



ORIGINAL RESEARCH ARTICLE

Antimicrobial activities of selected medicinal plants of the Tugens of Koibatek, district, Kenya.Maara T.N.^{1*}, Macharia² and Ahenda J.O.³¹Department of Natural Resources, Egerton University, Njoro, Kenya.²Department of Biological Sciences, Egerton University, Njoro, Kenya.³Kenya Plants Health Inspectorate Services, Nairobi, Kenya.

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Abstract: Based on the number of ailments a plant is used to treat and the difficulty in propagating them from seeds, nine top ranked species were selected for this study. Plant barks were extracted, air dried, ground to moderately fine powder and Soxhlet extracted with methanol. The extracts were screened for alkaloids, sterols, saponins, flavanoids and anthraquinones. Antimicrobial tests were performed with five bacteria and one fungus strains using agar diffusion method. Brine Shrimp Lethality test was also used to test for bioactivity. Probit analysis on a Finney computer program was used to determine the lethal concentration (LC₅₀) for half of the test organisms. Ethnobotanical survey results indicated that the local people used 47 medicinal tree species and their uses were identified. Two to four secondary compounds were recorded in extracts of all species. Extracts exhibited antibacterial activity against one to four types of bacteria with inhibition zones ranging from 18 – 34 mm. Extracts from all species except *Myrica salicifolia*, *Toddalia asiatica* and *Rauwolfia caffra* showed antifungal activity.

Key words: Antimicrobial tests; Brine Shrimp Lethality test; Ethnobotanical survey

Introduction

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world (Prabhat and Avnish, 2010). It is the oldest form of healthcare known to mankind (Girma, 2005; Manzoor and Maksuda, 2000) and remains an important element of human and livestock healthcare systems in many developing countries (Lambert *et al.*, 2005). Presently approximately 60 - 80 % of the world population and 90 % in Africa use herbal medicine for their primary health care (Ramzi *et al.*, 2008; Musyimi *et al.*, 2008; Parekh *et al.*, 2006; Kim, 2005, Hostettmann *et al.*, 2000; Hamann, 1991). In China for example, herbal medicine is an integral part of the formal health system (Akerle *et al.*, 1991). Industrialized countries also use medicinal plants, as many pharmaceuticals are based on or derived from plants compounds. Plant products have played an important role in the discovery of new therapeutic agents since ancient times. For example, quinine which is obtained from *Cinchona* species has been successfully used to treat malaria (Sujata *et al.*, 2004).

At least over 1,200 flora of Kenya have been recorded to have ethno-medical application by the 42 ethnic groups (Ogweno *et al.*, 2002). These plants have also been reported to contain a vast array of substances that can be used to treat chronic and infectious diseases (Sumathi and Parvathi, 2010). This has resulted in herbal

medicine taking a vital part in our health care system that is characterized by mushrooming of herbal medicine operators. Similarly, local and international trade in herbal medicine has grown to a multi-billion-dollar industry. In recognition of the important role played by herbal medicine, the government in its National Biodiversity Strategy of 1996 emphasized on conservation of such resources. This led to the process of registration of herbalists through the Ministry of Social Services.

The medicinal value of the plants lies in some chemicals that produce a definite physiological action on human body (Duraipandiyar *et al.*, 2006). Plants naturally synthesize several carbon compounds basically for physiological functions or for use as chemical weapons against disease organisms, insects and predators (Fatope, 1995). The antimicrobial properties of medicinal plant extracts have also been related to the ability to treat chronic as well as infectious diseases (Ushimaru *et al.*, 2007; Veeramuthu *et al.*, 2006; Duraipandiyar *et al.*, 2006). These secondary metabolites produce some biological activity in man and animals. Screening of methanol extracts of some plant materials have revealed presence of alkaloids, tannins and flavonoids (Dash and Murthy, 2011). The most important of these bioactive compounds includes alkaloids, flavanoids, tannins and phenolic compounds.

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Traditionally, the older people have been the custodians of indigenous knowledge on herbal medicine. However, this knowledge is rapidly being lost because the main mode of transmission from one generation to another is oral; there has been little documentation. Herbalists and their patients use herbal medicine without knowledge of active compounds present in a particular plant species. That is partly why most herbal medicines are administered as concoctions. Disease causing pathogens are also becoming increasingly resistant to conventional drugs; consequently, people are reverting to herbal remedies. This scenario necessitates investigations into the bioactive compounds in the plants and their antimicrobial activity.

