IN VITRO ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS FROM PLANTS *BRYOPHYLLUM PINNATUM* AND *KALANCHOE CRENATA*

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Abstract

Extracts from the leaves of *Bryophyllum pinnatum* and *Kalanchoe crenata* were screened for their antimicrobial activities. Solvents used included water, methanol, and local solvents such as palmwine, local gin (Seaman’s Schnapps 40% alcoholic drink,) and “omi ekan-ogi” (Sour water from 3 days fermented milled maize). Leaves were dried and powdered before being soaked in solvents for 3 days. Another traditional method of extraction by squeezing raw juice from the leaves was also employed. All extracts were lyophilized. These extracts were tested against some Gram-negative organisms (*Escherichia coli* ATCC 25922, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella paratyphi*); Gram-positive organisms (*Staphylococcus aureus* ATCC 25213, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*) and a fungus (*Candida albicans*). Agar well diffusion and broth dilution methods were used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) at concentrations of 512mg/ml to 4mg/ml. All the organisms except *Candida albicans* were susceptible to the extracts obtained from the traditional method. The squeezed-leaf juice of *Kalanchoe crenata* was the most active one with MIC of 8 mg/ml against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis*, 32 mg/ml against *Shigella flexneri*, 64 mg/ml against *Escherichia coli* and 128 mg/ml against the control strain *Staphylococcus aureus* while its MBC is 256 mg/ml against these organisms except *Bacillus subtilis* and *Klebsiella pneumoniae*. The Gram-positive organisms were more sensitive to the methanol and local gin-extract of *Bryophyllum pinnatum*. Extracts from other solvents showed moderate to weak activity.

Key words: Antimicrobial, *Bryophyllum pinnatum*, *Kalanchoe crenata*, Local solvents, Gram-positive organism, Gram-negative organism

Introduction

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant (WHO, 2001, Aibinu et al., 2003; Aibinu et al., 2004). The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams, 2000). Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius et al., 2003, Moreillion et al., 2005).
Bryophyllum pinnatum and Kalanchoe crenata belong to the plant family Crassulaceae. They show close proximity in usage, habitat, preparation and identification. The external morphological features of Kalanchoe crenata resemble that of Bryophyllum pinnatum. An inexperienced taxonomist and even unseasoned traditional practitioner can readily confuse the two plants. This is attested to by the same local name that is being used by Yorubas in Southwest Nigeria. Some People refer to both plants as “Odundun”. Ethnobotanically, most often they are prepared and administered the same way. Bryophyllum pinnatum is used in ethnomedicine generally for the treatment of ear-ache, cough, diarrhoea, dysentery, abscesses, ulcers, insect bites, heart-troubles, epilepsy, arthritis, dysmenorrhea and whitlow (Gill, 1992). In southern Nigeria, it is used to facilitate the dropping and healing placenta wound of newly born babies. The plant leaf is mildly exposed to heat and the juice is squeezed out and applied as poultice to the baby’s placenta on daily basis. Also, the crushed leaves as well as the extracted juice are mixed with shea butter or palm oil and rubbed on abscesses or other swellings. This is also applied on ulcers, burns and on the bodies of young children when they are ill. The leaves of this plant contain bryophyllin, potassium, malate, ascorbic, malic, and citric acids (Oliver, 1989). The plant is rich in both macro and micro elements, vitamins, calcium, phosphorus, ascorbic acid, inulin (Okwu and Josiah, 2006) and other compounds like saponin, flavonoids, anthraquinones, xanthenes, bryophyllin A and B (Iwu, 1993). Anti-inflammatory, hypoglycaemic, anti diabetic and anticancer properties have been reported (Gill, 1992).

The external applications of Kalanchoe crenata are the same as those of Bryophyllum pinnatum. The juice obtained by squeezing the leaves that have been passed over fire slightly, is most commonly used for the treatment of headache, general debility, dysentery, smallpox and convulsion. One or two drops of the leaf juice is dropped into the ear for earache. A poultice of the leaves is applied over wounds and sores. The leaves can be boiled in water and the extract is given as a sedative for asthma and palpitation. Also the leave juice mixed with salt and honey is a remedy for chronic cough. The extract of dried leaves is applied to septic wound (Sofowora, 1993).

