH_2CL_2	160	-	6.50±0.70 AI: 0.36	-	-	-
		H_2O	200	-	-	-	-	-
Standards				Amp: 13.00±1.41	Amp: 11.00±7.07	Gent: 20.00±0.00	Gent: 18.00±2.83	Fluc: 21.00±0.00

Key: AI: Activity Index = Inhibition zone of the test sample divided by inhibition zone of a standard drug.

Table 3: Growth inhibition zone means ±SD and AI of plant crude extracts observed in Disc diffusion assays.

Plant name	**Part extracted**	**Extract**	**Conc (mg/ml)**	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***
Harungana madagascariensis	Leaves	Pet ether	150	-	8.50±2.12 AI: 1.06	-	-	-
		CH_2CL_2	130	-	9.50±0.70 AI: 1.19	-	-	-
		H_2O	80	25.00±0.00 AI: 1.85	14.00±0.00 AI: 0.78	-	-	-
Jatropha curcas	Leaves	Pet ether	160	8.50±0.70 AI: 0.63	7.00±0.00 AI: 0.34	-	8.50±0.70 AI: 0.28	-
		CH_2CL_2	130	-	-	14.5±0.70 AI: 1.73	-	9.00±0.00 AI: 0.32
		H_2O	85	40.00±0.00 AI: 2.96	8.50±0.70 AI: 0.47	39.00±00 AI: 1.50	15.00±1.41 AI: 1.54	9.00±0.00 AI: 0.32
Lantana trifolia	Leaves	Pet ether	25	-	-	-	-	-
		CH_2CL_2	40	-	-	-	-	-
		H_2O	80	-	-	-	-	-
Plectranthus barbatus	Leaves	Pet ether	130	10.00±1.41 AI: 0.74	7.00±0.00 AI: 0.34	-	19.00±1.41 AI: 0.64	9.00±00 AI: 0.32
		CH_2CL_2	70	-	-	-	21.00±1.41 AI: 0.71	6.50±0.70 AI: 0.23
		H_2O	60	14.00±1.41 AI: 1.03	9.00±2.82 AI: 0.50	-	-	8.00±0.00 AI: 0.29
Pseudospondias microcarpa	Leaves	Pet ether	90	9.00±0.00 AI: 0.70	5.50±0.70 AI: 0.31	7.50±2.12 AI: 0.29	-	-
		CH_2CL_2	160	-	-	-	6.50±0.70 AI: 0.22	-
		H_2O	100	-	-	-	-	-

Table 3: Growth inhibition zone means ±SD and AI of plant crude extracts observed in Disc diffusion assays coontinued

Psorospermum febrifugum	Bark	Pet ether	100	7.00±1.41 AI: 0.52	7.00±1.41 AI: 0.34	9.00±00 AI: 0.35	-	7.00±00 AI: 0.25
		CH_2CL_2	170	-	10.00±0.00 AI: 0.55	8.00±0.00 AI: 0.31	-	-
		H_2O	100	-	-	-	-	-
Teclea nobilis	Leaves	Pet ether	170	-	-	7.50±0.70 AI: 0.29	-	-
		CH_2CL_2	170	-	-	-	-	-
		H_2O	170	-	-	-	-	-
Vernonia adoensis	Leaves	Pet ether	70	-	-	-	-	-
		CH_2CL_2	160	-	-	-	-	-
		H_2O	200	20±0.00 AI: 1.48	10.00±0.00 AI: 0.55	46.00±1.41 AI: 1.76	-	-
Standards				Amp: 13..50±1.29	Amp: 18.00±4.24	Gent: 26.00±4.61	Gent: 29.75.00±2.06	Fluc: 27.75±2.62

Key: AI: Activity Index = Inhibition zone of the test sample divided by inhibition zone of a standard drug.
- Amp: Ampicillin (10µg/disc), **Gent:** Gentamicin (15µg/disc), **Fluc:** Fluconazole 0.4mg/disc).

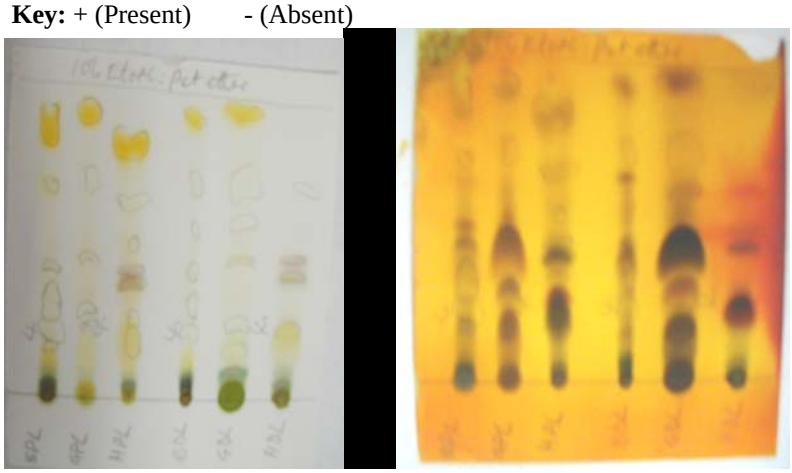
Table 4: Detection of secondary metabolites by different colour indications

Secondary metabolites	**Reagent used**	**Positive colour indication**
Alkaloids	Dragedorff's reagent	Orange
Terpenoids	Anisaldehyde	Purple to blue
Triterpenes	10% Ammonium hydroxide	Purple
Phenolics	5% Aqueous Iron (III) Chloride	Green to blue
Tannins	Ferric chloride	Blue-black to green
Flavonoids	Ammonium hydroxide vapour	Deep yellow
Anthraquinones	10% Ammonium hydroxide	Violet
Flavonols/Flavones and/or Chalcones	Aluminium chloride	Yellow
Sterols	50% Acetic anhydride in H_2SO_4	Green to blue
Saponins	Shaking in water	Froth formation

