In vitro Antimicrobial Activity of Extracts from Some Cameroonian Medicinal Plants


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Abstract: In order to valorise the pharmaceutical potential of natural products, 10 plants of nine different families collected on ethnobotanic informations around Yaoundé in Cameroon were extracted. Seventeen organic and hydro-organic extracts were tested for their biological effects. The in vitro evaluation of antibacterial and antifungal activity was carried out by the well agar diffusion method on seven Gram positive and Gram negative bacteria (P. aeruginosa, E. coli, S. faecalis, S. choelera, P. mirabilis and M. morganii) and two groups of fungi (Filamentous, Yeast). The results showed that 12 of the 17 extracts demonstrated antibacterial activity against the seven pathogenic bacteria tested. The growth inhibition halos were ranged from 8.00 to 32.33. Among them, extracts of Solanum aculeastrum (Solanaceae) and Syzygium guinensis (Myrtaceae) showed the higher antibacterial activity. For the antifungal activity, growth inhibition halos varied from 8.00 to 17.55 mostly against Geotrichum candidum and Penicillium species. The extracts S. aculeastrum demonstrated antibacterial and antifungal activity.

Key words: Antimicrobial activity, growth inhibition, pharmaceutical potential

INTRODUCTION

Traditional medicine practice has been around for centuries and still remains very common in the developing world. It is estimated that about 80% or more of the world’s population rely primarily on traditional medicine for their healthcare (Kurt et al., 2000). Plant extracts as well as other alternative forms of medical treatment have made large contributions to human health and well-being (Chukwuje-kwu et al., 2005). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkebia, 2004). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996; Iwu et al., 1999). The use of medicinal plants in Cameroon has been demonstrated (Table 1) in the centuries (Betti, 2004).

In recent years, secondary plant metabolites (phytochemicals and phytotherapy), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin, 1985). The aim of this study was to evaluate the in vitro antimicrobial activity of extracts from 12 plants against several Gram-positive and Gram-negative bacterial as well as fungi isolates.

MATERIALS AND METHODS

Extract preparation: Plant materials dried at room temperature (30°C±2) were ground to fine powder. Each powder (250 g) was separately macerated by mixing in 1000 mL organic or a mixture of hydro-organic 4:1 (v/v) 72 h. Each resulting solution was filtered using Whatman filter paper No. 1, concentrated with rotavapor following in an air circulating oven at 54°C until total dryness. The experiment was repeated twice with the same powder and the crude extract obtained was stored at 5°C. Each extract was suspended in the DMSO for the antibacterial and antifungal assay.

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Table 1: Ethnobotanic information of some plants traditionally used in Cameroon and some area in Africa

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Vernacular name</th>
<th>Part of plants</th>
<th>Traditional used of plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia senegal</em> (Nees)</td>
<td><em>Acaciaeae</em></td>
<td>Fonndzup (Barnoun); Ngick (Basasi); Ndole (Bamoun); Bar bar (Lanoo)</td>
<td>St-Le</td>
<td>Spain, gonorrhea, dysentery, chronic ulcer, intestinal helminthesis and pharyngitis.</td>
</tr>
<tr>
<td><em>Bothriochloa zonata</em></td>
<td><em>Poaceae</em></td>
<td>Longio Diep (Bamena); Peper (Bakossi)</td>
<td>Le</td>
<td>Dysenteri, abdominal pain, haemorrhoids, urinary infections and gonorrhea.</td>
</tr>
<tr>
<td><em>Boojoba acaulis</em> (SW DC)</td>
<td><em>Poaceae</em></td>
<td>Owondo bekone (Balu)</td>
<td>Le</td>
<td>Asthma, respiratory tract infections, coughs, chronic bronchitis and other pulmonary disorders.</td>
</tr>
<tr>
<td><em>Euphorbia hirta</em> L.</td>
<td><em>Euphorbiaceae</em></td>
<td>Okoul bifies (Ewoondo)</td>
<td>Pt-Le</td>
<td>Toothache, inflammatory diseases and analgesic.</td>
</tr>
<tr>
<td><em>Lophira alata</em></td>
<td><em>Ocitaceae</em></td>
<td>Oikoga (Ewoondo)</td>
<td>Ba</td>
<td>Haemorrhoids, constipation, inguinal hernia, intestinal parasitosis, bacteriuria, skin infections, syphilis, diabetes, gastrintestinal, jaundice, Eczema, Thyphoidemins, Ringwound food poison.</td>
</tr>
<tr>
<td><em>Pohon acaulis</em> (L.) Roxb</td>
<td><em>Leguminosae-cessalpinaceae</em></td>
<td>Ngom (Ewoondo); Seu nana (Bamoun)</td>
<td>Le-St-</td>
<td>Haemorrhoids, constipation, inguinal hernia, intestinal parasitosis, bacteriuria, skin infections, syphilis, diabetes, gastrintestinal, jaundice, Eczema, Thyphoidemins, Ringwound food poison.</td>
</tr>
<tr>
<td><em>Solanum nigrum</em></td>
<td><em>Solanaceae</em></td>
<td>Sirerka (Bamu)</td>
<td>Fr</td>
<td>Sipper wounds, gonorrhea, cancer, particularly of subcutaneous, tubal blockage, gastritis and cellulitis.</td>
</tr>
<tr>
<td><em>Syzygium guineense</em> DC.</td>
<td><em>Myrtaceae</em></td>
<td><em>Leucaena</em></td>
<td>Le</td>
<td>Antitussive, coughs. Pneumonic inflammatory disease.</td>
</tr>
<tr>
<td><em>Leucaena leucocephala</em></td>
<td><em>Rubiaceae</em></td>
<td>Totum (Bolu)</td>
<td>Le</td>
<td>Spleen in children, edematitis pelvic inflammatory disease.</td>
</tr>
<tr>
<td>Morinda lucida Berth</td>
<td><em>Rubiaceae</em></td>
<td>Aken (Ewoondo)</td>
<td>Le</td>
<td>Fever, abdominal pain, dysenteri and splenomegaly.</td>
</tr>
</tbody>
</table>

