

Full Length Research Paper

## ***In-vitro* evaluation of the anti-bacterial properties of crude extracts of *Dorstenia mannii* (Moraceae)**

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Bacterial resistance to classical antibiotics nowadays is becoming a health concern in Cameroon. Patients suffering from bacteria attack spend money to carry out laboratory tests for effective diagnosis, with very little positive results. This is due to the fact that most of our hospitals lack the up to date laboratory equipments, to detect the real antibiotic that will kill a particular bacterium. This has led to more patients spending a lot of money on bacterial infection treatment, and some even end up dying with the disease. It is on this basis that this work was designed to research the antibacterial properties of the extracts namely crude extract, hexane-acetate 25%, hexane-acetate 50%, hexane-acetate 75%, hexane, acetate and methanol of the leaves of *Dorstenia mannii*, a plant that is readily available from our natural environment at little or no cost. These extracts were tested on the following bacteria species namely; Gram-negative bacteria; *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica*; Gram-positive bacteria; *Staphylococcus aureus* and *Bacillus* sp. After some laboratory analysis using the antibiogramme sensitivity test, it was discovered that the extracts of the leaves of *D. mannii* were only sensitive on three of the eight bacteria used which were, *E. coli*, *S. enterica* and *Klebsiella* sp. The other five namely; *P. aeruginosa*, *E. aerogenes*, *P. mirabilis*, *S. aureus* and *Bacillus* sp, were resistant to the extract. The sensitivity level of the three bacteria, that showed positive response where however low. Therefore, more research has to be carried out using different concentrations of the leaves extract for a more conclusive result. This concentration could permit to appreciate the degree of sensitivity and also to see if other bacteria that have not manifested any sensitivity can show some positive response to the extracts. Also, different laboratory methods and more bacteria should be used to prove that the leaves of *D. mannii* have the potential as a promising antibacterial medicinal plant to either render bacteria inactive or kill them completely.

**Key words:** Antibacterial properties, *Dorstenia mannii*, strain sensitivity, gram positive, gram negative bacteria.

### INTRODUCTION

Antibacterial properties are the ability of a drug or a plant extract to stop a bacterium from growing, render it inactive or kill it completely. These properties are derived

from synthetic compounds or drugs and from natural plants products (Walker et al., 2004). The goal in surveying plants for biologically active compounds should be to isolate the constituent (s) responsible for a particular activity, although the constituent may act additively or synergistically (Horan et al., 2003). Knowledge of the phytochemicals present may help explain or predict a variety of events relating to the

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antimicrobial efficacy and toxicity of herbal preparations (De Smith and Revier, 1989).

The study of antibacterial properties of plants is an important aspect of biochemistry. The isolation and characterization of secondary metabolites like coumarins and flavonoids, from a plant prove scientifically that the plant contains antibiotic healing substances is a discipline of phytochemistry (Abegaz et al., 2003).

The species of the genus *Dorstenia* have been largely used in traditional pharmacopoeia in Africa. Research has been carried out by (Kueté et al., 2007a; Ngameni et al., 2007) on the antimicrobial properties on many genus of *Dorstenia* but findings have very little studies on the antibacterial properties of *D. Mannii*.

Presently, there are many chronically debilitating or life threatening diseases that urgently require improved or new medicinal treatments. With new diseases and increasing resistance to existing drugs, there is need to discover and develop new innovating drugs with diminished side effects to combat bacteria infections (Cirzrt et al., 2005; Zhu et al., 2008). The use of herbs to treat diseases is almost universal among non industrialised societies (Brater and Daly, 2000). Pharmaceutical products are quite expensive for most of the world's population, half of which live on less than 2 US\$ per day (Eldredge, 2003). In this context, any resources that can help alleviate the burden of these deadly diseases, including the search for new chemical entities from plants that derived biologically active ingredients, are worthy of investigation, in view of helping the society with little or no cost on drugs purchasing.