In the study area, with a population of 217,480 and an annual growth rate of 5.3 %, over 200,000 people are dependent on herbal medicine for their health care. Determining the active compounds in plant extracts and their antimicrobial activity will eventually improve the prescription and dosage of medicines dispensed once the compounds are isolated and tested against the causal organisms. This will increase the efficacy of herbal medicines. Such endeavors in future will also identify specific plants to be used to treat specific ailments, therefore reducing the wanton destruction of these plants. Results from this study will serve as a guide to identify priority species for conservation/ domestication which will ensure availability of their products for future use.

Materials and Methods

Location of the study Area

Plant materials were collected in Eldama Ravine and Esageri Divisions of Koibatek District in the Rift Valley Province (Fig. 2). The district is located between longitudes 35° 30" and 35°15" East and between latitudes 0°10" South and 0° 25" North and the altitude ranges from 1550 m to 2100 m above sea level. The district covers an area of 2,536 km² and the equator crosses it at the southern tip. Koibatek District has a forest cover of 51,229.5 ha. The exotic forest plantations cover an area of 25,068 ha while the rest is under natural forest. The vegetation cover includes indigenous and exotic forests with dispersed areas of grasslands, bush lands and bamboo. The topographical features include river valleys, plains, highlands and hills, lakes and the floor of the Great Rift Valley.

The district has a population of 217,480 with an annual growth rate of 5.3 % (Republic of Kenya, 1997); the population density is 79 persons per km². Poverty levels are 61% and 44 % below poverty line in urban and rural areas respectively. Eldama Ravine and Esageri have 43 and 40 % people living below poverty line. The infant

mortality rate in the district is 63/1000 while the literacy level is 67 % (Republic of Kenya, 1997).

Rainfall in Koibatek District is seasonal and fairly reliable. The long rains start at the end of March and end in July with peak rains in May. The short rains start at the end of September and end in November with maximum rains in October. Minimum rainfall is experienced in January and the average rainfall varies from 400 mm per annum in lowlands to 1200mm in the highlands. Average annual minimum and maximum temperatures are 24° C and 30° C (Republic of Kenya, 1997).

The common diseases reported in the area include respiratory system infections, malaria, skin diseases, diarrhea, pneumonia, urinary tract infections, ear infections, rheumatism and dental disorders (Republic of Kenya, 2007). This coupled with the high percentage of people living below poverty line in the district necessitated the study in the area.

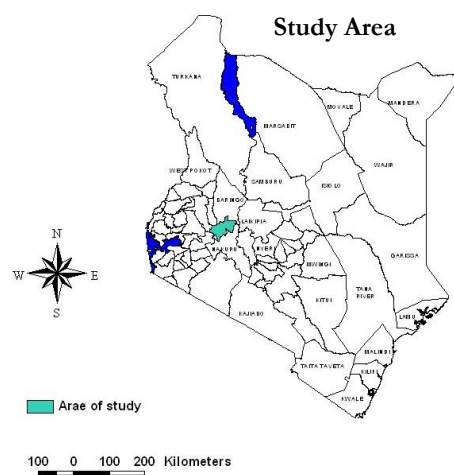


Figure 1: Map of Kenya showing the location of Koibatek District

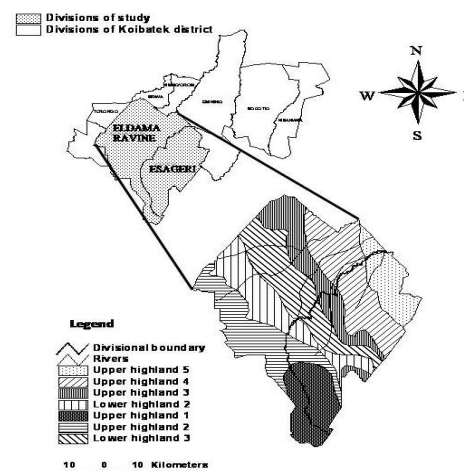


Figure 2: Agro-ecological map of Eldama Ravine and Esageri Divisions of Koibatek District

