

## Short Communication

## ***In vitro* antimicrobial investigation of *Zanthoxylum chalybeum* stem bark**

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The antibacterial properties of ethanolic, dichloromethane (DCM) and acetone of *Zanthoxylum chalybeum* stem bark were evaluated with the gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*) and gram-positive bacteria (*Staphylococcus aureus*) and *Candida albicans*. All tested bacteria have been shown to be resistant to the acetone extracts whereas, only *S. aureus* growth has been inhibited by DCM extract (14 mm). Moreover, the ethanolic extract inhibited growth of *S. typhi*, (17 mm) *P. aeruginosa* (23 mm) *S. aureus* (16 mm) around agar well. However, *E. coli* and *C. albicans* have not been inhibited by ethanolic extract. For minimum inhibitory concentrations (MICs) determination, acetone extract has no activity against all microorganisms tested whereas both ethanol and DCM exhibited strong activity to the *S. aureus* at 32 µg/ml of MIC value. The ethanol extract has exhibited low activity against *S. typhi*, and *P. aeruginosa* with respectively 250 and 500 µg/ml of MIC values.

**Key words:** *Zanthoxylum chalybeum*, antimicrobial activity, crude extracts.

### INTRODUCTION

In the last decades of human history, we explored the use of an enormous variety of plants which are benefit in health. Natural substances of plant origin have obviously contributed in alleviating of several diseases from human and animal. Nevertheless, the natural products activity exerted against microorganisms that invade the human and bovine has interested the research in discovering novel pharmacological active compounds. Medicinal plants have been thought to contain significant amounts of bioactive compounds which provide relevant biological activity.

*Zanthoxylum chalybeum*, commonly known as intareyirungu in Rwanda belongs to the Rutaceae family.

The plant is a tree up 12 m and is widespread in western province of Rwanda. Their dried leaves and bark extract decoctions are respectively employed to treat malaria and fever (Maundu, 1999; Kokwaro, 2008). Chemical investigations of *Z. chalybeum* seed have found alkaloid skimmianine (Sofowora et al., 1975). The *Zanthoxylum* spp. has been showed to contain chelerythrine, berberine and canthin-6-one which have been reported to exhibit antibacterial activity (Sofowora and Isaacs 1971; Islam et al., 2001). Patchanee et al. (2008) have reported that over 150 species belong to the *Zanthoxylum* genus are medicinally and commercially useful.

The *Zanthoxylum gillettii* ethanolic extract has been

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shown to inhibit the growth of *Candida albicans* and *Pachypodanthium staudtii* (Kamanzi et al., 2002). Furthermore, the essential oils were expected to possess antibacterial activity against Gram positive (*Staphylococcus aureus*), and Gram negative (*Klebsiella pneumoniae* and *Salmonella Setuba*) bacteria (Wellington et al., 2003).

In Rwanda, the practitioners use many medicinal remedies decoctions as treatment in bloody diarrhea associated with the bacteria or protozoal. *Z. chalybeum* stem bark has been identified as the most delivered due to its effectiveness. Their dried leaves are used by Eastern people of Rwanda as decoction to alleviate cough, asthma, and fever. This prompted us to investigate the antimicrobial properties of *Z. chalybeum* stem bark. The present study is aimed to provide validity for the use of *Z. chalybeum* stem bark as antimicrobial agent.

## MATERIALS AND METHODS

### Plant material collection and identification

Fresh stem barks of *Z. chalybeum* plant were collected at Ndego, Eastern region of Rwanda. The plant was identified in Department of Biodiversity and Botany, Institut de Recherche Scientifique et Technologique by Christopher Ruffo. Stem bark was air dried at room temperature for 15 days.

### Extraction of the plant material

Extraction was carried out as described by Eloff (1998) with small modification. Separately, hundred gram of each powdered stem were extracted using respectively 70% ethanol, DCM, and acetone (500 ml) for 24 h. Each of the extract was evaporated to dryness using a rotary evaporator at 50°C.

### Test organisms

The pure clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. aureus* and *C. albicans* were collected from Laboratoire du Département de Biologie Médicale de Butare du Centre Hospitalier Universitaire and were used for microbiological evaluation.

### Evaluation of antimicrobial activity

The agar diffusion method using Mueller Hinton agar seeded with the microorganisms was used (Pelczar et al., 1993). A solution of each dried extract was prepared in a mixture of sterile distilled water and dimethyl sulphuroxide (DMSO) (1%). The stock concentration of the extract was prepared to obtain 1000 µg/ml. All extracts were sterilized by filtration through 0.45 µm paper filter (Millipore). The agar wells (8 mm) were cut using sterilized steel borer into solidified agar plates which have been earlier seeded with appropriate organisms. Thereafter, the holes were filled with 1 ml of extract. The plates were incubated at 37°C for 24 h and diameter of clear zone of inhibition was measured.

