Effects of Leaf and Root Extracts of Aspilia Plant
(Aspilia mossambicensis) (Oliv) Wild on Some Selected
Micro-Organisms

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Abstract: Phytochemical and anti-microbial properties of the methanol extracts of
Aspilia mossambicensis (family, Compositae) were studied. The plant has been used by
traditional healers to treat skin diseases, wounds, gonorrhea, abdominal pains, respiratory
problems and malaria. Bacteria used for antimicrobial analysis consisted of clinical strains
of Streptococcus pyogenes (gram positive) and Salmonella typhi (gram negative) bacteria and
one strain of fungi (Aspergillus niger). Phytochemical screening of the plant leaves and roots
of A. mossambicensis revealed that the plant contains active compounds of flavonoids,
alkaloids, saponin, steroids, carbohydrates and anthraquinones. The methanol extract
showed antimicrobial activity against the three microorganisms at relatively lower
concentrations. Leaves extracts showed higher level of growth inhibition of S. pyogenes,
followed by S. typhi and A. niger, respectively. Root extracts showed higher growth
inhibition for A. niger, followed by S. typhi and S. pyogenes in that order. In comparison
leaves extracts showed better microbe growth inhibition than root extracts and was attributed
to absence of aldehydes in the roots, or a more concerted synergistic activity of active
compounds present in the leaves. The study has shown that species of
Aspilia mossambicensis have considerable activity against gram positive bacterium
Streptococcus pyogenes, gram negative bacterium Salmonella typhi and one fungal strain,
Aspergillus niger. The study supports the previous claims by traditional healers of the plant
to heal several diseases in traditional communities in Kenya. The study provides a strong
evidence for new sources of antimicrobial drugs from this plant.

Key words: Aspilia mossambicensis, antimicrobial properties, A. niger, S. pyogenes, S. typhi

INTRODUCTION

People inhabiting different ecological zones use different plants and plant parts in their treatment
arsenal (Bussmann et al., 2006; Nguroge and Bussmann, 2006). About 70-80% of people in the world
depend on traditional herbal medicine for primary health care needs (Hamayun et al., 2006). While
plant species used in traditional medicines continue to be reliable sources for discovery of useful
compounds, the pharmaceutical value and concentration of active ingredients in each plant vary
depending on climatic and edaphic factors (Rajakaruna et al., 2002; Adeboye and Opabode, 2004;
Bussmann et al., 2006; Duraiappahyan et al., 2006). Few published studies on phytochemical
compounds have aimed at elucidating the degree of specificity of the effect of the compounds among

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the various types of their bioactivity. Several factors such as phonological age of the plant, percent humidity of the harvested material and the method of extraction have been identified as possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Rajakaruna et al., 2002). Moreover, these chemical compounds seem to possess distinct molecular formula, a certain molecular weight and certain physicochemical properties.

Aspilia plant (Aspilia mossambicaeensis) is widely spread in south, south west and west of Kenya from coast to Lake Victoria. As a result of variable ecological conditions, the chemical composition of the plant is known to vary and this may have been the reason as to why the plant has been used to treat different diseases in different places (Agnew and Shirley, 1994). The plant belongs to the family of Compositae (Asteraceae) and genus Aspilia. Few reports indicate that the plant possess antimalarial activity against Plasmodium falciparum (Offulla et al., 1996). The plant has been used traditionally by many African communities to treat several diseases including, cystitis and gonorrhea, abdominal pains and backache. Root decoction is normally given to breast feeding mothers to increase milk production. Pounded leaves decoction is drunk in order to treat intestinal worms including hookworms (Kokwaro, 1976). Among the Kamba community of Kenya the pounded leaves decoction is applied to circumcised young men for faster healing of the wounds (D.M. Musyimi personal communication). Some phytochemical constituents have been reported to have medicinal or antimalarial properties (Kokwaro, 1976; Offulla et al., 1996). There were no reports in the literature of earlier studies carried out to test antimicrobial activity of A. mossambicaeensis, hence this study. The objective of this study was to isolate, identify and to evaluate the antimicrobial properties of the compounds present in the leaves and roots of Aspilia mossambicaeensis (Oliva) wild species in Kenya.