Ethno-botanical survey

Socio-ecological survey and focus group discussions were conducted to acquire general information on the ethno-botanical factors of the area (knowledge on medicinal plants, plants used and their availability, common ailments in the region and known herbalists) (Mouk *et al.*, 2001; Dery, *et al.*, 1999) (Appendix 1). Three hundred households were interviewed, 160 for Esageri and 140 from Edama Ravine divisions respectively (Fig. 3). Differences in age, socio-economic status and sex were taken into consideration in selecting the households. Herbalists were ranked based on the respondents scoring and the ten highly recorded were selected for further interview. The selected herbalists were asked for their consent to share their knowledge only for the purpose of this study. A structured questionnaire (Appendix 2) with both closed and open-ended questions and interviews were used to gather indigenous knowledge on the use of plants, parts used, preparation methods and ailments treated. The herbalists ranked the priority species based on their perspectives on frequency of use and difficulty in propagation. Based on frequency of use, scores from 10 to 1, where 10 was most frequently used and 1 for the least used. On the difficulty of propagation, a numerical scale of 1 to 10 was used: 10, most difficult to propagate and one (1) for the least difficult. Nine species which appeared among the nine in both rankings were selected for testing for bioactive compounds and propagation experiments. Samples were collected and identified in the department of botany, Egerton University. To determine the most threatened medicinal plants in the study area, herbalists were asked to rank the plants on a scale of 1 to 10, where 10 represented the most threatened and 1 representing the least threatened.

Collection and preparation of plant extracts

Plant stem barks were collected and dried at room temperature for three weeks. The dried materials were milled and stored in polythene bags. Ten grams of each plant sample were extracted with 400 ml methanol by using a Soxhlet apparatus for 8 hours. The residue was dried overnight and then extracted with 250 ml water on a water bath-shaker at 70° C for 2 hrs. The methanol and water extracts were filtered using a rotary evaporator then evaporated in a freeze dryer to recover the crude dried extracts. The dried extracts were stored at 20° C until used. The graded concentrations (5 and 20 mg/ml) of the extracts were prepared for the bioassay.

Screening for phytochemical compounds

Plant crude extracts were screened for the presence of alkaloids, sterols, saponins, flavanoids and anthraquinones according to standard methods (Harborne, 1973). To quantify the amounts of compounds, the following notations

were used - = not present, + = Low, ++ = medium and +++ = high quantity. Turbidity was used for alkaloids, color for sterols, flavonoids and anthraquinones and froth for sterols. Half a gram of extract from each plant was used.

Alkaloids

Half a gram of each plant extract was stirred in 2 ml of 1 % aqueous HCL and heated in a boiling water bath for 10 minutes. The mixture was filtered while hot and treated with Dragendorff's reagent. Turbidity or precipitation was used as a preliminary test for alkaloids.

Sterols

The extract was stirred in hexane to remove most of the coloring materials. The residue was extracted with 1 ml dichloromethane. The dichloromethane solutions were then dehydrated over anhydrous sodium sulphate. Then 1 ml portions of the dichloromethane were mixed with 0.5 ml acetic anhydride followed by two drops of sulphuric acid. A gradual appearance of green to blue color was taken as an indication of sterols.

Saponins

The extracts were shaken with cold water in a test tube. Persistent froth for at least half an hour showed a positive test for saponins.

Flavonoids

The extract was defatted by washing severally in hexane. The defatted residue was washed in 2 ml 80 % methanol and filtered. One ml of 1 % aluminium chlorite in methanol was added to the filtrate and development of yellow color indicated the presences of flavonoids.

Anthraquinones

The plant extract was shaken in 2 ml of benzene and filtered. One millilitre of 10 % ammonium hydroxide solution was added and the mixture shaken. Development of violet colour during the ammonium stage indicated the presence of free anthraquinones. To test for bound anthraquinones 0.5 g of benzene washed extract was boiled with 2 ml of 1 % HCL and filtered while hot. The filtrate was subsequently shaken with 1 ml of benzene and thereafter the benzene layer removed followed by addition of 1 ml of 10 % ammonium hydroxide. Development of violet color indicated presence of bound anthraquinones.

Brine shrimp tests

A rapid general bioassay for bioactive compounds was carried out using the Brine Shrimp Lethality procedure (McLaughlin and Lingling, 1998; Meyer *et al.*, 1982). Sea salt solution was prepared by dissolving 20 g of sea salt in 400 ml of water. The sea salt water was then poured into a 400 ml container after which a half top part of the container was covered and the other half left open.

