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## EVALUATION OF METHANOLIC EXTRACTS OF SIX MEDICINAL PLANTS USED BY HERBAL PRACTITIONERS IN CENTRAL PROVINCE- KENYA

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### ABSTRACT

The antimicrobial effects of selected medicinal plants commonly used by herbal practitioners in central province Kenya was evaluated on different bacterial strains- *Staphylococcus aureus* (Gram +ve cocci) - ATCC 20591, *Salmonella typhi* (Gram -ve rod) - ATCC 2202, *Escherichia coli* (Gram-ve rod) - STD. 25922, *Klebsiella pneumoniae* (clinical isolate) and *Pseudomonas aeruginosa* (Gram-ve rod) - ATCC 25852. Also *Candida albicans* ATCC EK138 was used as a fungal isolate. Methanol was used as the only solvent in the extraction. The *in vitro* antimicrobial activity was performed by agar disc diffusion method. The most susceptible Gram-positive bacteria was *S. aureus* (between 19.33-23.33mm), while the most susceptible Gram-negative bacteria was *P. aeruginosa* (14.66-19.33mm). All the extracts showed sufficient inhibitory activity to the test strains. The Gram positive strain (*S. aureus*) was more sensitive to the extracts (range 23.33-19.33mm) than the Gram negative strains (range 21.00-14.66mm). The mean inhibition value was between 15.997mm and 19.995mm. Statistical analysis revealed that *Hyptis spicigera* and *Crotalaria quartiniana* produced significantly different ( $P \leq 0.05$ ) zones of inhibition in all the test strains. Other extracts average zones of inhibition showed no significant difference among the test strains. The significant antibacterial activity of active extracts was compared with the standard antimicrobials (Fluconazole for *C. albicans*, and amoxicillin for bacterial isolates) and dried methanol discs, giving a pooled SD of 2.349mm. The results obtained in the present study suggest that the extracts can be used in treating diseases caused by the test organisms.

#### Keywords:

Medicinal plants,  
Antimicrobial activity,  
MIC,  
MBC,  
Methanol extract

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**INTRODUCTION:** Diarrhoea is a killer disease worldwide<sup>1, 2</sup> and unfortunately, it is among the symptoms of many other diseases. In most communities of developing countries it poses serious problems particularly for children due to amongst other reasons, lack of adequate sanitation and pipe borne water<sup>3</sup>. The disease burden worldwide from water, sanitation and hygiene together has been calculated to be 4% of all deaths and 5.7% of the total disease burden. Amongst the many known water borne diseases, diarrhoeal diseases kill more than 1.8 million people every year, mostly children from developing countries<sup>4</sup>. The major causative agents of diarrhoea in man include: *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*<sup>5</sup>. *Candida albicans* has also been known to cause diarrhoea in humans<sup>6</sup>.

Man has used plants to treat and prevent various common infectious diseases including diarrhoeal ailments since time immemorial<sup>7</sup>, and some of the traditional medicines are still included as part of the habitual treatment of various maladies in various communities in the world<sup>8,9</sup>. It is estimated that 20,000 species from several families are useful for these purposes<sup>10</sup>. Furthermore, about 80% of the world's population is dependent (wholly or partially) on plant-based products<sup>11</sup>. Scientific interest in medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of modern medicine.

There is also the continuing emergence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials which have reduced the efficacy of antimicrobial agents currently in use. For instance, more than 70% of the bacteria causing infections are resistant to at least one of the drugs commonly used for their

treatment<sup>4</sup>. This situation has been worsened by HIV/AIDS pandemic, poverty, an upsurge of new and re-emerging infectious diseases, high costs and side effects of available drugs<sup>12, 13</sup>. This has resulted in increased severity of infectious diseases and high mortality rates from certain infections.

The emergence of multiple drug resistance in human pathogenic bacteria and fungi is stimulating research directed towards the discovery of novel antibacterial and antifungal agents from other sources including plants<sup>14</sup>. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents<sup>15</sup>. Thus, it is possible that phytochemicals with adequate antimicrobial efficacy will be used for the treatment of microbial infections<sup>16</sup>. The aims of the policy of World Health Organization (WHO) on medicinal plant materials include ascertaining their safety, efficacy and specifications<sup>18</sup>. This study is therefore aimed at assessing the effect of six methanol extracts on the selected bacterial strains and *C. albicans*. Preliminary phytochemical assessment was undertaken to provide clues on active secondary compounds in the plants.