Micro-organisms: The micro-organisms used for the antibacterial evaluation are clinical isolates and provided by the Centre Pasteur de Cameroon of Yaoundé. These include *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella cholera*, *Proteus mirabilis* and *Morganella morgani*. Filamentous fungi (*Fusarium sp.*, *Penicilium sp.*, *Helminthosporum sp.* and *Aspergillus flavus*) and yeast (*Candida albicans*, *Candida kefyr* and *Geotrichum candidum*). The microbes used for antifungal activity were isolated from clinical specimens and identified in the Mycology laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé.

**Antibacterial assay:** The antimicrobial assay was performed by well agar diffusion method (Parekh and Chanda, 2007). The molten Mueller Hinton agar was inoculated with 100 µL of the inoculum (1×10⁶ cfu mL⁻¹) and poured into the Petri plate. For agar well diffusion method, a well was prepared in the plates with the help of a corkbore (0.6 cm). Fifty microliter of the test extract (50 mg mL⁻¹) was introduced into the well. The plates were incubated overnight at 37°C for 18 h. Microbial growth was determined by measuring the diameter of the inhibition zone. The pure solvents were used instead of the extract as the negative control while the gentamicine (0.5 mg mL⁻¹) was utilised for the positive control. The result was obtained by measuring the diameter of the inhibition zone with a caliper-square. The experiment was done three times and the mean values are presented.

**Antifungal assay:** Cultures of filamentous fungi and yeast were treated with different extracts (50 mg mL⁻¹) as previously described in the antibacterial assay. Ketoconazone was used as a positive control at the concentration of 2 mg mL⁻¹. Following incubation at 28°C for 48 h, the diameters (mm) of the growth inhibition halos were measured using a caliper-square.

**Statistical analysis:** The results were expressed as mean±SD (mm). Data were analysed by one way analysis of variance (ANOVA) followed by student-Newman-Keuls test (p<0.05).

**RESULTS AND DISCUSSION**

The antibacterial activity of 17 extracts of plant species were assayed in vitro by agar well diffusion method against 7 bacteria species and fungi isolates. A total of seventeen and eleven extracts of plants are tested, respectively, for their in vitro antibacterial and antifungal activities (Table 2, 3). The diameter of inhibition of the extracts varied from 8.00 to 32.33 mm. The effects of the extracts on seven bacteria growth showed that 11 (64.7%) extracts produced significant antibacterial activity. Six (35.29%) extracts were active on more than three of seven bacteria used in the experiment. Most of the bacteria tested were less sensitive to CH₃Cl, and MeOH extracts of *A. montanus* and *L. guineensis* respectively. For the antifungal activity, only 5 (45.5%) of eleven extracts showed no antifungal activity. Only EtOH extract of *S. aculeastrum* demonstrated a weak inhibition growth of five fungal species.
The remaining extracts (5, 45.5%) showed an inhibition halo on one or two fungi species. With respect of the distribution of the biologically active constituents present in the extracts, results indicated that the polar extracts are more effective than the non polar ones. According to the growth inhibition halos of the extracts, the results were interpreted as follow: no activity (<7 mm halo), weak activity (7-10 mm halo), moderate activity (11-16 mm halo) and high activity (>16 mm halo) (Monks et al., 2002).