A lamp was placed above the open side of the container to attract hatched shrimps. Ten grams of shrimp eggs were added to the covered side of the tank. The shrimp eggs were allowed two days to hatch and mature as nauplii (*Artemia salina*). Twenty milligrams of each plant sample were weighted and 2 ml of solvent added (20 mg/2 ml) from which 500, 50, and 5 ml were transferred to vials corresponding to 1000, 100, or 10 mg/ml, respectively. The solvent was evaporated under nitrogen and then placed under high vacuum for about 30 min for volatile solvents to evaporate. Four milliliters of the prepared seawater was added to each vial in quadruplicate and 10 shrimps per vial introduced; the volume of seawater was adjusted to 5 ml per vial. The vials were uncovered and placed under the lamp. The numbers of surviving shrimps per vial were counted after 24 hr and the live and death percentages computed.

Determination of antimicrobial activity

Test organisms: The test organisms (*Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) were obtained from Biological Sciences laboratory and Dairy Department at Egerton University.

Preparation of culture media and inoculum

Twenty grams of nutrient agar was dissolved in 500 ml of distilled water. The solution was stirred as it heated to boiling to enhance faster gelling of the media in plates. After boiling, the media was autoclaved at 121°C and 1 bar for 15 minutes and allowed to cool. The nutrient agar media was then poured into petri dishes (halfway full) and allowed to gel. The same procedure using the dextrose agar for culturing fungi was followed. Using a cotton swab, the six microorganisms were spread uniformly on the surface of the agar on the plates in four replicates.

The antimicrobial test was performed using the agar diffusion method (Nair & Chanda, 2005).

Discs (5mm) cut from disinfected filter paper were made using a paper punch and sterilized by autoclaving. Using forceps (disinfected by heating in red-hot flame), four discs were placed in a test tube containing extracts (antimicrobial under test) of the designated concentration and then carefully transferred onto the surface of the agar plate media already inoculated with the microbes. Streptomycin at a concentration of 1 mg/ml was used as the standard antibiotic (positive control). The plates were then incubated at 37°C and the zones of inhibition recorded after every 24 hr.

Minimum inhibitory concentration (MIC) bioassays

The minimum inhibitory concentration (MIC) of the extracts was determined using a two-fold dilutions method (Russell & Furr, 1977).

Results and Discussion

Bioactive compounds

The entire tree species contained one to four compounds in varying concentrations. Extracts of *M. salicifolia* had only flavonoids and anthraquinones in low concentrations (Table 9). *Faurea saligna* extracts contained higher concentration of terpenes and flavanoids (Plate 4) and low concentration of alkaloids. *Toddalia asiatica* and *P. vilidiflorum* extracts had moderately high levels of terpenes, saponins and anthraquinones while those of *P. thonginii* had moderately high levels of terpenes but anthraquinones and high levels of saponins. High levels of terpenes, moderate levels of flavonoids and low concentration of anthraquinones were recorded in *R. Cafra* and *M. kummel*. *Haploculum folioloum* extracts had the highest concentration of alkaloids, sterols and flavanoids. *Albizia anthelmintica* extracts had low concentrations of terpenes, saponins, flavanoids and moderately high concentrations of anthraquinones.

Table 1: Bioactive compounds found in plant extracts

| Species | Bioactive compounds | | | | | |
|-------------------------|---------------------|---------|----------|----------|------------|----------------|
| | Alkaloids | Sterols | Terpenes | Saponins | Flavanoids | Anthraquinones |
| <i>M. salicifolia</i> | - | - | - | - | + | + |
| <i>F. saligna</i> | + | - | +++ | - | +++ | - |
| <i>T. asiatica</i> | - | - | ++ | ++ | - | ++ |
| <i>P. vilidiflorum</i> | - | - | ++ | ++ | - | ++ |
| <i>P. thonginii</i> | - | - | ++ | +++ | - | ++ |
| <i>R. cafra</i> | - | - | +++ | - | ++ | + |
| <i>H. folioloum</i> | +++ | +++ | - | - | +++ | - |
| <i>M. kummel</i> | - | - | +++ | - | ++ | + |
| <i>A. anthelmintica</i> | - | - | + | + | + | ++ |

