

ANTIBACTERIAL ACTIVITY OF *MOMORDICA CHARANTIA* L. GEMMOTHERAPIC EXTRACT

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Abstract

The antibacterial activity of a gemmotherapeutic extract of Momordica charantia L., was investigated against 5 species of bacteria, Bacillus subtilis, Streptococcus faecalis, Serratiamarcescens, Pseudomonas aeruginosa and B. cereus v. mycoides, using the well diffusion assay. The extract was prepared from young buds of M. charantia, in according to the gemmotherapeutic principles. The result revealed that the extract had a significant inhibitory activity against Bacillus subtilis and Pseudomonas aeruginosa but moderate on the other species at a concentration of 50mg/mL. The results are indicating that the gemmotherapeutic extracts can be a viable alternative to the modern extraction techniques.

Keywords: *Momordica charantia*, antibacterial activity, gemmotherapeutic extracts, well diffusion assay.

INTRODUCTION

Gemmotherapy or plant stem cell therapy uses a wide variety of embryonic plant parts, collected in the spring at a critical stage in the plants growth when much of the plants energy is directed to the growing areas. Gemmotherapy is an important subsection of phytotherapy.

Gemmotherapeutic extracts are known for their higher content in active compounds (Rozenchwajg, 2008). They are prepared according to gemmotherapeutic principles from the French Pharmacopoeia, (as cited in Nicoletti, 2012), which consists in maceration of plant buds with equal thirds of water, alcohol and glycerin.

In recent years, there have been a revival of natural, plant based antimicrobial agents. This trend is the consequence of the limited effectiveness of synthetic products to fight against newer, drug resistant bacteria. For this purpose, the antimicrobial properties of many plant compounds from a wide variety of plant species have been assessed (Karuppusamy, 2009). Further, about 80% of the drugs used in modern medicine are the products of plant origin (Patwardhan et al., 2004). Also, food preservatives derived from plants and herbs are of growing interest, since plant compounds often possess antimicrobial properties

that protect them from infection (Lou et al., 2010).

In this study, we tested a gemmotherapeutic extract from young buds of *M. charantia* based on the principle that gemmotherapeutic extracts have a more intense inhibitory activity compared with the traditional extracts (Braca, 2008).

M. charantia is a climber belonging to family Cucurbitaceae, is commonly known as bitter gourd or bitter melon. The plant is native to Asia and it was recently introduced in culture in Romania. It is cultivated in the western part of Romania since the year 2006 (Crisan, 2007). The antimicrobial activity of *M. charantia* is well documented, the plant possessing high inhibitory effects on many species of bacteria and fungi (Abalaka et al., 2011), anti-inflammatory activities (Koboriet al., 2008), (Lii et al., 2009) and antioxidant (Wu and Ng, 2008).

MATERIALS AND METHODS

Plant material collection

Plants of *M. charantia* were cultivated in a garden at the University of Agricultural Sciences in Timisoara. Early buds were

collected from very young plants and put immediately in alcohol of 96% concentration.

Preparation of gemmotherapeutic extracts

The solutions were made with equal thirds of alcohol, glycerol and distilled water. The fresh buds were collected, cleaned, washed with distilled water and then put in the solution for extraction. The process of extraction took place for a week in a dark place at 10°C, using an orbital shaker. The extract was then filtered, concentrated by using a rotavap and weighted. The dry material was diluted for the tests and filtered through a sterile membrane filter. Two concentrations were tested: one of 50 mg/mL and the second, of 1:10 dilution factor solution.

Microorganisms

The bacteria used in this experiment are two Gram-negative bacteria species: *Pseudomonas aeruginosa* and *Serratiamarcescens* and three Gram-positive bacteria: *Bacillus subtilis*, *Bacillus cereus* var. *mycooides* and *Streptococcus faecalis*. The bacterial cultures were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck).

Bacteria counting

The bacteria were counted using a Burkert chamber. The values are shown in (Table 1).

Table 1: The Burkert chamber count data

Bacteria species	Number of cells/ml
<i>Pseudomonas aeruginosa</i>	1.6x10 ⁸
<i>Serratia marcescens</i>	1.7x10 ⁸
<i>Bacillus subtilis</i>	8x10 ⁷
<i>Bacillus cereus</i> var. <i>mycooides</i>	15x10 ⁵
<i>Streptococcus faecalis</i>	2.2x10 ⁸

Well diffusion assay and antibacterial activity

The antibacterial activity was determined using the hole in plate assay procedure (Perez et al., 1990). All bacterial cultures were maintained on nutrient agar slants at temperature of 4°C and sub cultured onto nutrient agar broth for 24 hours prior to testing. The pure cultures of the microorganisms were inoculated onto Muller-Hilton nutrient broth incubated at temperature of 37°C for 24 hours. 25 ml of nutrient agar was poured into the 100 mm plate, with an even depth of 4 mm on a level surface shaken and allowed to cool.