The study of herbs dates back over 5000 years to the Sumerians (Kasem, 1992). Through this study, our ancestors through traditional culture have left a chain of traditional knowledge on how we can use medicinal plants in an empirical manner (Swain et al., 1991). The exploitation of our own natural resources to treat diseases is of great importance for a low poor developing nation like Cameroon. In this light, this piece of work had as its goal, to prove the antibacterial properties of the crude extracts of the leaves of *D. Mannii*, to determine the extract with greatest antibacterial properties, and what bacteria species amongst the studied bacteria was more sensitive to the extract.

Many of our present pharmaceuticals are derived directly or indirectly from higher plants (Horan et al., 2003), and the treatment of diseases with plant extracts is called herbalism or phytotherapy (Houghton, 1995). Herbal medicines are not pure products with a single active ingredient and the heterogenous nature of herbal medicines makes safety monitoring essential (Huxtable, 1980).

Even though many bacteria are resistance to antibiotics, there exists little publication on the bacteria that show resistance to synthetic antibiotics (Kueté et al., 2007b). In the genus of *Dorstenia*, there are many plant species that have been proven scientifically that they

have antimicrobial properties (Ngadjui et al., 2000; Abegaz et al., 2002). But no work has been done to prove the antibacterial properties of *D. mannii*. This study was therefore orientated towards the research on the antibacterial properties of *D. mannii* like proven in other *Dorstenia*'s. The question here is to know whether *D. mannii* presents general antibiotic properties on microbes. Can these extracts react on the following; protozoans, fungi, virus or bacteria? Bacterial infections have become a frequent pathological problem and need serious attention. Bacteria infections can cause diarrhoea, dysentery, and typhoid, food borne illnesses and sometimes sterility in adults (Schell and Jorgensen, 2001; WHO, 2008a). Most of these common bacteria are antibiotics resistant. The species of the genus *Dorstenia* are largely used in traditional Pharmacopoeia (Swain and Downum, 1980). Studies have shown that many species from the *Dorstenia* genus present in different degree, antimicrobial activities (Swain et al., 1991; Abegaz and Ngadjui, 1999). Amongst others, in Cameroon, twigs of *Dorstenia angusticornis* have been tested for their *in vitro* antimicrobial activity (Kueté et al., 2007c). The twigs of *Dorstenia elliptica* have been tested for their *in vitro* antimicrobial activity against bacteria and fungi (Kueté et al., 2007c).

Outside these activities observed from this genus, traditional pharmacopoeia reveals other particular activities of certain species, such as anti-diabetic, anti-cholesterol, anti-oxidant and anti-malarial properties (Kueté et al., 2007a). Thus the leaves of *Dorstenia multiradiata* are used in Nigeria for their antimolluscicide activity (Haerdi, 1964). The twigs of *Dorstenia bacteri* are used in Cameroon for their antimalarial properties (Iwu et al., 1992). In many African countries, the roots of *Dorstenia barmianiana* are used in the treatment of skin diseases (Iwu et al., 1992). The juices of the leaves of *D. elliptica* are used as eye drops in Congo (Bouquet, 1969). The decoction of leaves of *Dorstenia poinsettifolia* is used to treat infected wounds (Tsopmo et al., 1998). *Dorstenia contrajerva* is used in Bolivia like an antidote against snake and insect bites and also to remove worms like tenea (Okunji, 1998). It is equally used in Guatemala to treat diarrhea, in Costa-Rica, Brazil and Mexico to provoke menstruation (Logan, 1973). This same specie is used in Argentina like a tonic. The roots of *Dorstenia Klanei* are used as infusion against stomach ache, in gargarisme against tooth ache or in fraction mixed with the stems of *Pycnanthus angolensis*, plus small portion of white clay, to calm down head ache and fights rheumatism (Walker and Sillans, 1961). The roots of *Dorstenia drakena* present an anti-secretory effect, a diuretic activity, lachrymal activity, mydratic and spasmolytic activities (Kyerematen et al., 1985). The decoctions of the leaves and roots of *Dorstenia psilurus* are used in the treatment of rheumatism, snake bites, against head ache and stomach ache (Bouquet, 1969). The roots of *D. psilurus* contain odorous substances and

are thus used like cooking spices in the western region of Cameroon (Noumi, 1984).