### The minimum inhibitory concentrations determination

The minimum inhibitory concentration (MIC) value of the extract of the plant was carried out by two-fold serial method dilution of extracts in small volume in 96-microwell plates. In this method, broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 4000 µg/ml in sterile distilled water/DMSO (1%) and serially diluted (two-fold) to a working concentration ranging from 1000 to 1.953 µg ml<sup>-1</sup> using nutrient broth. The cultures were then further diluted with 100 µl suspension of approximately 10<sup>6</sup> bacteria ml<sup>-1</sup>. The experiment was performed in triplicate. The plates were incubated at 37°C for 24 h after which the wells were observed for the presence of turbidity. The lowest concentration where no turbidity was observed was noted as the minimum inhibitory concentration.

## RESULTS AND DISCUSSION

Results of antibacterial activity of the stem bark extracts is presented in Table 1. We used extracts from various solvents (DCM, acetone and ethanol). In particular, ethanolic extract has shown to be active to a large number of microorganisms tested. Some of them, *S. typhi*, *P. aeruginosa*, *S. aureus*, were inhibited around agar well at diameter of 17, 23 and 16 mm of inhibition zone, respectively. Interestingly, we observed ethanol extract decrease growth of gram negative *S. typhi* and *P. aeruginosa* with MICs values of 250 and 500 µg/ml, respectively. A particular interesting activity is observed with ethanol extract with MIC values of 32 µg/ml against gram-positive *S. aureus*.

This is in agreement with the traditional usage of the remedy as aqueous decoction to treat their patient. This ethanol extract activity contained the wide range of active compounds isolated in the *Zanthoxylum* species. Several species of the Rutaceae family have reported to contain alkaloids, tannins and flavonoids (Adesina, 2005). Furthermore, DCM extract has inhibited growth of gram-positive *S. aureus* (14 mm) and it has shown to be active with MIC value of 32 µg/ml. At 1000 µg/ml of MIC, all extracts were unable to inhibit *C. albicans*. Despite acetone being a better solvent to extract tannins, its extract has not shown any activity against all bacteria. This suggested that *Z. chalybeum* properties may be due to the phenol canthine-6-one alkaloids which mostly occurred in root and stem bark of several *Zanthoxylum* spp. This compound has been reported to possess strong antibacterial activity (Odebiyi and Sofowora, 1979; Tsuchiya et al., 1996) and antifungal, leishmanicidal and trypanocidal properties (Ferreira et al., 2011).

The recent findings by O'Donnell and Gibbons (in press) reported potential activity of canthin-6-one alkaloids against methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant (MDR) to exhibit MIC value ranging from 8 to 64 µg/ml. The high polar ethanol has shown to inhibit a large number of tested microorganisms, in particular gram-positive bacteria *S. aureus*. Such polar

**Table 1.** Summary of inhibition evaluation of extracts of *Z. chalybeum* stem bark.

| Species              | Inhibition zone diameter (mm) |     |         |     | MICs ( $\mu\text{g/ml}$ ) |       |         |
|----------------------|-------------------------------|-----|---------|-----|---------------------------|-------|---------|
|                      | Ethanol                       | DCM | Acetone | CPX | Ethanol                   | DCM   | Acetone |
| <i>E. coli</i>       | 0                             | 0   | 0       | 39  | >1000                     | >1000 | >1000   |
| <i>S. typhi</i>      | 17                            | 0   | 0       | 26  | 250                       | >1000 | >1000   |
| <i>P. aeruginosa</i> | 23                            | ND  | 0       | 35  | 500                       | >1000 | >1000   |
| <i>S. aureus</i>     | 16                            | 14  | 0       | 30  | 32                        | 32    | >1000   |
| <i>C. albicans</i>   | 0                             | 0   | 0       | 16  | >1000                     | >1000 | >1000   |

DCM: Dichloromethane, ND: not determined, CPX: ciprofloxacin. The extracts obtained from ethanol, DCM and acetone have been tested to the clinic isolates gram-negative (*E. coli*, *S. typhi*, *P. aeruginosa*) gram-positive (*S. aureus*) and *C. albicans*.

extracts contain chemical compounds which have been associated with the antibacterial activities, thus they have the curative properties against pathogens (Nweze et al., 2004).

The phytochemical compounds which may exert antibacterial activity in polar natural products belong to the phenolic class. They are thought to exhibit promising activity against a wide range of bacteria; it has often been suggested that the high molecular weight might exhibit strong effect against gram-negative bacteria by membrane-damaging activity or by fusing cell into clusters (Ikigai et al., 1993). Further accuracy bioguided investigations are needed in order to determine the type of compounds responsible for these antibacterial properties of those extracts.

This study provides some scientific bases for the use of this plant as a remedy for abdominal disorders associated with bacteria, parasites and fungal infections in folkloric medicine, whose causative agents are some of the pathogens studied.

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