



Full Length Research Paper

***In vitro* antibacterial activity of *Ipomoea reptans* extracts**

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This study was carried out to determine the antibacterial activity of *Ipomoea reptans* extracts which the plant collected from Sudan. All previous literatures and studies carried out but this the first time to study this plant from this area. In this present study, *in vitro* antibacterial activity of nine *Ipomoea reptans* extracts were performed to determine the more active fraction against several Gram-positive and Gram-negative bacterial strains. The results exhibited that hexane (IR1), dichloromethane-ethyl acetate (IR6-1, 6-2 and 7), and ethyl acetate fractions are considered more active whereas dichloromethane-ethyl acetate (IR7, 1:1, v/v) showed more activity against the two tested Gram-positive bacteria (*Bacillus cereus* and *Micrococcus ruseus*). Consequently, five *I. reptans* extracts (IR1, IR61, IR62, IR7 and IR8) demonstrated moderately to highly antibacterial activity against the tested Gram-positive bacteria with diameters of inhibition zones ranged from 13 to 17 mm and MIC values ranged from 40 to 120 ppm. Unfortunately, no activity was detected against Gram negative bacteria for all extracts.

Keywords: *Ipomoea reptans* - Antibacterial activity - *Bacillus cereus* - *Micrococcus ruseus*

INTRODUCTION

Natural products have a history of providing novel chemically useful anticancer drugs. There are many compounds used in medicine today whose original derivatives were of plant origin. Modern researches have shown that the action of medicinal plants is due to a relatively small number of constituents called the active principles produced by the plants. The biotransformation study is one of the means that will enable us to understand the role of these natural products. The

primary interest in the chemistry sesquiterpene lactones however, stemmed from their pharmacological properties. Among these are compounds possessing antitumor, hypnotic, antifungal, antimicrobial, antielmentic, and activity. Historically, plants have provided a good source of anti-infective agents. Plant-derived medicines have been a part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as a source of agents to fight microbial diseases (Zia-Ul-Haq, 2011). Many species of the genus *Ipomoea* such as *I. obscura*, *I. carnea*, *I. sepiaria* and *I. aquatic* are well known for their activity against both Gram-positive and

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Table 1. Inhibition zones diameter of *Ipomoea reptans* extracts against Gram-positive bacteria (*Bacillus cereus* and *Micrococcus ruseus*).

Test organism	<i>Ipomoea reptans</i> extracts (concentration = 200 ppm)				
	IR1	IR6-1	IR6-2	IR7	IR8
	Inhibition zone (mm)				
<i>Bacillus cereus</i>	14	16	-	17	15
<i>Micrococcus ruseus</i>	-	-	13	16	-

Gram negative bacteria (Mungoleet *et al.*, 2010; Adsul *et al.*, 2012; Das *et al.*, 2011; Sivaraman *et al.*, 2013; Meira *et al.*, 2012).

MATERIALS AND METHODS

1. Plant material

The fresh plant of *I. reptans* (leaves, stems, and flowers) was collected from Darfur, west of Sudan during March 2010. This area is a closed without any studies or researches subject included medicinal plants since 1927.

2. Preparation of plant extraction

The whole plant of *Ipomoea reptans* (5 kg) were dried and powdered in a mechanical grinder. The powdered material was extracted by maceration with 100% methanol for 24 h. The extract was filtered and the solvent evaporated under reduced pressure using rotatory evaporator to get a crude extract.

3. Sample preparation

The methanol crude extract was subjected to silica gel flash column chromatography (60-120 mm mesh) and eluted with increase polarity using 100% *n*-hexane, mixture of hexane with dichloromethane until 100% dichloromethane, then increase the polarity by using dichloromethane with ethyl acetate until 100% ethyl acetate and finally increase the polarity with methanol until 100% methanol. Each fraction was evaporated to dryness and kept in freezer.