These large utilisation of the genus *Dorstenia* has led to the studies of the anti bacterial properties of *D. mannii* which so far has not yet been investigated and analysed scientifically to show that bacteria cultured *in vitro* can be resistant to the extracts or not, and also to find out what fraction of the extract possesses the highest degree of anti bacterial property.

The resistant problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria, thus an alternate source of treatment from plant sources (Ngadjui et al., 2000).

Faced with these situations, this work has as objective; to investigate sensitivity of the following bacteria strains; Gram negative bacteria- *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Salmonella enterica* *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*; Gram positive bacteria- *Bacillus* sp. and *Staphylococcus aureus*, on exposure on the crude extracts of the leaves of *D. Mannii* (Moraceae).

## MATERIALS AND METHODS

### Materials used for plant harvesting

The materials used for harvesting of the plant sample were a sharp knife to cut the plant, a camera to film the plant, a book and a pen to record information and a bag to put the plants inside.

The first phase of this work was harvesting of the plant sample, identification and conservation. Harvesting was done at the Nkoljobe hill in August 2008, in Yaounde Centre Region of Cameroon. The identification of the plant was established by Mr Nana and Dr Guedje Nicole, Botanist and Ethno-botanist respectively, at the National Herbarium in Yaoundé, Centre Region. The plant was conserved under the voucher specimen reference number (No. 2135) at the National Herbarium. The second phase of this work is drying and chemical extraction of the leaves of *D. mannii*.

### Drying of the sample

Before drying could start, the plants were washed to remove sand and dust particles. The leaves were separated from the rest of the plant and dried separately under shade in an airy condition.

### Chemical extraction and isolation of the plant

The air dried and powdered leaves of the *D. mannii* (1 kg) were soaked in methanol for 48 h at room temperature. Then the extract was filtered using a funnel and a filter paper, and then kept in a flask. Removal of the solvent from the obtained extract under reduced pressure yielded 45 g of a dark-green residue that constituted the crude extract. A mass of 40 g of this organic extract was submitted to flash liquid chromatography on silica gel 60 (220 g), and eluted with hexane, hexane-ethyl acetate gradients (3:1), (1:1), (1:3), pure EtOAc and finally with pure MeOH to give 6 fractions as seen in Figure 1.

Aluminium sheets pre-coated with silica gel 60 GF254 Merck was used for thin layer chromatography and the isolated spots were visualised using both ultra-violet light (254 and 366 nm) and by

spraying with ammonium molybdate solution and heating. The extracts were then kept separately in small bottles and put in the fridge to be used in the bacteriology laboratory in extraction procedure of the leaves of *D. mannii* as shown in Figure 1.

### Bacterial strains

Some seven species of bacteria presented as multi antibiotics resistant namely (Gram negative bacteria) *P. aeruginosa*, *E. coli*, *S. enterica*, *E. aerogenes*, *Klebsiella* sp. and *P. mirabilis*. (Gram positive bacteria) *S. aureus*, *Bacillus* sp. were used. These strains were clinically isolated and cultured in the bacteriology laboratory of C.H.U. in Yaounde, where the antibacterial tests were performed. The strains were incubated at 37°C for 24 h cultured on nutrient agar, which was prepared using the Mueller Hinton media.

### Preparation of bacteria media- Mueller Hinton media

Preparation of bacteria media was done using the Mueller Hinton media.