#### **Methodology:**

- **Plant material collection, identification and extract preparation:** Mature fresh parts of six medicinal plants were collected from Twiga, Marera and Tatu regions in Central province, Kenya. They were collected based on the ethnobotanical survey that was carried out in August 2009. The plants were authenticated by a plant taxonomist from the Department of Pharmacy and Complimentary Alternative Medicine (CAM), Kenyatta University, Nairobi, Kenya, in whose herbarium the voucher specimens are deposited. The various plant parts collected were chopped into small pieces,

shade dried and ground using a hammer type milling machine (Meecon, CM/L-1364548, India). The powdered materials were transferred into 250ml quick fit flasks and extracted in the soxhlet extractor using methanol for 72 h<sup>18</sup>. The extracts were filtered through a Whatmann filter paper No. 42 (125 mm) and concentrated using a rotary evaporator (Laborota 4000, SN 090816862, Germany) with the water bath set at 40 °C<sup>19</sup>, then dried in a dessicator over anhydrous CuSO<sub>4</sub>. The powdered residue were transferred into vials and stored at 4 °C in airtight vials till the time of analysis.

- **Antimicrobial screening/bioassay:**

**a. Test cultures:** The type culture isolates were obtained from Kenyatta National Hospital in Nairobi, Kenya which included *Staphylococcus aureus* (Gram +ve cocci) - ATCC 20591, *Salmonella typhi* (Gram -ve rod) - ATCC 2202, *Escherichia coli* (Gram-ve rod) - STD. 25922, *Klebsiella pneumoniae* (clinical isolate) and *Pseudomonas aeruginosa* (Gram-ve rod) - ATCC 25852. They were selected on the basis of their role in causing diseases and their cell wall properties. Also *Candida albicans* ATCC EK138 isolates were used as fungal test cultures.

The test strains of the bacteria were kept refrigerated on Muller-Hinton (Merck, Germany) agar slants during the experimental period and were subcultured and incubated for 24h at 37°C then tested biochemically for purity before use. Fungal strains were also maintained on Potato Dextrose Agar (PDA) at 4°C and were also subcultured in PDA broth before they were tested for purity prior to their usage.

**b. Disc diffusion method:** The antimicrobial assay was performed by agar disc diffusion method for methanol extracts<sup>20, 21</sup>. The Mueller Hinton agar (Biotec) was prepared following the manufacturers

instructions and was inoculated with 100 µl of the inoculum that is equivalent to MacFarland turbidity standard of 0.5 ×10<sup>6</sup> CFU/ml which were spread plated. Then a disc (6 mm) was saturated with 100 µl of the plant extract, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter from the end of growth to the disc at one end to the beginning of growth at the other end by use of a transparent ruler. For each bacterial strain, 250 µg of amoxicillin (Hangzhou Ruijian chemical Co., Ltd., batch 490805241) was used as positive control and methanol as the negative control. The results were again obtained by measuring the zones of inhibition. The experiment was repeated three times and the mean values recorded.

The fungal isolate was cultured by taking 0.1mls from the broth and culturing by spread plate method and then incubating at 25°C for 72h. By use of the cork boarer, a section with the young mycelium was picked and placed on a PDA plate. The dry disc (6mm) treated with 0.1ml of the plant extracts that were made by dissolving 300mg of the extracts in 1ml of methanol were placed at a distance around the section of the mycelium extracted and were incubated at 25°C for 48-72h. Fluconazole (Pfizer Ltd., UK batch 30) was impregnated into sterile discs (6mm diameter) by dispensing 0.1ml of the dissolved 300mg drug in ethanol. The discs were then left to dry before they were also mounted onto the PDA with the extracted mycelium. Dry discs treated with methanol were used as the negative control.

**c. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC):** This was done only where the plant extract showed strong antibacterial activity by the disk diffusion method (≥9 mm) (22). A micro-dilution technique using 96

well micro-titre plates<sup>23, 3</sup>, was used to obtain MIC values of the crude extracts against all the test bacteria or fungi. Each plant extract was serially diluted to obtain 75mg/ml, starting from the first well. Similar serial dilutions were performed for Fluconazole (300mg) for *C. albicans* and Amoxicillin (250mg) for bacterial strains as positive controls. An equal volume of 50µl of fresh bacterial or fungal cultures in nutrient and potato dextrose agar respectively were adjusted to match 0.5 MacFarland standards and added to each of the wells.