Antibacterial activity screening

The antibacterial activity of different *Ipomoea reptans* extracts (IR1, IR2, IR3, IR4, IR5, IR6-1, IR6-2, IR7 and IR8) was determined by agar well diffusion method as described by Pandey, *et al.*, (2004) and Sen, *et al.*, (1995). The partially purified extracts were dissolved in ethyl alcohol, 20 mL of sterilized nutrient agar (NA) medium were seeded with 50 µl of the pathogenic bacteria (*Bacillus cereus*, *Micrococcus ruseus*,

Escherichia coli and *Pseudomonas aeruginosa*, respectively, swirled gently and aseptically poured into Petri dishes and allowed to solidify. Sterile cork borer (6 mm diameter) was used to bore wells in the plate, extracts with different concentrations (40, 80, 120, 160 and 200 ppm) were then carefully dispensed into the bored holes. The extract was allowed to diffuse for 1 h before incubating aerobically at 37 °C for 18-24 h. The presence of a zone of inhibition around each well was indicative of antibacterial activity. The diameter of the zones of complete inhibition was measured to the nearest whole in millimeters. The entire test was conducted in duplicate. (Nath, *et al.*, 2012; Nicola, *et al.*, 2005).

The minimum inhibitory concentration (MIC) was determined according to the National Committee for Clinical Standard (1999). Extracts were added to tubes, each containing 2ml of sterile nutrient broth to obtain final concentrations (25, 50, 100, 150 and 200 ppm) of each extract. The test organism was inoculated into the labeled tube except the control; the tubes were incubated at 37 °C for 72 h. The MIC was recorded as the lowest concentration that prevented visible growth. The above procedure was repeated in triplicates for each of the tested organisms.

RESULTS AND DISCUSSION

Among of tested fractions, (IR1, IR6-1, IR6-2, IR7 and IR8) were exhibited different activity from moderate to high antibacterial activity against the two tested Gram-positive bacteria (*Bacillus cereus* and *Micrococcus ruseus*) as shown in figures 1 and 2, while fractions (IR2, IR3, IR4 and IR5) are considered inactive. The Gram negative bacteria (*Escherichiacoli* and *Pseudomonas aeruginosa*) were unsusceptible to all the eight extracts. Diameters of inhibition zones ranged from 13 to 17 mm, and they are clearly shown in table 1 and figure 1-A. Similarly, Sivaraman *et al* (2010), recorded that leaf extract of *Ipomoea aquatica* Forsk has shown zone of inhibition of (15-25mm) against tested Gram-positive and Gram negative bacteria. MIC values are presented in table 2 and figure 1-B. IR9-2 showed the lowest MIC against *Bacillus cereus* (40 ppm) indicating high antibacterial activity. Aliyu *et al* (2011), reported that the MICs of *Ipomoea asarifolia* for aqueous extract were 100

Table 2. MIC values of *Ipomoea reptans* extracts against the test organisms.

Test organism	Fractions of <i>Ipomoea reptans</i> extract				
	IR1	IR6-1	IR6-2	IR7	IR8
<i>Bacillus cereus</i>	80	80	-	40	80
<i>Micrococcus ruseus</i>	-	-	120	120	-