Table 5: Distribution of secondary metabolites from the extracts of the selected plant species

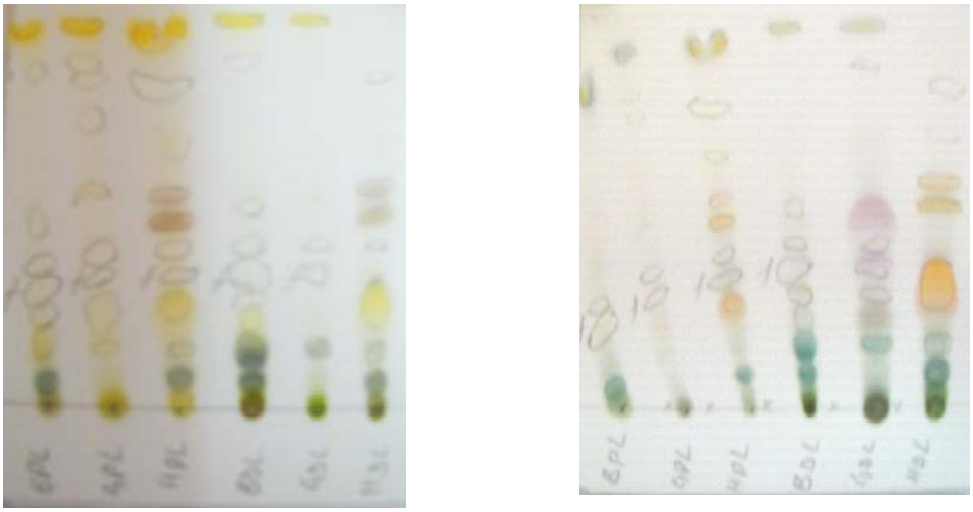
Compounds	***Jatropha curcas* (Leaves)**	***Plectranthus barbatus* (Leaves)**	***Pseudospondias microcarpa* (Leaves)**
Alkaloids	-	-	-
Terpenoids	+	+	-
Triterpenes	-	+	-
Phenolics	+	+	+
Tannins	+	+	+
Flavonoids	-	+	-
Anthraquinones	-	+	-
Flavonols/Flavones/ Chalcones	-	+	-
Sterols	+	+	+
Saponins	-	-	-

Key: + (Present) - (Absent)



A **B**

Figure 3: Test for Tannins using Ferric chloride: **(A)-**Reference plate, **(B)-**Treated plate (Solvent system: EtOAc: Pet ether/10:90)



A **B**

Figure 4: Test for Flavones, Flavonols or Chalcones using Aluminium chloride: **(A)-**Reference plate (**B)-**Treated plate. (Solvent system: EtOAc: Pet ether/10:90)

Table 6: Examples of Secondary metabolites against reported medicinal uses*

Plant name	Traditional use	Class of compounds	Reported medicinal uses of the compounds
Harungana madagascariensis	Chronic diarrhoea, head ache, malaria, dysentery, Jaundice	Flavonoids	a, b, c, d.
		Tannins	e, f, d, g.
		Saponins	h, i.
		Alkaloids	j, k.
Jatropha curcas	Body sores, skin infections, wounds, chest diseases, kidney failure.	Terpenoids	l
		Phenolics	m, n, o.
		Tannins	e, f, d, g.
		Sterols	p, d.
Lantana trifolia	Chronic cough, rheumatism, indigestion, hepatic diseases, colds, flue, anaemia.	Flavonoids	a, b, c, d.
Plectranthus barbatus	Oral thrush, skin rashes, ring worms, Diarrhoea, Colics, purgative	Terpenoids	l
		Triterpenes	g, q, k.
		Phenolics	m, n, o.
		Tannins	e, f, d, g.
		Flavonoids	a, b, c, d.
		Flavonols/flavones or chalcones	b, k, g.
		Sterols	p, d.
		Anthraquinones	g, d, e.
Pseudospondias microcarpa	Chronic cough	Phenolics	m, n, o.
		Tannins	e, f, d, g.
		Sterols	p, d.
Psorospermum febrifugum	Skin infections, pimples, scabies, leprosy	Phenolics	m, n, o.
Teclea nobilis	Weight loss, Chronic cough, rheumatism, fever, pneumonia, anthelmintic, malaria	Alkaloids	j, k.
Vernonia adoensis	TB	Terpenoids	l

Key: a-Extend activity of vitamin C, **b**-Antioxidant, **c**-Anti-inflammatory, **d**-Skin injury, wounds, dermal ulcers, skin eruptions, dressing burns and skin lesions, **e**-Prevention of diarrhoea, typhoid, purgative**, f**-Controlling haemorrhages, **g**-Treating peptic ulcers, abdominal pains, **h**-Stimulating immune system, **i**-Arthritis, rheumatism, **j**-Bitter tonics, and stomachics, **k**-Malaria and fever, **l**-Improvement and maintenance of body health, **m**-Stimulating intestinal peristalsis, **n**-Diuretic, **o**-Reducing blood pressure, **p**-Reducing cholesterol, concentration and maintaining good heart health, **q**-asthma.

*Additional information on traditional uses of the plants and the classes of compounds is from secondary sources: Amonkar *et al.* (1981), Rwangabo (1988), Kokwaro (1993), Beentje (1994), Mona (1997), Kisangau (1999), Craig (1999), Okoli *et al.* (2002), Repetto and Llesuy (2002), Farombi (2003), Wagner and Emadfa (2003), Richelle *et al.* (2004), Berger *et al.* (2004), Njoroge *et al.* (2004), Moulari *et al.* (2006),

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