In East Africa, the slightly heated leaves (heated over fire) are rubbed over the body as treatment for stiff joint and rheumatism (Gill, 1992). Other parts of the plant especially the root is prescribed for gonorrhhea, vermifuge and abortion (Sofowora, 1993). Alkaloids and saponins are present in the aqueous and alcoholic extracts of leaves and lectins in the juice from the fresh leaves (Nguelefack et al., 2006). The green callus of the plant contain malic acid, quinones and tocopherol (Sofowora, 1993; Oliver, 1989).

Other works have also shown that this plant possesses analgesic, anticonvulsion, antiinflammatory, antiarthritic and antispasmolytic properties (Theophil et al., 2006). The conventional method to extract plant materials is to use methanol, ethanol, acetone and so on as extracting solvents, but the ethnobotanical approach like the use of Palmwine, “Omidun/ekan-ogi”(the water derived from three days fermented milled maize), local gin as extracting solvent and ways in which they are prepared locally, has received less attention. The type of solvents and methods of preparation affect antimicrobial activity of plants (Ellof, 1998; Adesanya, 2005). On the basis of this background, in-vitro antimicrobial activities of the extracts Bryophyllum pinnatum and Kalanchoe crenata from various solvents were tested against clinically important pathogens.

Materials and Method
Plant Materials

The leaves of Bryophyllum pinnatum and Kalanchoe crenata were collected from Oke-igbo in Ondo State. These plants can be collected during rainy season particularly between March and July. The samples were identified by Mr. T.K., Odewo of the Herbarium Department, Forestry Research Institute of Nigeria (FRIN) at Ibadan, Oyo State. The leaves were dried in an oven at 45°C for fourteen days. The dried leaves were powdered and stored in a sterile bottle at room temperature (Shahidi Bonjar, 2004). Both aqueous and methanol extraction were carried out by using Soxhlet extractor (Quickfit U.K). Powdered dried leaves (50 g) were extracted with 250 ml of solvent (Akinyemi et al., 2005).

Traditional methods of extraction includes:
Steeping.

The powered leaves (50 g) were suspended in the following solvents – Palm-wine, “Omidun” and the local gin (Seaman’s Schnapp from Nigeria Distilleries Ltd), inside a conical flask. The conical flasks were shaken intermittently for three days at room temperature. The resulting extracts were decanted, filtered using Muslin cloth and Whatman No 1 filter paper and stored in sterile bottles (Atata, 2003).
Squeezing of the leaf part

Fresh leaves (300g) were passed over heat using the bursen burner; similar to the way they are prepared traditionally. The leaves were then squeezed and the resulting juice was filtered using a sterile Muslin cloth and Whatman No 1 filter paper and stored in sterile bottle. Extracts from all these extraction methods were tested for purity by plating them on Mueller Hinton Agar (MHA) (Oxoid, UK) and incubated for 24 hours at 37°C. All the resulting extracts were lyophilized in a freeze dryer (Edwards, USA). The powdered forms were weighed, labeled, stored in sterile bottles and kept in refrigerator.

Preparation of Test Samples

In the study of the antimicrobial activities of these plants, concentrations of 512 mg/ml of each extract were used for the screening. This was done by dissolving 2.1 g of the extracts in 4 ml of each of the extracting solvents. For the squeezed juice that was lyophilized, distilled water was used to suspend the sample. Each of the solid extracts was reconstituted in its respective solvent to obtain a stock solution of 512 mg/ml (NCCLS, 2000).

Sources and maintenance of organisms

Gram-positive organisms (Staphylococcus aureus ATCC 25213, Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis). Gram-Negative organisms (Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli ATCC 25922, Escherichia coli, Shigella flexneri, Salmonella paratyphi, Citrobacter species) and a fungal yeast Candida albicans were obtained and confirmed at the research laboratory of the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos. They were maintained on Mueller-Hinton Agar medium (Oxoid, UK). Twenty-four hour old pure cultures were prepared for use each time.

Culture media

Mueller-Hinton Agar (Oxoid, UK) was prepared according to the manufacturer’s instruction, autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

Antimicrobial Bioassay

Suspension of micro-organisms was made in sterile normal saline and adjusted to 0.5 Macfarland standard (10^8Cfu/ml) (NCCLS, 2000). From the stock of 512mg/ml extract, serial dilutions were made to 256, 128, 64, 32, 16, 8, 4 mg/ml (NCCLS, 2000). Each labelled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5mm diameter was used to make wells on the medium. 0.1ml of the various extract concentration were dropped into each, appropriate labeled well (Atata 2003; Shahidi Bonjar 2004). Other solvents used for extraction apart from water were tested neat for each organism. The inoculated plates were kept in the refrigerator for 1 hour to allow the extracts to diffuse into the agar (Atata, 2003). The Mueller Hinton Agar plates were incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. 0.05% of Ciprofloxacin was used as control (NCCLS, 2000).