The seventeen extracts of eleven medicinal plants selected were tested for the in vitro antimicrobial activity by assessing their ability to inhibit bacteria and fungi growth (Table 2, 3). Regarding the potency of antibacterial activity of these extracts at the concentration tested, these extracts could be separated into three groups.

The first group includes six (35.29%) extracts showed the higher inhibition growth on more than three antibacterial. It concerns S. aculeastrum (MeOH and EtOH extracts), S. alata (aqueous-EtOH extract), E. hirta (MeOH extract) and A. floribunda (CHCl₃ extract). All these plants are used for the treatment of various diseases in Africa (Adjanahoun et al., 1991; Igoli et al., 2005). The results in this study demonstrated that the MeOH extract of S. aculeastrum is the most active. S. aculeastrum is widely used in traditional medicine for the treatment of human and livestock diseases (Hutchings et al., 1996), jigger wounds and gonorrhea (Agnew and Agnew, 1994). The berries and leaves of S. aculeastrum demonstrated antiproliferative on three human tumour cell lines HT29, Hela and CMF7 (Koduru et al., 2006a). The antimicrobial and antioxidant activities of S. aculeastrum have been demonstrated by Koduru et al. (2006a). The oil from S. aculeastrum revealed the presence of terpenoids, alkanes, aldehydes, ketones, fatty acids and esters, diterpenes, aromatic hydrocarbons and miscellaneous compounds (Koduru et al., 2006b). Alkaloids, steroids and terpenoids have been reported to have good activities (Cowan, 1999).
In this study both MeOH and EtOH extracts of S. aculeastrum demonstrated significant inhibition growth of all bacteria tested except M. morganii. The aqueous-EtOH extract S. alata exhibited moderate antibacterial and antifungal (C. albicans, G. candidum). Traditional healers have been using the leaves of Senna alata (L.) Roxb. for a long time for the treatment of tinea versicolor and ringworm infection and other diseases in Africa (Koch, 1981; Adjamahoun et al., 1991; Igo, 2005; Wuthi-Udomlert et al., 2005). Its EtOH extract was reported to inhibit D. congoensis growth (Ali-Emmanuel et al., 2003). Makinde et al. (2007) in contrary showed higher antifungal activity of aqueous-MeOH extract of S. alata on fungi (Microsporum canis, Blastomyces dermatitidis, Trichophyton mentagrophytes, Candida albicans, Aspergillus flavus) and weak inhibition activity on bacteria (Dermatophilus congoensis, Proteus vulgaris, Staphylococcus aureus, Corynebacterium parvum, Actinomyces bovis, Nocardia asteroides, Clostridium septicum and Bacillus pumilus). Weak antifungal activity of aqueous-EtOH, HCl, EtOH, lyophilized and aqueous extracts of leaves of S. alata on dermatophyte and C. albicans have been demonstrated Wuthi-Udomlert et al., (2005).

The screening of bioactive molecules of leaves of S. alata revealed the presence of anthraquinone aglycone, anthraquinone glycosides (Wuthi-Udomlert et al., 2005), phenolics and terpenoids, alkaloid salt, alkaloid (Makinde et al., 2007). Study results demonstrated that the MeOH extract of E. hirta is more active to all bacteria than the CH2Cl2 ones confirming that polar extract is more active. Similar extracts were obtained with the EtOH extract of leaves of E. hirta on E. coli, S. aureus, B. subtilis and P. aeruginosa at the concentration of 200 mg mL−1 (Ogbulie et al., 2007). The antiplasmodial activity of CH2Cl2 and EtOH extracts of E. hirta have been reported Tona et al. (1999). Physicochemical studies of E. hirta reported in the literature showed the presence of terpenes, saponins, alkaloids, steroids and cardiac glycosides (Oliver-Bever, 1986; Parekh and Chanda, 2007). Both MeOH extracts of S. guineensis and A. floribunda demonstrated weak antibacterial on S. cholera. A. floribunda is commonly used by the population of Baka pygmies in Cameroon and Central Republic Africa for the treatment of cough (Betti, 2004).