Micro titer-plates were covered and incubated at 37°C overnight for the bacterial strains and at 72h for the fungal strain. The MIC values were determined as the lowest concentrations of the extract showing no growth. All the wells where no growth (not turbid) was observed were subcultured, and the lowest concentration of the plant extracts that did not yield any colony on the solid nutrient medium for the bacterial cultures and PDA for the fungal cultures after sub-culturing and incubating for 24h for the bacterial cultures and 72h for the fungal cultures were taken as the MBC or MFC respectively. All tests were performed in triplicate<sup>24</sup>.

- **Phytochemical screening:** Qualitative phytochemical analysis of the crude powder of the plants collected was determined by established methods<sup>16, 19</sup>.
- **Statistical analysis:** The mean zones of inhibition for each test culture were analyzed by one-way analysis of variance (ANOVA) to get the differences among group means. A P value < 0.05 was considered as significant. The means were separated by the Tukey's test. The computer software STATISTICA® was employed for the statistical analysis.

**RESULTS:** Following the ethnobotanical survey six medicinal plants were collected (**Table 1**). The medicinal plants belonged to different families such as Euphorbiaceae, Verbenaceae, Labiatae, Fabaceae, Leguminosae and Asteraceae. There was no predominant family in terms of the species of the medicinal plants collected. The herbal practitioners' harvests various parts like the bark, whole plant and leaves. The leaves were found to be the most common part harvested.

The antibacterial activities of 6 plant species were assayed *in-vitro* by agar disc diffusion method against 5 bacterial strains and 1 fungal isolate (**Table 2**). Table 2 summarizes the average microbial growth inhibition of the methanolic extracts of the screened plant species against the test cultures. All the extracts had significant activity against both the bacterial and fungal isolates. From the findings, the Gram positive isolate was more sensitive to the methanol extracts than the Gram negative isolates. *Lippia kituensis* (23.33mm) and *Tithornia diversifolia* (21.66mm) produced the highest average zones of inhibition against *S. aureus*.

However the methanol extracts were active against *C. albicans* producing average inhibition zones. *Crotalaria quartiniana* produced the highest average zone of inhibition against *C. albicans* (19.66mm). All the extracts produced better average zones of inhibition than the positive control (Fluconazole-13mm) against *C. albicans*. *Hyptis spicigera* and *Crotalaria quartiniana* produced significantly different zones of inhibition in all the test strains.

All of the methanol extracts produced high MICs and MBCs/MFCs (**Table 3**). Most of the methanol extracts produced MICs and MBCs/MFCs ranging from 18.75 mg/ml to 37.5 mg/ml. The MICs concentrations were not the same concentrations for the MBCs/MFCs in most of the extracts.

**TABLE 1: SELECTED MEDICINAL PLANTS USED BY COMMUNITIES IN CENTRAL PROVINCE, KENYA**

Botanical Name	Family name	Where collected from	Part(s) used	Diseases treated
<i>Eurphobia hirta</i> L.	Euphorbiaceae	Twiga	Whole	Diarrhoea, asthma
<i>Lippia kituiensis</i> Vatke	Verbenaceae	Marera	Leaves	Diarrhoea, chest problems
<i>Erythrina melanacantha</i> Harms	Leguminosae	Twiga	Bark	Stomach aches, chest pains
<i>Tithornia diversifolia</i> (Hemsl.) A. Gray	Asteraceae	Twiga	Leaves	Diarrhoea
<i>Hyptis spicigera</i> Lam	Labiatae	Marera	Leaves	Stomach ache, pulmonary troubles
<i>Crotalaria quartiniana</i> A.Rich.	Fabaceae	Tatu	Leaves	Diarrhoea