Determination of Minimum Inhibitory Concentration (MIC)

To measure the MIC values, various concentrations of the stock, 512, 256, 128, 64, 32, 16, 8, 4mg/ml were assayed against the test bacteria. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth (Prescott et al., 1999; Shahidi Bonjar, 2004).

Determination of Minimum Bactericidal Concentration (MBC)

Equal volume of the various concentration of each extract and Mueller Hinton broth (Oxoid, UK) were
mixed in micro-tubes to make up 0.5ml of solution. 0.5ml of McFarland standard of the organism suspension was added to each tube (Shahidi Bonjar, 2004). The tubes were incubated aerobically at 37\(^{\circ}\)C for 24 h. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculum. The MBC was determined by sub culturing the test dilution on Mueller Hinton Agar and further incubated for 24 h. The highest dilution that yielded no single bacterial colony was taken as the Minimum bactericidal Concentration (Akinyemi et al., 2005). This was carried out on some of the extracts with high antimicrobial activity and some of the highly sensitive organisms

**Results**

Antimicrobial activities of the extracts obtained from *Bryophyllum pinnatum* and *Kalanchoe crenata* leaves, using different solvents and extracts from the squeezed leaves, against the tested organisms are shown in Table 1-3. The “omi ekan-ogi” extract of *Kalanchoe crenata* showed no activity. The zones of inhibition of each organism and the respective minimum inhibitory concentrations of the extracts, which range between 8mg/ml to 512mg/ml, are shown in Tables 1 and 2. The minimum bactericidal concentrations of some of the most active extracts, which range between 128mg/ml to 512mg/ml, are also shown in Table 3. Extracts from the squeezed leaves of *Kalanchoe crenata* was particularly active against tested organisms except *Candida albicans*. This was followed by the activities of extract from the local gin, aqueous extract and methanol extract. The methanol extract of *Bryophyllum pinnatum* showed greater antimicrobial activities than the local gin extract, followed by the squeezed leaves extract, then the aqueous extract, “Omidun” extract and palmwine extract. These extracts were active against tested organisms but the activity of the Palm-wine extract was poor.

**Discussion**

The result of this study showed that *Bryophyllum pinnatum* and *Kalanchoe Crenata* extracts have varied antibacterial activities against the tested organisms except “Omidun” extract of *Kalanchoe crenata* which showed no activity (Table 2). This suggests that the extracts of these plants are broad spectrum in their activities. This correlates with the observation of previous workers that plants contain substances that are antimicrobial (Olukoya, 1986). Extracts from the squeezed leaves of *Kalanchoe crenata* is the most active. It showed better antimicrobial activity than *Bryophyllum pinnatum* leaves that were prepared in the same way. It was active against both the Gram-positive and Gram-negative organisms with better activity against the Gram negative organisms. The palm wine extract of *Kalanchoe crenata* was highly active against the *E. coli* organisms tested, *Salmonella paratyphi* and *Citrobacter* spp. This implies that the use of palm-wine as a solvent releases some active ingredients which have high activity against some enteric organisms. There was no activity observed against *Candida albicans* (Table 2). The various antimicrobial activities of this plant extract in different solvents as shown from the result of this study, confirms its use traditionally in treating antimicrobial infections like dysentery, wound infection, sore, ear infection and abscesses. Both the aqueous extract of the dried leaves and methanol extraction of *kalanchoe crenata* showed moderate antimicrobial activities.