Key: concentrations

+ Low, ++ medium and +++ high concentrations of bioactive compounds

Brine shrimp test

The plant extracts exhibited low to very high degrees of toxicity against brine shrimp larvae (Table 10). Six out of the 9 extracts had LC₅₀ values ranging from 126.59 to 556.33 µg/ml. The

order of toxicity was *M. Salicifolia* (LC₅₀ 126.59 µg/ml), *F. saligna* (LC₅₀ 257.29 µg/ml), *P. vilidiflorum* (LC₅₀ 446.34 µg/ml), *T. asiatica* (LC₅₀ 452.3 µg/ml), *P. thonginii* (LC₅₀ 527.44 µg/ml) and *A.*

anthelmintica (LC₅₀ 556.33 µg/ml). Lethality was directly proportional to the concentrations of the extracts. Maximum mortalities occurred at 1000 mg/ml concentration for all the species with *M. salicifolia* extracts killing all the larvae. The least mortalities were at 10 mg/ml concentration.

At the highest concentration of 1000µl, *M. salicifolia*, *F. Saligna*, *T. Asiatica* and *P. vilidiflorum* extracts killed over 50 % of the brine shrimp while *P. thonginii*, *R. cafra*, *H. folioloum*, *M. kummel* and *A. anthelmintica* extracts killed between 46 – 6 % of brine shrimp. At 100 µl *M. salicifolia*, *F. saligna*, *T. asiatica*, *P. vilidiflorum*, *P. thonginii* and *A. anthelmintica* extracts killed between 6-24 % while those of *M. kummel*, *R. cafra* and *H. folioloum* showed no action against the organisms. At the lowest concentration of 10µl. *M. salicifolia*, *F. saligna*, *T. asiatica* *P. vilidiflorum*, *P. thonginii* and *A. anthelmintica* extracts killed between 2-18 % while those of *M. kummel*, *R. cafra* and *H. folioloum* showed no action against the organisms.

Table 2: The Brine shrimp lethality for extracts

| Species | Concentrations (µl) | | | |
|-------------------------|---------------------|-------|------|------------------|
| | 1000µl | 100µl | 10µl | LC ₅₀ |
| | % Deaths | | | |
| <i>M. salicifolia</i> | 100 | 20 | 18 | 126.59 |
| <i>F. saligna</i> | 86 | 24 | 6 | 257.29 |
| <i>T. asiatica</i> | 56 | 16 | 2 | 452.3 |
| <i>P. vilidiflorum</i> | 60 | 6 | 2 | 446.34 |
| <i>P. thonginii</i> | 46 | 12 | 2 | 527.44 |
| <i>R. cafra</i> | 10 | 0 | 0 | >1000 |
| <i>H. folioloum</i> | 6 | 0 | 0 | >1000 |
| <i>M. kummel</i> | 16 | 0 | 0 | >1000 |
| <i>A. anthelmintica</i> | 44 | 16 | 6 | 556.33 |

Table 3: Microbial inhibition zones (mm)

| Species | <i>E.coli K.pneumonia B.subtilis S.aureus P.aeruginosa C.albicans</i> | | | | | | | | | | | |
|-------------------------|---|----|--------------------|----|-------------------|----|-----------------|----|---------------------|----|-------------------|----|
| | <i>E.coli</i> | | <i>K.pneumonia</i> | | <i>B.subtilis</i> | | <i>S.aureus</i> | | <i>P.aeruginosa</i> | | <i>C.albicans</i> | |
| | 5 | 20 | 5 | 20 | 5 | 20 | 5 | 20 | 5 | 20 | 5 | 20 |
| | Concentration mg/ml | | | | | | | | | | | |
| <i>M. salicifolia</i> | 20 | 28 | 32 | 34 | 0 | 12 | 24 | 26 | 33 | 32 | 0 | 0 |
| <i>F. saligna</i> | 18 | 28 | 29 | 32 | 20 | 20 | 18 | 21 | 0 | 0 | 0 | 2 |
| <i>P. vilidiflorum</i> | 20 | 22 | 0 | 0 | 20 | 24 | 20 | 22 | 0 | 0 | 2 | 2 |
| <i>T. asiatica</i> | 0 | 0 | 31 | 32 | 30 | 31 | 28 | 29 | 26 | 28 | 0 | 0 |
| <i>P. thonginii</i> | 0 | 0 | 0 | 0 | 10 | 20 | 19 | 20 | 0 | 0 | 13 | 12 |
| <i>R. cafra</i> | 0 | 0 | 0 | 0 | 22 | 24 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>H. folioloum</i> | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 24 | 12 | 22 | 13 | 15 |
| <i>M. kummel</i> | 0 | 0 | 0 | 0 | 19 | 20 | 0 | 0 | 18 | 24 | 2 | 2 |
| <i>A. anthelmintica</i> | 0 | 0 | 0 | 0 | 19 | 20 | 0 | 0 | 0 | 0 | 13 | 15 |
| Chloramphenicol | 18 | 20 | 20 | 20 | 18 | 19 | 24 | 20 | 0 | 0 | - | - |
| Amphotericin | - | - | - | - | - | - | - | - | - | - | 28 | 32 |