MATERIALS AND METHODS

Field Collection and Extraction

Plant roots and the leaves were collected in February 2007, from areas surrounding Maseno University, Kenya. Specimens were cleaned off adhered soil or dust in the field by shaking and were placed inside polythene paper bags and taken to the Maseno University Botany laboratory where the study was undertaken. The specimens were cleaned of all dust and allowed to dry under shade. The voucher specimens in duplicate were deposited in the herbarium of Maseno University. Leaves and roots of Aspilia mossambicaeensis (Oliv) wild species were air dried at room temperature for 2 weeks. The roots and leaves were subsequently ground into fine powder using an electric blender yielding 300 and 500 g, respectively and cold extracted with methanol then filtered under vacuum using a 0.2 mm Nalgene filter unit. The resulting extracts were concentrated to remove the methanol using the rotary vapour at 40°C. Roots and leaves extracts were dried and subsequently weighed and yielded a crude extract of 31.8 and 19.7 g, respectively. Extracts were then stored in refrigerator until they were needed for analysis. All work was carried out in accordance with the general guidelines for methodologies on research and evaluation of traditional medicines (WHO, 2000).

Phytochemical Screening

The qualitative chemical analysis of the powders was carried out for the determination of the presence of anthraquinone, saponins, steroids and alkaloids, Aldehydes, ketones and carbohydrates. Phytochemical screening was done according to Akinyemi et al. (2005).

Isolation of Pure Compounds

Methanol extract (15 g) was chromatographed on silica gel column eluting with n-hexane:ethyl acetate (90:10) according to Kaberia et al. (1999). Most of the chemicals used were of analytical grade.
Silica gel column (60 g, si 60, 40-63 µm) was from Merck, Germany and commercially prepared TLC plate (GmbH & co. D-3440) from Eschwege, Germany. The R_f-values of the bioactive fraction in the sample and the standard were determined (El-Olemy et al., 1994).

**Test Microorganisms**

The clinical isolates were obtained from biomedical school of Maseno University, Kenya. The microorganisms consisted of one gram positive bacteria (*Streptococcus pyogenes*) and one gram negative bacteria (*Salmonella typhi*) and one fungal strain (*Aspergillus niger*).

**Preparation of the Culture Media**

Nutrient agar pH 7.4 and Sabouraud dextrose agar pH 5.4, all products of ooxid laboratories, England were used in this study. Thirty nine gram of Sabouraud dextrose agar and 28 g of nutrient agar were dissolved in 1 L of water and heated to dissolve the contents completely. The nutrient agar and Sabouraud dextrose agar petri dishes were prepared by pouring 15 mL of molten media into sterile petri dishes and allowed to solidify for 5 min. The media, distilled water and petri dishes were sterilized separately by autoclaving at 120°C at 1 bar pressure for 20 min.

**In vitro Antibiosis**

Extracts from the two plant parts (leaves and roots) were diluted in hexane (2 g of the dried filtrate was reconstituted with 10 mL of 100% hexane to prepare stock solution). Different concentrations of the plant extracts were prepared by diluting the stock extract to hexane according to the following ratios 1.9 (10%), 1.18 (5%) and 1.32 (2.5%). Control experiment had n-hexane alone without any extract to nullify the effect of the solvent on the test organisms. Two methods were used: plate and disc methods according to Murthy and Nagodra (1977). Susceptibility testing was carried out by measuring the inhibitory zone diameters on the Nutrient Agar (NA) and Sabouraud dextrose agar using conventional paper disc method and pit method. The inhibitory zone distances and rounded off to the nearest whole numbers (mm) for analysis.

**Pit Method**

A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar media, into which 0.1 mL of the prepared plant extracts of various concentrations were added. The microorganisms were introduced on the media around the pit using applicator sticks bearing cotton wool at the tips. Three replicates for each microbe and plant extract concentration were used. The petri dishes inoculated with bacteria and fungi were kept for incubation for 24 h at 37 and 25°C, respectively. The clear zone of growth inhibition formed around each pit was measured to the nearest millimeter using a transparent ruler.

**Disc Method**

Circular paper discs 8 mm diameter were cut out from Whatman No. 1 filter paper using a paper punch and each dipped in a known concentration of the plant extracts for about 2 min, then gently transferred at the center of the inoculated agar media. Petri dishes inoculated with bacteria and fungi were kept for incubation for 24 h at 37 and 25°C, respectively. The petri dishes inoculated with bacteria and fungi were kept for incubation for 24 h at 37 and 25°C, respectively. The diameter of inhibition zones were measured with a ruler and compared with the n-hexane control disk to nullify the effect of the solvent on the growth of the test organisms.
Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using SAS statistical package. Means were separated and compared by Duncan's multiple range test at (p<0.05).