The second group of five (29.45%) extracts included which showed moderate antibacterial activity on at least 4 bacteria tested. They included MeOH extracts of S. guineensis and M. lucida and L. alata aqueous-EtOH extracts of L. guineensis, D. adscendens and aqueous-MeOH extract of C. asiatica. This results showed that Monnarda lucida Benth possess antibacterial property. This plant is used in tropical Africa for their therapeutic value in the treatment of antiparasitic diseases (Kambu, 1990; Tona et al., 1999). Some biological properties of leaves of M. lucida such as Schistosomicidal (Adewummi and Adesogan, 1986a), Molluscicidal (Adewummi and Adesogan, 1986b) and trypanosomicidal (Asuzu and Chineme, 1990) have been reported. Adomi (2006) demonstrated that the aqueous bark of M. lucida was sensitive only on S. aureus and P. aeruginosa while its ethanol extract showed the great inhibition halos on S. aureus, S. typhi, K. pneumoniae, P. aeruginosa, E. coli, B. subtilis, Flavobacterium sp. and Centella asiatica has been used in traditional medicine in Asia for hundreds of years as a fresh eaten vegetable (Hamid et al., 2002).

This plant has been effectively used in folk for the treatment of inflammation, anemia, asthma, blood disorders, bronchitis, fever, urinary discharge and splenomegaly (Duke, 2002). It is also believed, to have beneficial effects in improving memory and treating mental fatigue, anxiety and eczema (Duke, 2002). In this study C. asiatica demonstrated potential antimicrobial activity. This property is attributed to triterpene, saponins, asiaticoside, sapogenin asiatic acid, madecassoside and madecassic acid major constituents isolated from the plant and which the antibacterial activity has been demonstrated by Cowan (1999) and Somchit et al. (2004).

The aqueous extract of C. asiatica possesses antioxidant, cognitive-enhancing, antiepileptic, antinociceptive and anti-inflammatory properties (Gupta et al., 2003; Somchit et al., 2004). An aqueous-EtOH extract of L. guineensis showed no antifungal activity. S. faecalis is found to be the most sensitive among seven bacteria tested. The chemical composition of aqueous extract of L. guineensis reported in the literature indicated the presence of alkaloids, saponins and reducing sugars (Falodun et al., 2007). In this antifungal screening, an aqueous-EtOH extract of D. adscendens, M. lucida as well as C. asiatica showed poor inhibition on Penicillium sp. Several studies reported the antibacterial (Chukwujelewu et al., 2005; Ogbulie et al., 2007), anti-inflammatory and antimalarial (Chukwujelewu et al., 2005) activities of extract of M. lucida. A bark extract of a tree of Lophira alata is used as a folk medicine in western Africa for inflammations and analgesic activity. Three monooesters related to lophirosides and 4 lophirosides were isolated from Lophira alata (Tih et al., 1994).
The last group of these molecules exhibited weak anti-bacterial activity against Micrococcus luteus but do not act as defensive substances against insects and microorganisms (Fleming, 1999).

The last group of five (29.41%) including CH₂Cl₂ extract of A. montanus, C. asiatica, E. hirta, MeOH extract of L. guineensis and D. adscendens. All these extracts exhibited moderate inhibition the growth of 29.24% of bacteria tested. Poor antifungal activity of MeOH extract of E. hirta was noted in this study. The leaf decoction of Acanthus montanus (Nees) T. Anders (Acanthaceae) is used by Nigeria healers to treat chesty coughs (Obute, 2006). In general, all bacteria used in this study involved diseases such as urogenital and respiratory tract infection, chancr, nosocomial pathogens, diarrhoea and opportunistic infections (Atlas, 1988). The observed antibacterial properties corroborate its use in traditional medicine. The relatively high zone of inhibition exhibited by the extracts against E. coli is also of significance, since E. coli and S. cholera is a common cause of diarrhoea in developing countries. The large zones of inhibition exhibited by the extract against S. aureus and P. aeruginosa justified their use by traditional medical practitioners in the treatment of sores, bores and open wounds. S. aureus and P. aeruginosa have been implicated in cases of boils, sores and wounds (Ogbulie et al., 2007). The inability of some extracts to inhibit M. morganii and P. mirabilis may be that these bacteria to posses mechanisms by which they convert substances that inhibit their growth to non-toxic com-pounds.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Continued, further exploration of plant-derived antimicrobials is needed today.

The present study, of in vitro antimicrobial evaluation of some plants, forms a primary platform for further phytochemical and pharmacological studies. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds. In conclusion, EtOH extract of S. aculeastrum possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. Its MeOH extract showed weak antifungal property. Further research will be carried out on the fractionation of these interesting extracts in order to identify the active.

REFERENCES