It is a liquid nutritive media used to research sensibility of germs to antibiotics. It was composed of beef infusion, casein, starch and distilled water and composition made following manufacturers instruction. In the preparation 21 g of powder was dissolved in 1 L of distilled water and sterilized in autoclave at 121°C for 15 min. (Koch, 2003)

### Antibiotic chemicals

Some eight different antibiotic discs that are regularly used for the treatment of bacteria infections were used. Gentamicine (GM), Cotrimoxazole (SXT), Amoxicillin (AMC), Ciprofloxacin (CIP), Amoxycillin + Clavulanic acid (AMC), Cefuroxime (CXM), Cetazolin (CZ), and Ceftriaxole (CRO) were used as reference antibiotics.

### Preparation of test samples

#### Preparation of discs

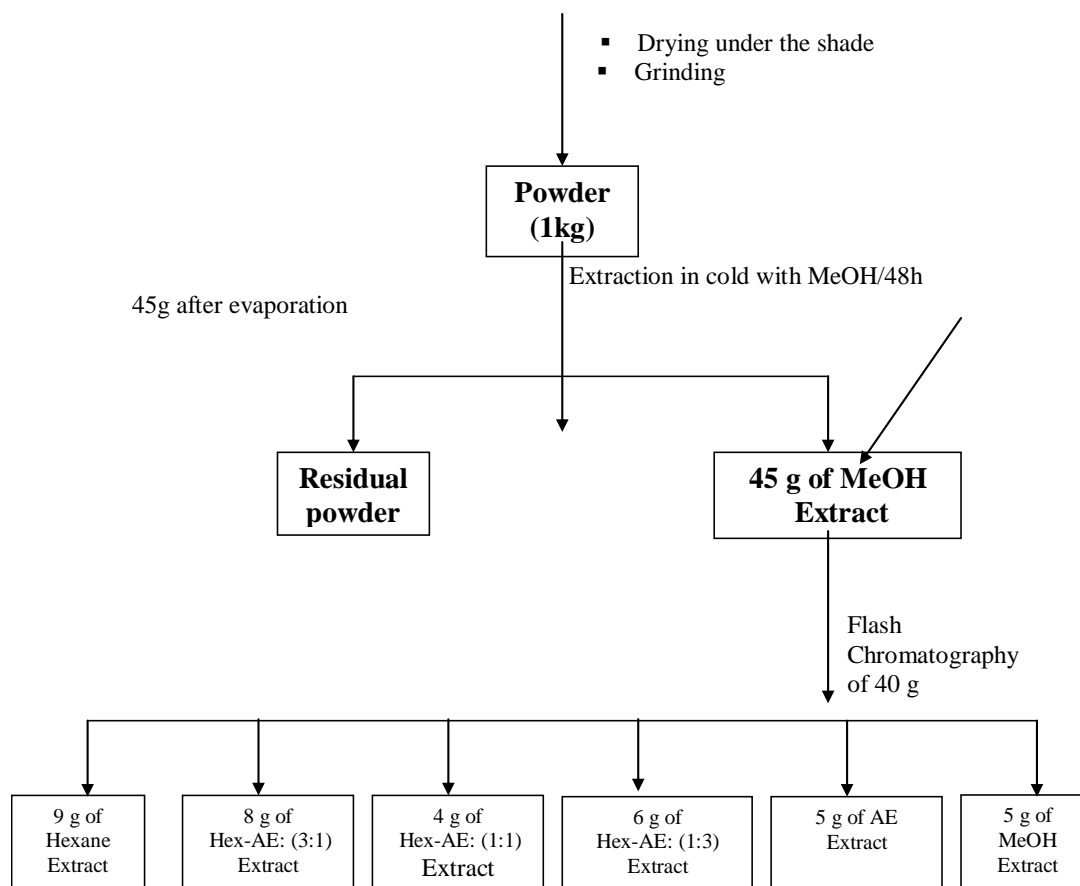
Scheicher and Schüll filter papers were perforated and discs of 6 mm diameter were impregnated with the solution of crude extract or fractions prepared using Dimethyl sulfoxide solution (DMSO).