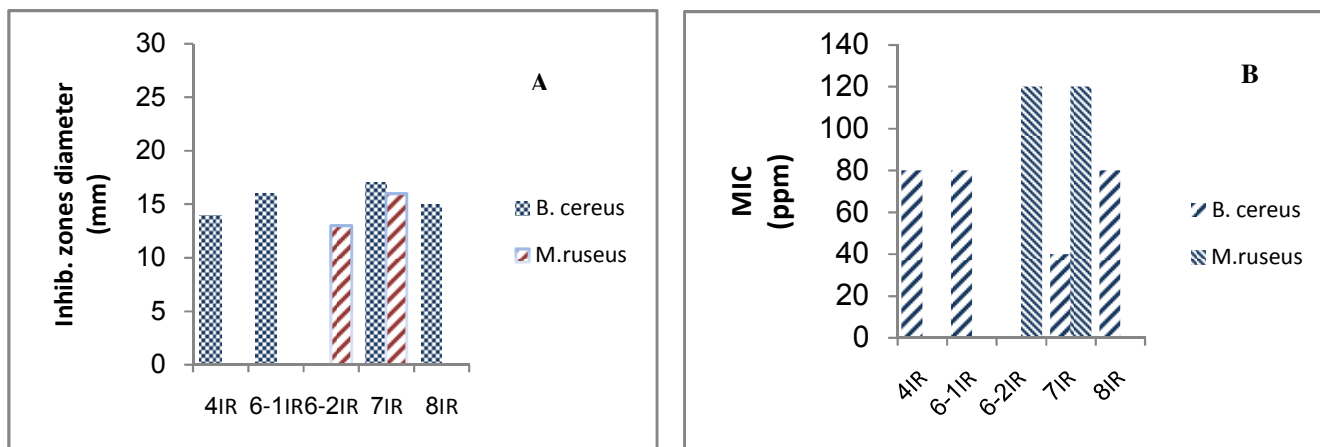


Figure 1. Antibacterial activity of *Ipomoea reptans* extracts against *B. cereus* and *M. ruseus*), A) Inhibition zones diameter and B) MIC values

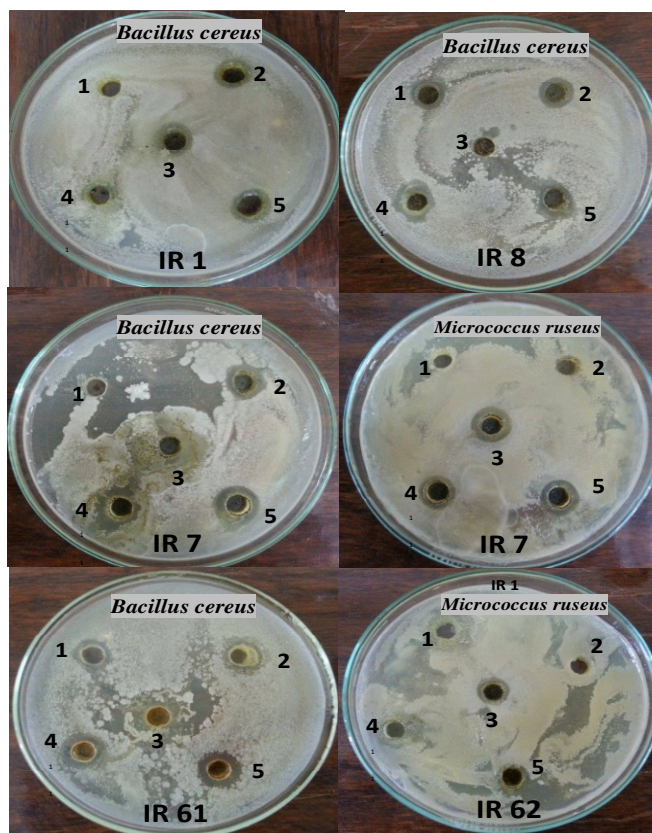


Figure 1. Antibacterial activity of *Ipomoea reptans* extracts against *Bacillus cereus* and *Micrococcus ruseus*, where 1, 2, 3, 4, 5 are 25, 50, 100, 150 and 200 ppm respectively.

ppm against *S. aureus*.

The present study indicates that the five extracts of *I. reptans* are effective against two Gram-positive bacteria (*Bacillus cereus* and *Micrococcus ruseus*). This study also indicates that *Ipomoea reptans* can be studied for further assay to evaluate effectiveness as an antimicrobial agent. These findings come to agreement with Wei et al. (2008) who reported antimicrobial activity of *Ipomoea reptans* extract against pathogenic *Citrobacter freundii*, *Vibrio alginolyticus*, *V. harveyi*, *V. parahaemolyticus* and *V. vulnificus* with inhibition zones 7, 9.5, 7, 7 and 8 mm, respectively. On the other hand, Adsul et al., (2012) illustrated antibacterial activity of *Ipomoea carnea* extracts against *Bacillus cereus* ATCC-11778. Moreover, extracts of *Ipomoea hederacea* performed potent activity against Gram-positive bacteria, *Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus subtilis* (Zia-Ul- Haq, 2011).

CONCLUSION

In conclusion, The extract of *Ipomoea reptans* is antibacterial active against tested Gram positive Bacteria while it has no activity against tested Gram negative bacteria. Also it may be useful as medicinal treatment.

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