**TABLE 2: THE AVERAGE ZONES OF INHIBITION (MM) OF THE SELECTED MEDICINAL PLANTS AGAINST THE TEST CULTURES**

Extract	1	2	3	4	5	6	Mean
<i>Eurphobia hirta</i>	21.00	18.66	19.66	16.33	16.33	14.33	17.718ab
<i>Lippia kituiensis</i>	23.33	16.00	18.33	17.66	15.33	16.66	17.885ab
<i>Erythrina melanacantha</i>	19.00	15.66	17.33	14.66	16.66	16.33	16.607ab
<i>Tithornia diversifolia</i>	21.66	19.00	14.66	19.33	18.00	13.66	17.718ab
<i>Hyptis spicigera</i>	19.33	15.33	13.00	15.66	17.66	15.00	15.997b
<i>Crotalaria quartiniana</i>	19.33	21.00	20.66	18.66	20.66	19.66	19.995a
Positive control	23.00	21.33	20.22	17.33	17.66	13.00	18.757ab
Negative control	6.00	6.00	6.00	6.00	6.00	6.00	6.000c

Key: 1- *S. aureus*, 2- *S. typhi*, 3- *E. coli*, 4- *P. aeruginosa*, 5- *K. pneumoniae* 6- *C. albicans*. Positive controls - Fluconazole for *C. albicans* and amoxicillin for bacterial isolates. Negative control- dried methanol discs. Values are means of triplicates

**Note:** Mean inhibition values denoted by similar letters are not significantly different

**TABLE 3: THE MICs AND THE MBCs/MFCs (MG/ML) OF THE MEDICINAL PLANTS AGAINST THE TEST CULTURES**

BOTANICAL PLANT NAME	<i>S. aureus</i>		<i>S. typhi</i>		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>K. pneumoniae</i>		<i>C. albicans</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
<i>Eurphobia hirta</i>	18.75	18.75	37.5	75	37.5	75	18.75	18.75	18.75	37.5	37.5	75
<i>Lippia kituiensis</i>	37.5	37.5	18.75	37.5	18.75	18.75	37.5	37.5	37.5	37.5	37.5	37.5
<i>Erythrina melanacantha</i>	37.5	75	37.5	75	37.5	37.5	37.5	37.5	18.75	18.75	37.5	37.5
<i>Tithornia diversifolia</i>	37.5	37.5	18.75	37.5	18.75	18.75	18.75	18.75	18.75	37.5	75	75
<i>Hyptis spicigera</i>	37.5	37.5	18.75	37.5	18.75	18.75	37.5	37.5	18.75	75	37.5	75
<i>Crotalaria quartiniana</i>	37.5	37.5	37.5	75	37.5	37.5	37.5	37.5	18.75	18.75	18.75	37.5
Positive controls	18.75	18.75	18.75	37.5	37.5	75	37.5	75	37.5	75	75	75

*K. pneumoniae* was most affected by most extracts where its growth was inhibited at low concentrations. Higher concentrations of the extracts used were found to inhibit the growth of *C. albicans* compared to the ones observed for the bacterial isolates. *Lippia kituiensis* was observed to have inhibited the growth all the test isolates at the lowest concentrations that ranged from 18.75mg/ml to 37.5mg/ml. Various phytochemicals were found to be present in the six medicinal

plants screened as summarized in **Table 4**. The tested phytochemicals were tannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids. *Hyptis spicigera* and *Crotalaria quartiniana* were found to possess all the phytochemicals under test with the rather having moderate to high concentrations of most of the screened phytochemicals.

**TABLE 4: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE MEDICINAL PLANTS**

Extract	Tannins	saponins	flavonoids	Steroids	Cardiac glycosides	Alkaloids (Wagner's test)
<i>Eurphobia hirta</i>	++	+	-	+	++	+
<i>Lippia kituiensis</i>	++	-	++	++	++	+
<i>Erythrina melanacantha</i>	++	+	-	+	+	-
<i>Tithornia diversifolia</i>	+++	++	+	-	-	+
<i>Hyptis spicigera</i>	+	+++	++	+	++	+
<i>Crotalaria quartiniana</i>	+++	++	++	+	+	+++

+++ = Present in high concentration, ++ = Moderately Present, + = Trace, - = Absent

Tannins were found to be the most common phytochemical in the extracts screened as it was present in all the extracts in different concentrations. Saponins were also found to be present in most extracts in substantial amounts except for *Lippia kituiensis*. Cardiac glycosides and steroids were found in all the extracts in substantial amounts except in *Tithornia diversifolia*. Flavonoids were also found to be absent in *Eurphobia hirta* and *Erythrina melanacantha*.