Out of all the extracts from *Bryophyllum pinnatum* leaves, the methanol extract was the most active. It showed marked antibacterial activities against Control strain of *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis* and *Pseudomonas aeruginosa* The zones of inhibition observed in the Gram-positive organisms were almost the same with the control antibiotic (Ciprofloxacain) used (Table 1). This result is similar to the work of Akinpelu (2000) and Ofokansi (2005) that showed strong activities of methanol extract of *Bryophyllum pinnatum* against some Gram-positive organisms. The antimicrobial effect of methanol extract against these organisms may be due to the ability of the methanol to extract some of the active properties of these plants like phenolic compounds, saponin, bryophyllin and other secondary metabolites which are reported to be antimicrobial (Cowan, 1999; Okwu and Josiah, 2006). Extracts from the squeezed leaves of *Bryophyllum pinnatum* showed significant effect on some of the Gram positive and Gram-negative organisms (Table 2). This justifies the traditional use of the juice obtained from the slightly heated fresh leaves of this plant against antimicrobial infections like skin infections, wound infection, abscess and gastrointestinal disorder (Gill, 1992). This plant’s leaf juice is commonly used in treating ear infection and navel of newborn babies, which not only heals fast but also prevent infection at this site. This practice is common among the women in the Southern part of the country. Extracts of this plant in which “omidun” and the local gin were used as solvent were also found to have good activity on both Gram-positive and Gram-
Table 1: Antimicrobial Activities of Plants Extracts against Some Microorganisms

<table>
<thead>
<tr>
<th>ORGANISMS</th>
<th>ZONE OF INHIBITION (MM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MB</td>
</tr>
<tr>
<td>1 Staphylococcus aureus</td>
<td>20</td>
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<tr>
<td>Control strain</td>
<td></td>
</tr>
<tr>
<td>2 Staphylococcus aureus</td>
<td>21</td>
</tr>
<tr>
<td>3 Enterococcus faecalis</td>
<td>18</td>
</tr>
<tr>
<td>4 Bacillus subtilis</td>
<td>23</td>
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<tr>
<td>5 Escherichia coli</td>
<td>16</td>
</tr>
<tr>
<td>6 Escherichia coli ATCC 25922</td>
<td>14</td>
</tr>
<tr>
<td>7 Pseudomonas aeruginosa</td>
<td>17</td>
</tr>
<tr>
<td>8 Klebsiella pneumoniae</td>
<td>12</td>
</tr>
<tr>
<td>9 Shigella flexneri</td>
<td>18</td>
</tr>
<tr>
<td>10 Salmonella paratyphi</td>
<td>17</td>
</tr>
<tr>
<td>11 Citrobacter spp</td>
<td>14</td>
</tr>
<tr>
<td>12 Candida albicans</td>
<td>17</td>
</tr>
</tbody>
</table>

**KEYS**

MB = Methanol extract of *Bryophyllum pinnatum* leaves

OB = “Omi ekan ogi extract of *Bryophyllum pinnatum* leaves

SB = Extract from Squeezed leaves of *Bryophyllum pinnatum* slightly passed over heat

AB = Aqueous extract of *Bryophyllum pinnatum* leaves

PB = Palmwine extract of *Bryophyllum pinnatum* leaves

LB = Local gin extract of *Bryophyllum pinnatum* leaves

MK = Methanol extract of *Kalanchoe crenata* leaves

SK = Extract from Squeezed leaves of *Kalanchoe crenata* slightly passed over heat

AK = Aqueous extract of *Kalanchoe crenata* leaves

PK = Palmwine extract of *Kalanchoe crenata* leaves

OK = “Omi ekan ogi” extract of *Kalanchoe crenata* leaves

LK = Local gin extract of *Kalanchoe crenata* leaves

CIP = Ciprofloxacin

Negative isolates tested. Other extracts of *Bryophyllum pinnatum* showed moderate to weak activity against tested organisms (Tables 1 and 2). This may be as a result of loss of some of the plant’s active principle when drying or the inability of the solvents to dissolve some of the active principles of this plant (Ellof, 1998).

In this study, the various extracts from the two plants demonstrated varying activity but generally the traditional method of preparation of squeezing the leaves showed significant activity than other local and conventional methods of preparation. An exception was the methanol extract of *Bryophyllum pinnatum*. This study does not only show the scientific basis for some of the therapeutic uses of this plant in traditional medicine, but also confirms the fact that ethno botanical approach should be considered when investigating antimicrobial properties of plants (Adesanya et al., 2005; Iwu, 1993). It also explains why these plants give similar therapeutic result when they are used interchangeably in spite of their uniqueness (Sofowora, 1993).

The implication of the broad spectrum action of some of these extracts is that they can be useful in antiseptic and disinfectant formulation as well as in chemotherapy if the active principle can be isolated (Olukoya et al., 1993). The anti-pseudomonal and anti-staphylococcal activities of some of the effective extracts of these plants can be further explored.