RESULTS

Phytochemical screening of the plant (Table 1) indicated a number of constituents were present and included flavonoids, alkaloids, steroids and anthraquinones, ketones and aldehydes. Root extracts did not have aldehydes unlike the leaves which had both ketones and aldehydes. Flavonoids were only present in the roots. There were a lot of sterols in the roots extract than in the leaves extract. Higher concentrations of alkaloids were present in both root and leaves extracts. Meta and Ortho hydroxyls groups were present in both roots and leaves extracts while Para hydroxyl was only present in leaves, confirming the presence of carbohydrates, particularly the monosaccharides. Elution of the column with n-hexane and ethyl acetate led to isolation of fifteen Rf values of uncharacterized active compounds (data not shown). Seven active compounds were isolated from the leaves while nine active compounds were from the roots. All plant extracts were found to possess microbial growth inhibitory activities (Table 2 and 3). Leaves extracts caused the higher degree of growth inhibition on the test microorganisms as compared to the root extracts (Table 2 and 3). The zones of inhibition obtained in respect to the leaf extract and test organisms were higher for S. pyogenes, followed by S. typhi and least in A. niger. Root extracts inhibited the growth of A. niger at a greater degree, followed by S. typhi and least inhibition was experienced by S. pyogenes. The two bioassay methods also influenced the growth inhibition results of the microbes. There were significant (p<0.0001) interactions between the plant parts used, extract concentration, microorganisms and bioassay methods used (Table 4).

Table 1: Phytochemical screening of secondary metabolites present in the plant parts extracts of *capsila mossambicensis*

<table>
<thead>
<tr>
<th>Phytochemical groupings</th>
<th>Leaves</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Anthraquinone glycosides</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Steroid glycosides</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Safonin glycosides</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Carbonyl compounds</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ketones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

---: Absent, +: Present, ++: Low concentration, +++: Moderate concentration, ++++: High concentration

Table 2: Effect of different concentrations of leaf and root extracts of *capsila mossambicensis* on the microorganisms. Data presented are means of three replicates±SE

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Organisms</th>
<th>Control treatment</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td><em>Salmonella typhi</em></td>
<td>0.2±0.0*</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2±0.06</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus pyogenes</em></td>
<td>4.5±0.6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger</em></td>
<td>0.0±0.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Root</td>
<td><em>Salmonella typhi</em></td>
<td>0.0±0.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus pyogenes</em></td>
<td>4.8±0.6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger</em></td>
<td>0.1±0.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*: Diameters zone of inhibition (mm)

Table 3: General comparison of method and growth inhibitory effects of root and leaf extracts of *capsila mossambicensis* on the micro-organisms. Data presented are means of three replicates±SE

<table>
<thead>
<tr>
<th>Parts</th>
<th>Extract</th>
<th><em>Salmonella typhi</em></th>
<th><em>Streptococcus pyogenes</em></th>
<th><em>Aspergillus niger</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>leaf</td>
<td>pt</td>
<td>8.5±0.4*</td>
<td>10.3±0.4</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td></td>
<td>disc</td>
<td>1.8±0.4</td>
<td>3.8±0.4</td>
<td>2.8±0.4</td>
</tr>
<tr>
<td>root</td>
<td>pt</td>
<td>6.3±0.4</td>
<td>2.6±0.4</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td></td>
<td>disc</td>
<td>3.8±0.4</td>
<td>2.2±0.4</td>
<td>5.3±0.4</td>
</tr>
</tbody>
</table>

*: Diameters zone of inhibition (mm)
DISCUSSION

Medicinal plants have been the subject of human curiosity and need (Bussmann et al., 2006; Khalil and Dababneh, 2007). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Phytochemical screening of the leaf and root extract of the plant (Table 1) revealed that the plant contains flavonoids, saponins, anthraquinones, alkaloids, carbohydrates, aldehydes and ketones in agreement with studies reported for some other plants (Weimann and Heinrich, 1997; Atindehou et al., 2002; Oomah, 2003; Edeoga et al., 2005). The most important of these bioactive compounds are alkaloids, flavonoids and phenolic compounds (Atindehou et al., 2002; Edeoga et al., 2005), since are plant metabolites known for exhibiting physiological and antimicrobial activity. The zone of inhibition using leaf extracts was higher for S. pyogenes, a gram positive bacterium than for the other two microbes. Interestingly, S. pyogenes experienced the least inhibition with the root extracts compared to the other two microbes (Table 2 and 3). It is possible that the growth of the microbes was inhibited by different chemical compounds. For instance, roots lacked aldehydes hence aldehydes found in the leaves extracts could be responsible for greater growth inhibition of S. pyogenes. Generally methanol leaves extracts were more active than methanol roots extracts; probably because of higher concentration of the active chemical compounds in the leaves. The greater growth inhibition of A. niger by methanol root extracts, may be due to absence of aldehydes in the roots as evidenced from the study (Table 1). Interestingly, root extracts had higher number of active compounds determined by thin layer chromatography (data not presented); perhaps the comparatively higher activity of the leaves than roots was due to active constituents in the leaves acting in a more synergistic manner. It is worth noting that leaves phytochemical compounds had greater inhibitory activity on A. pyogenes than the other organisms. The antimicrobial activity of the methanol extracts appears to have broad spectrum of activity, especially since both gram positive and gram-negative bacterial and fungi were sensitive to the extracts. The antimicrobial activity of the methanol extract appears to have broad spectrum of activity, especially since both gram positive and gram-negative bacterial and fungi were sensitive to the extracts. Phytochemical compounds are heterogeneous mixtures of single substances and biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic activities. Mixtures of chemicals show a broad spectrum of biological effects and pharmacological properties (Robinson, 1967; Coelho-de-Souza et al., 1998; Atindehou et al., 2002).