Table 4: Minimum inhibition concentrations of extracts (mg/ml)

| Plant Species | Microorganisms | | | | | |
|-------------------------|----------------|--------------------|-------------------|-----------------|---------------------|-------------------|
| | <i>E.coli</i> | <i>K.pneumonia</i> | <i>B.subtilis</i> | <i>S.aureus</i> | <i>P.aeruginosa</i> | <i>C.albicans</i> |
| <i>M. salicifolia</i> | 5.0 | 5.0 | 0.31 | 0.63 | 5.0 | - |
| <i>F. saligna</i> | 2.5 | 5.0 | 2.5 | 2.5 | - | - |
| <i>T. asiatica</i> | - | 0.625 | 1.25 | 0.31 | 0.625 | - |
| <i>P. vilidiflorum</i> | 0.17 | - | 0.17 | 0.31 | - | 2.5 |
| <i>P. thonginii</i> | - | - | 0.31 | 0.31 | - | 2.5 |
| <i>R. cafra</i> | - | - | 0.17 | - | - | - |
| <i>H. folioloum</i> | - | - | - | 0.625 | 0.625 | 0.625 |
| <i>M. kummel</i> | - | - | 0.31 | - | 0.31 | - |
| <i>A. anthelmintica</i> | - | - | 0.17 | - | - | 0.313 |
| Chloramphenicol | 1.25 | 1.25 | 1.25 | 1.25 | - | - |
| Amphotericin | - | - | - | - | - | 0.625 |

Antimicrobial activity

Plant extracts differed in their anti-microbial activities (Table 11). At concentrations of 5 mg/ml and 20 mg/ml the inhibition zones ranged from 12 mm to 32 mm (Plates 5 and 6). *Myrica salicifolia* extracts inhibited all bacteria species but not the fungus. *Faurea saligna* and *T. asiatica* extracts inhibited growth of four out of the five bacteria tested. *Ravolfia cafra*, *H. folioloum*, *M. kummel*, and *A. anthelmintica* extracts inhibited growth of only two types of bacteria (Table 11). *Myrica salicifolia*, *F. saligna* and *P. vilidiflorum* extracts showed activity against *Escherichia coli* (Table 11). *Myrica salicifolia*, *F. saligna* and *T. asiatica* extracts inhibited the growth of *K. Pneumonia*. *Bacillus subtilis* was inhibited by extracts of all the species except those of *H. folioloum* with *M. salicifolia* extracts showing activity only at 20mg/ml concentration. Extracts of all species except those of *R. cafra*, *M. kummel* and *A. anthelmintica* inhibited the growth of *S. Aureus*. *Myrica salicifolia*, *T. asiatica*, *H. folioloum* and *M. kummel* extracts inhibited growth of *P. aeruginosa*. *Aibizja anthelmintica*, *M. Kummel*, *H. folioloum*, *P. thonginii*, *P. vilidiflorum* and *F. saligna* extracts showed microbial activity against *C. albicans* which was lower than that of the control. Chloramphenicol, the control drug inhibited growth of all the tested bacteria expect *Pseudomonas aeruginosa*. *Myrica salicifolia*, *F. saligna*, *P. vilidiflorum* and *T. asiatica* extracts had higher bacteria inhibition than the control drug. amphotericin which was the fungal control drug inhibited growth of only *C. albicans*.