### Preparation of bacterial inoculation

The bacteria media were vortex and the Petri dishes were inoculated with the bacterial strains, using a sterile swab. The impregnated discs of 7 filter papers were placed in the Petri dishes at equidistant points of the inoculated Petri dishes for the extracts. In the same manner, the disc antibiotics were placed in another seven swabbed Petri dishes that had been inoculated with bacteria media. The Petri dishes were then covered with the lid sealed with Para-film to prevent contamination then incubated at 37°C for 24 h. Anti bacterial activity was evaluated by measuring the diameter of the inhibition zone around the disc. The assay was repeated twice and the results were expressed using signs as follows (-) for no activity and (+) for activity of the crude extracts respectively.

## RESULTS

Out of the eight different bacteria strains used in this



**Figure 1.** Schematic representation of the extraction procedure of *Dorstenia Mannii*.

study, three showed positive response to the antibacterial activity of the crude extract of the leaves of *D. mannii*, while the five other bacterial strains were negative. The three bacteria strain that showed sensitivity were all Gram negative bacteria- *E. coli*, *klebsiella* sp. and *S. enterica*, in response to extracts (AE 100%), (Hex-AE 75%), and crude extract respectively), as shown in Table 1 and Figures 2 to 4. The *S. enterica* showed sensitivity with extract 1 (crude extract) as shown in the Figure 2, while *Klebsiella* sp showed sensitivity with extract 5 (Hex-AE 75%) as shown in the Figure 3. *E. coli* showed sensitivity in one of the extract a shown in Figure 4 and Table 1.

The antibacterial activity of the eight classical antibiotics used showed the following results. *Klebsiella* sp. showed a general resistance to all the eight classical antibiotics used as shown in the Figure 3 and Table 2.

*P. aeruginosa* also showed resistance to all the eight classical antibiotics used in the experiment as shown in Table 2. The antibiogramme sensitivity test showed that *Klebsiella* sp. and *P. aeroginsa* all Gram negative bacteria, were resistant to all the reference antibiotics as shown in Table 2. *S. aureus* a Gram positive bacterium was also resistant to all the reference bacteria.

## DISCUSSION

The antibacterial activity of the crude extract of the leaves of *D. mannii* showed the following results. Out of the eight different bacteria strains used in this study, three showed positive responds while the five other bacterial strains were negative. The three bacteria strain that showed sensitivity were all Gram negative bacteria- *E. coli*, *klebsiella* sp. and *S. enterica*, in response to extracts (AE 100%), (Hex-AE 75%), and the crude extract respectively. The high cost of drugs and increase in drug resistance to common diseases like malaria, bacteria infections and other sexually transmitted diseases has caused the approach to alternative traditional medicine as an option for search for new chemical entities (NCE) (Sofowora, 1982; UNESCO, 1994; Tuley, 1997; WHO, 2008b).

Earlier studies on the antimicrobial activity of different species of *Dorstenia*, for example, the twigs of *D. angusticornis* (Kuetee et al., 2007a) has revealed that most of the bacteria used above showed sensitivity when tested with the extracts of the twigs of *Dorstenia angusticornis*. Antibacterial properties are the ability of a drug or a plant extract to stop a bacterium from growing,