**Acknowledgement**

This study was supported by a grant from University of Lagos (CRC 2005/08). We appreciate the assistance of members of staff of the Herbarium Department of Forestry Research Institute of Nigeria (FRIN). The laboratory assistance of Mrs Sola Oyerinde of the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos is gratefully acknowledged.
Table 2: Minimum Inhibitory Concentrations (mg/ml) of the Extracts

<table>
<thead>
<tr>
<th>ORGANISMS</th>
<th>MB</th>
<th>SB</th>
<th>OB</th>
<th>PB</th>
<th>LB</th>
<th>SK</th>
<th>MK</th>
<th>PK</th>
<th>OK</th>
<th>AK</th>
<th>LK</th>
</tr>
</thead>
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<tr>
<td>1 Staphylococcus aureus Control</td>
<td>128</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>128</td>
<td>128</td>
<td>256</td>
<td>-</td>
<td>-</td>
<td>256</td>
<td>128</td>
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<tr>
<td>strain</td>
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</tr>
<tr>
<td>2 Staphylococcus aureus</td>
<td>256</td>
<td>128</td>
<td>256</td>
<td>-</td>
<td>128</td>
<td>32</td>
<td>128</td>
<td>512</td>
<td>-</td>
<td>-</td>
<td>256</td>
</tr>
<tr>
<td>3 Enterococcus faecalis</td>
<td>512</td>
<td>512</td>
<td>256</td>
<td>-</td>
<td>128</td>
<td>32</td>
<td>128</td>
<td>512</td>
<td>-</td>
<td>-</td>
<td>256</td>
</tr>
<tr>
<td>4 Bacillus subtilis</td>
<td>128</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>128</td>
<td>32</td>
<td>8</td>
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<td>5 Escherichia Coli</td>
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<td>-</td>
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<td>64</td>
<td>256</td>
<td>256</td>
<td>-</td>
<td>512</td>
</tr>
<tr>
<td>7 Pseudomonas aeruginosa</td>
<td>256</td>
<td>512</td>
<td>512</td>
<td>256</td>
<td>128</td>
<td>64</td>
<td>8</td>
<td>512</td>
<td>512</td>
<td>-</td>
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</tr>
<tr>
<td>8 Klebsiella pneumoniae</td>
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<td>8</td>
<td>512</td>
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<tr>
<td>9 Shigella flexneri</td>
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<td>256</td>
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<td>256</td>
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<td>128</td>
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<td>64</td>
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<tr>
<td>12 Candida albicans</td>
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<td>128</td>
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</tbody>
</table>

**KEYS**

MB = Methanol extract of *Bryophyllum pinnatum* leaves  
SB = Extract from Squeezed leaves of *Bryophyllum pinnatum* slightly passed over heat  
AB = Aqueous extraction of *Bryophyllum pinnatum* leaves  
PB = Palmwine extraction of *Bryophyllum pinnatum* leaves  
LB = Local gin extract of *Bryophyllum pinnatum* leaves  
MK = “Omidun” extract of *Bryophyllum pinnatum* leaves  
OB = Methanol extract of *Kalanchoe crenata* leaves  
AK = Aqueous extract of *Kalanchoe crenata* leaves  
SK = Extract from Squeezed leaves of *Kalanchoe crenata* slightly passed over heat  
PK = Palmwine extract of *Kalanchoe crenata* leaves  
LK = Local gin extract of *Kalanchoe crenata* leaves  
- = No inhibitory activity

Table 3: Minimum Bactericidal Concentration (mg/ml) Of Some Extracts on Susceptible Organisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>MB</th>
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<th>LB</th>
<th>PK</th>
<th>LK</th>
<th>SK</th>
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<tr>
<td>Salmonella paratyphi</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Bacillus subtilis</td>
<td>128</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>256</td>
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<td>Citrobacter spp</td>
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<td>-</td>
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<td>512</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
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</tr>
</tbody>
</table>

**KEYS**

0 = No bactericidal activity  
- = Not tested for minimum bactericidal activity  
MB = Methanol extract of *Bryophyllum pinnatum* leaves  
SB = Extract from Squeezed leaves of *Bryophyllum pinnatum* leaves  
LB = Local gin extract of *Bryophyllum pinnatum* leaves  
SK = Extract from Squeezed leaves of *Kalanchoe crenata* leaves  
PK = Palmwine extract of *Kalanchoe crenata* leaves  
LK = Local gin extract of *Kalanchoe crenata* leaves
References

12. Inc., Florida, U.S.A