The inhibitory activity exhibited by the secondary metabolites agrees with reports linking the antibacterial properties of plants to the presence of secondary metabolites (Musyimi *et al.*, 2008). Activity of *A. antbelmintica* extracts has been reported with inhibition zones ≥ 10 mm (Runyoro *et al.*, 2006) and that the inhibition zone was dependent on the concentration. Hugo and Russel (1998) also indicated that the position of the zone edge (diameter of inhibition) is dependent on by the initial population density of the organism, their growth rate and the rate of diffusion of the antimicrobial agent.

Toxicity against brime shrimp and antimicrobial activity was also variable. Presence of the isolated compounds in other medicinal plants has also been reported (Mann *et al.*, 2008; Musyimi *et al.*, 2008; Veeramuthu *et al.*, 2006; Runyoro *et al.*, 2006). The most common of these bioactive compounds are alkaloids, flavanoids, tannins and phenolics compounds (Oomah, 2003). Presence of saponins in *P.thonginii* reported by Jimoh and Oladiji, (2005) differs with the findings of this study. Based on the toxicity and activity against micro-organism results, the ranking tallies well with the ranking by the herbalists (Table 1). The most threatened species (Table 7 and plates 1, 2 and 3) were among also the most toxic and exhibited some of the highest anti-microbial activity.

Antimicrobial properties of medicinal plant extracts have been linked to the chemically active substances. The toxic phytochemical compounds could either damage DNA or inhibit the synthesis of proteins (Fatope, 1995). This is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Ionela and Ion, 2007). Alkaloids rank among the most useful therapeutically plant substances. Generally, they are extremely toxic but have marked therapeutic effect in minute quantities. Alkaloids and their synthetic derivatives are also used as basic medicinal agents all over the world for their analgesic, antispasmodic, and bactericidal effects.

Although *M.salicifolia* extracts had only low concentrations of flavonoids and anthraquinones (Table 9) they had one of the highest toxicity against brine shrimp (LC₅₀ 126.59, Table. 10) and antibacterial activity (Table 11). It exhibited antibacterial activity against all bacteria tested. Inhibition by extracts of this species compared well with the standard and had activity against *P.aeruginosa* which the standard did not. This justifies the traditional use of the species for treatment against the ailments shown in Table 2. This may suggest that the two compounds work synergically. *Faurea saligna*, *P.vilidiflorum* and *T. asiatica* extracts exhibited activity against at least three bacterial species and toxicity of LC₅₀ less than

500 um/ml. *Rafovia caffra*, *H. folioloum* and *M. kummel* extracts had very low toxicity (less 1000) had activity against less than two organisms. Microbial activities and toxicity against brime shrimp by some of these plant extracts has been reported against other organisms (Mbwambo *et al.*, 2007; Akinpelu and Obuotor, 2006; Voravuthikunchai *et al.*, 2004; Asuzu *et al.*, 1999).

The presence of terpenes in *F. saligna*, *R. cafra*, *M. kummel*, *T. asiatica*, *P. vilidiflorum*, *P. thonginii* and *A. antbelmintica* and their activity against *B.subilis*, *S. aureus* and *C. albicans* agrees with the report by Ionela and Ion (2007). Ranzi *et al.*, (2008), suggested that presence of saponins and flavonoids in some Yemen medicinal plants were responsible for the moderate antimicrobial and antioxidant activities. Flavanoids are synthesized by plants in response to microbial infection. Therefore, it is not surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Flavonoids may also disrupt microbial membranes. Anthraquinones may lead to inactivation of the protein and loss of function and may also render substrates unavailable to the microorganism (Ionela and Ion., 2007).

There are several factors which affect or reduce the efficacy of the medicinal plant extracts. These include time of collection, processing, storage and method of extraction (Girma, 2005). Generally, the reported data are difficult to compare because of the test methods, bacterial strains and sources of antimicrobial samples. The overall antibacterial activity of the tested extracts are, however, comparable to MIC values.

Conclusion

The plants used for herbal medicine contained two to four bioactive compounds that exhibit anti-microorganisms (bacteria and fungus) activity. The plant extracts tested also had toxicity against brine shrimp. This may justify their use as herbal medicine against the common diseases found in the area.

Recommendation

Recommendations based on results from the study:

1. Studies on isolation of the active compounds should be undertaken as these sources could form a basis for discovery of new natural bioactive compounds. These plants should also be screened and characterized to ascertain the genotypes and the concentration of bioactive compounds.

- Effectiveness of other methods of plant extraction should be investigated e.g. crude water extraction with testing to simulate how communities utilize the plant materials.

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