**DISCUSSIONS:** Just like other communities in the world it's evident that the community around the Twiga, Marera and Tatu regions in Central province, Kenya uses medicinal plants to treat common ailments like diarrhoea. In most of the medicinal plants, harvested leaves were found to be the major parts used by the herbal practitioners in the management of diarrhoeal ailments (Table 1).

The results obtained give a clear indication that the selected medicinal plants screened possess an inhibitory activity against the test cultures used. Our data show that, in general, the antibacterial activity of the extracts has more inhibitory effect on the Gram-positive test cultures than the Gram-negative types. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria and could have played a big role

in the general permeability of the cell walls of the extracts<sup>25</sup>. For instance *S. aureus* was more sensitive to *Lippia kituiensis* extracts (23.33mm) than other strains of bacteria screened (Table 2). Among the Gram negative bacteria *K. pneumoniae* was the least sensitive to the methanol extracts used. *Crotalaria quartiniana* produced a high inhibitory activity against the Gram negative bacteria than the Gram positive bacteria.

This may not be subscribed to the cell wall properties but may be due to synergistic properties of the phytochemicals that the extract may possess<sup>3</sup>. Fungi are mostly resistant to antimicrobial agents, however, from this study, *C. albicans* was inhibited by all the extracts of the plants. This means that the plants could be used for the treatment of *Candida* infections<sup>26</sup>. The methanol extracts also produced a substantial inhibitory activity against *C. albicans* with *Crotalaria quartiniana* producing the widest zone of inhibition (19.66mm).

All the extracts produced on average zones of inhibition that were wider than that produced by the positive controls, which is very encouraging and we are currently in the process of screening for isolation of active individual compounds. Most methanol extracts were bacteriostatic rather than bactericidal to most test isolates (Table 3). The extracts however produced good inhibitory concentrations where some produced lower or

same MICs as the positive control. For instance *Eurphobia hirta* produced MICs of 18.75mg/ml against *S. aureus* at a concentration which was the same as that produced by the positive control. For the *C. albicans* the methanol extract of *Erythrina melanacantha* produced the same MIC as that of the positive control- fluconazole.

Thus generally the extracts produced substantial activity against the test strains. It was also observed that the extracts possessed some phytochemicals like tannins, alkaloids, cardiac glycosides, saponins among others (Table 4). Such phytochemicals have been known to possess antimicrobial properties<sup>27</sup>. For instance *Hyptis spicigera* has been found to possess substantial amounts of saponins which have been found to possess the cell membrane lytic abilities<sup>28</sup> that could account for the high bactericidal activity of the methanolic extract<sup>27</sup>. Antibiotic resistance in beta-lactamase producers like *K. pneumoniae* is of growing concern clinically<sup>4</sup>.

In this study, *H. spicigera* leaf extracts showed strong bactericidal and bacteriostatic potency against antibiotic resistant strains of these organisms *in vitro*. These finding agrees with the findings of Ladan *et al.*,<sup>27</sup> who tested the leaves of *H. spicigera* against *K. pneumoniae*. Similarly, tannins which were detected in all the plant samples (Table 4) have been reported to have antimicrobial potential due to their potential to react with proteins, forming stable water soluble compounds, thereby killing the bacteria by directly damaging the cell membrane<sup>29, 30, 31</sup>.

In a study of *Tithornia diversifolia*<sup>26</sup>, it was observed that the methanol extracts of *Tithonia diversifolia* were more active against *Salmonella typhi*, *Pseudomonas aeruginosa* and *Candida albicans* a finding that is in contrast with findings in this study. It was further observed that the presence of saponins could be responsible for this

activity. Our data shows that *Tithonia diversifolia* extracts were active against *S. aureus* but this finding is contradicted by previous findings<sup>26</sup>. This could be as a result of the differences in extraction methods and the geographical localities where the medicinal plants were harvested from<sup>3</sup>.

**CONCLUSION:** The results of the investigation show that the medicinal plants contain pharmacologically active substances with antibacterial and antifungal properties. It also points out that there is a possibility of getting effective compounds from natural sources, which can be of value in the fight against most of the multidrug resistant strains of organisms and other infectious diseases. The study provides support for the use of these plants in the management of infectious diseases.

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