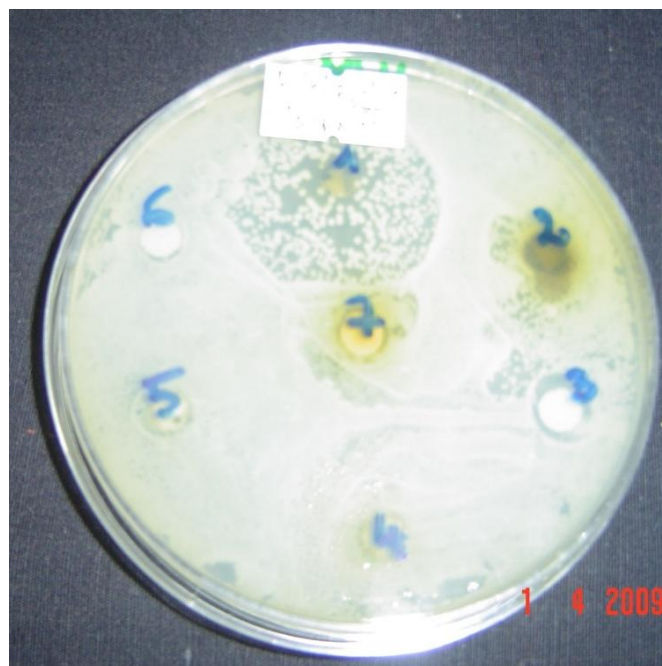
**Table 1.** Antibacterial activity of the crude extracts of leaves of *D. mannii*, determined by the antibiogramme sensitivity test.

Bacterial strains	Fractions						
	Crude extract	Hex (100%)	Hex-AE (25%)	Hex-AE (50%)	Hex-AE 75%	AE (100%)	MeOH (100%)
<b>Gram-negative bacteria</b>							
<i>Enterobacter aerogenes</i>	-	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-	+	-
<i>Klebsiella</i> sp.	-	-	-	-	+	-	-
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-
<i>Salmonella enterica</i>	+	-	-	-	-	-	-
<b>Gram-positive bacteria</b>							
<i>Bacillus</i> sp.	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-

(-): not active. (+): active. Hex-Hexane; Hex-AE=Hexane Ethyl acetate; MeOH= Methyl hydroxide.



**Figure 2.** Photo of *Escherichia coli* showing sensitivity with Extract 1 and 6.



**Figure 3.** Photo of *Salmonella enterica* showing sensitivity with Extract 1.

render it inactive or kill it completely. These properties are derived from synthetic compounds or drugs and from natural plants products (Walker et al., 2004). The goal in surveying plants for biologically active compounds should be to isolate the constituent (s) responsible for a particular activity, although the constituent may act additively or synergistically (Horan et al., 2003). Knowledge of the phytochemicals present may help

explain or predict a variety of events relating to the antimicrobial efficacy and toxicity of herbal preparations (De Smith and Revier, 1989). The knowledge of the local prevalence of pathogens and their antimicrobial sensitivity patterns is essential for clinicians in their routine work and to look for alternative treatments through the research in new chemical entities.

The species of the genus *Dorstenia* are largely used in



Figure 4. Photo showing *Klebsiella* sp. resistant to classical antibiotics.

Table 2. Antibacterial activity of the eight different classical antibiotics determined by the antibiogramme sensitivity test.

Bacterial strains	Antibiogramme sensitivity							
	AMX	AMC	CXM	CRO	GM	CIP	SXT	CZ
Gram-negative bacteria								
<i>Enterobacter aerogenes</i>	-	-	+	+	+	+	+	-
<i>Escherichia coli</i>	-	-	+	+	+	+	+	+
<i>Klebsiella</i> sp.	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i>	-	+	+	+	+	+	-	+
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-
<i>Salmonella enterica</i>	-	-	+	+	+	+	+	
Gram-positive bacteria								
<i>Bacillus</i> sp.	-	-	-	-	+	+	+	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-

(-): not active; (+): active.: Gentamicine (GM), Co-trimoxazole (SXT), Amoxicillin (AMC), Ciprofloxacin (CIP), Amoxycillin + Clavulanic acid (AMC), Cefuroxime (CXM), Cetazolin (CZ), and Ceftriaxole (CRO).

traditional Pharmacopoeia (Swain and Downum, 1980). Studies have shown that many species from the *Dorstenia* genus present in different degree, antimicrobial activities (Swain et al., 1991; Abegaz and Ngadjui, 1999). Amongst others, in Cameroon, twigs of *D. angusticornis* have been tested for their *in vitro* antimicrobial activity

(Kuate et al., 2007c). The twigs of *D. elliptica* have been tested for their *in vitro* antimicrobial activity against bacteria and fungi (Kuate et al., 2007c).