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The antimicrobial properties exhibited by the plant could be traced to its possession of alkaloids (Shelton, 1991, Akinyemi et al., 2005), flavonoids (Narayana et al., 2001) and Meta and Para-hydroxyl compounds present in the plant extracts (Mostahar et al., 2007). The presence of hydroxyl groups in methanol extracts enhances the likelihood of intramolecular reactions such as the formation of cyclic acetals and anhydroethers (Mann, 1994). Carbohydrates could have facilitated the growth of some of the microorganisms and hence antagonized the antibacterial activity of the active compounds in the root extracts. Many alkaloids have pharmacological effects and could be associated with inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Shelton, 1991). Alkaloids have been used to treat diseases like malaria, pain killers and managing heart diseases (Oorrah, 2003). Alkaloids are derivatives of amino acids and include morphine, atropine, quinine and nicotine. Flavonoids (such as, rutin and anthocyanins), allelopathic compounds (such as, caffeic acids, ferulic acid, other phenolics and polyphenolic compounds) and saponins (such as, protodioscin) are known to have biological and pharmacological activities on human health (Hartung et al., 1990, Akinyemi et al., 2005). Major flavonoid compounds such as rutin and its aglycone quercetin, have been reported to have beneficial biological effects, such as antagonizing the increase of capillary fragility associated with hemorrhagic disease (Griffith et al., 1944) and anti-carcinogenic activity (Yang et al., 2000). Biological activities of many other flavonoids, polyphenols or phenolics compounds were also reported (Edenharder and Grunhage, 2003, Wang et al., 2003).

The susceptibility of S. pyogenes, S. typhi and A. niger to the cold methanol extracts is an indication that the plant possess a great potential as a source of both antibacterial and antifungal compounds which may be comparable to those of commercially available drugs. The antimicrobial activities demonstrated by crude extracts of A. mosambicensis may, explain some of the previous claims by traditional healers, about this plant curing of diseases like dysentery and respiratory tract infections (Kolawo, 1976). The antimicrobial properties exhibited by the plant could be attributed to presence of alkaloids and flavonoids which have been noted for numerous biological activities such as anti-inflammatory, vasoprotective and antithrombotic effects (Oorrah, 2003, Mostahar et al., 2007). There are no reports on toxic effects of A. mosambicensis used in different African communities including Kenya for various ailments, hence phytochemical studies should be intensified to isolate, characterize and identify the specific active compounds in this plant responsible for the antimicrobial activity. We are in progress to find out the active compounds in the fractions of A. mosambicensis. Research is therefore needed to determine optimal doses and concentrations of the preparations and identify side effects of the traditional remedies.

CONCLUSIONS

Present study has provided evidence of antimicrobial activity of A. mosambicensis against gram negative bacterium S. typhi and gram positive bacterium S. pyogenes and fungal pathogen A. niger. The leaves extracts seem to have higher degree of growth inhibitory activity on S. pyogenes, followed by S. typhi and A. niger. On the other hand, roots extracts had higher growth inhibitory activity on A. niger, followed by S. typhi and S. pyogenes. Roots had the higher number of active compounds isolated by thin layer chromatography. The major compounds present in the methanol extracts includes, Flavonoids, saponins, anthraquinones and alkaloid compounds and seem to be responsible for the antimicrobial activity. The study has shown that methanol extracts of A. mosambicensis could be used as another source of cheaper conventional drugs since the plant is quite abundant in the forests. The study revealed that the plant contain chemicals that could help to control some of the problematic diseases caused by the microorganisms investigated. The results provide a background for rigorous screening of this plant for antimicrobial properties.
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