Outside these activities observed from this genus, traditional pharmacopoeia reveals other particular activities of certain species, such as anti-diabetic, anti-

cholesterol, anti-oxidant and anti-malarial properties (Kwete et al., 2007).

For the reference antibiotics used, it was observed that some of the bacteria namely *E. coli*, *P. aeruginosa* and *S. aureus* were resistant to many of the classical antibiotics that were used. The crude extracts from the leaves of *D. mannii* did not inhibit the growth of most of the tested bacteria using the antibiogramme sensitivity test. The resistance problem may be due to the fact that the sources of the bacteria were not determined. However, it is known that immuno deficient patients generally are resistant to antibiotics (Smith, 2002; Moyle, 2004). Patients that have long been hospitalised show resistance to antibiotics. In the hospital there are many nosocomial bacteria (bacteria that are very resistant to antibiotics). Therefore if the bacteria strain were collected from a patient with nosocomial infection there is a high possibility of resistance when tested with the extracts. The ages, weight and sex of the patients were not also known. The pathological products from where the bacteria were swabbed out were not known. All these could have helped us to either increase or decrease the dosage of the crude extracts. Methanol which is used in the extraction of the plant can reduce or kill the bactericidal power of the plant. This is because methanol (alcohol) is used as an antiseptic to render bacteria inactive or kill them completely.

The goal in surveying plants for biologically active compounds should be to isolate the constituent (s) responsible for a particular activity, although the constituent may act additively or synergistically (Horan et al., 2003). Knowledge of the phytochemicals present may help explain or predict a variety of events relating to the antimicrobial efficacy and toxicity of herbal preparation (De Smith and Revier, 1989).

*P. aeruginosa*, *S. aureus* and *Klebsiella* sp. were resistant to all the reference antibiotics used in this study. This indication was very useful information for future selection of antibiotics of reference for comparative studies with antibacterial agents from plants. The study of anti-bacterial agents from plant agents is a progressive research which aims at developing new chemical entities from plant (Ngameni et al., 2007). With the increasing bacterial resistance problems which poses serious problems, especially within our overcrowded hospital centres (nosocomial infections), and the use of cheap drugs with less active principle, there is the need for new alternative drug entities from plant material (Nicolle, 2008). Most drugs originate from plants or plant derivatives (Showed resistance to all the eight different classical antibiotics used during the experiment (Fokunang et al., 2000). *Bacillus* sp. was resistance to AMX, AMC, CZ, CXM and CRO. *S. enterica* was resistant to AMX, AMC and CZ. *Enterobacteries* was resistant to AMX, AMC and CZ. *E. coli* was resistance to AMX, AMC while *Proteus mirabilis* was resistant to AMX and SXT. At last all the bacteria were either resistant to two or more of

the classical antibiotics that were used in the laboratory.

## Conclusion

The extracts of the leaves of *D. mannii* were sensitive to three of the eight bacteria strains tested, and five bacteria showed resistance. This was a promising preliminary studies and an improved condition of extractions of the leaves of *D. mannii* using different concentrations may lead to a better result, to prove that the leaves of *D. mannii* are promising antibacterial agents for new chemical entities of plant origin. The antibiogramme sensitivity test has also provided vital information for future selection of reference bacteria for antimicrobial comparative studies with plant extracts.

The fight against the resistance problem of bacteria against antibiotics will depend on each and everyone's efforts. If the Cameroonian society is out to fight poverty by exploring our natural environment, then research should be focused on the use of plants for our welfare for it is said "a healthy man is a wealthy man".

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