The nutrient agar plates were seeded with 0.1 ml of standardized inoculums of each of the five test organisms. The inoculum was spread evenly over plate with a sterile glass spreader. Using a sterile cork-borer of 5 mm diameter, three holes were made into the Petri dishes seeded with bacterial culture. The bottoms of the holes were sealed with agar to avoid seepage. 50µl of extracts were introduced in the wells, using a micro liter syringe. Concentrations of 5 and 50 mg/ml extracts were reconstituted in distilled water and transferred into the wells. The plates were kept for 30 min at room temperature to allow diffusion of the extract, and then were incubated at temperature of 37°C for 24 hours. After the incubation period, the zones of inhibition were measured using a digital caliper. In this study, the measurement is taken including the 5 mm diameter of the hole.

Studies were performed in triplicates and the mean value was calculated. A solution of only alcohol, glycerol and water in equal ratios was used as a negative reference.

Statistical analysis

Data were averages of three results ± Standard Deviations (SD) by using Microsoft Excel.

RESULTS AND DISCUSSIONS

In the (Table 2) are presented the mean zone of inhibition measured after 24 hours and in the (Figure 1) is represented the graph with the measurements. The 1:10 dilution factor solution presents little or no visible inhibitory effect, most likely because the concentration here is too low as shown in the (Figure 2). All values were expressed as means ± standard error means.

From the measurements we obtained, it can be observed that *B.subtilis* and *P.aeruginosa* presented the highest sensitivity, the lowest being the *S.marcescens*. All bacteria species tested are susceptible to the extract, at 50 mg/mL concentration and intermediate at 5 mg/mL. The diameter of the zones of inhibition approximately doubles at a tenfold concentration.

These results are similar with those reported by Supraja (2013), in a test with a 50 mg/mL concentration of methanol extract of *M.*

charantia on *B.subtilis*, with a ZOI (zone of inhibition) of 16 mm diameter. In the present study, we obtained a 20 mm diameter at the same concentration.

Table 2: Antimicrobial activity of *Momordica charantia* by well diffusion method after 24 hours. Measurement taken including the 5 mm diameter of the hole

Bacteria/Zone of inhibition in mm*	50mg/ml	5mg/ml	Control
<i>Pseudomonas aeruginosa</i>	17.4±0.2	11.6±0.3	8.2±0.4
<i>Serratia marcescens</i>	11.4±0.5	10.2±0.4	9.0±0.2
<i>Bacillus subtilis</i>	20.8±0.2	12.4±0.4	7.8±0.3
<i>Bacillus cereus var. mycooides</i>	15.4±0.4	7.6±0.2	5.8±0.4
<i>Streptococcus faecalis</i>	13.6±0.2	11.4±0.3	6.2±0.2

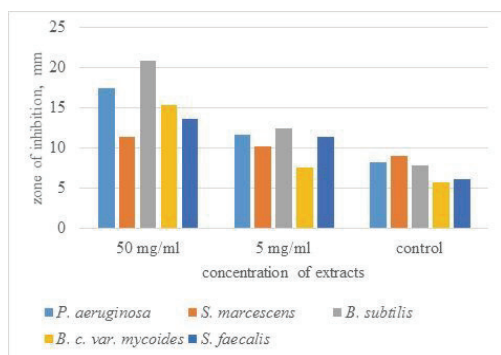


Figure 1. Antimicrobial activity of *Momordica charantia* solutions and the reference, zone of inhibition in mm

In another study made by Leelaprakash (2011), on a methanol leaf extract, he obtained a ZOI of 22 mm at *B.subtilis* but at 100 mg/mL concentration and an 18 mm at *P.aeruginosa* at the same 100 mg/mL concentration.

Although similar tests were performed with slightly different types of cork borers with diameter ranging from 5 to 8 mm, different extract volumes and different concentrations, the results from the present study, confirms the findings of Roopashree et al. (2008) and Costa et al. (2011) on crude alcoholic and water extracts of *M. charantia*.

The data in this study indicate that Gram-positive bacteria were more susceptible to inhibition as compared to Gram-negative bacteria. This finding confirms numerous

previous similar reports regarding this aspect (Somchit, 2010; Rahman, 2011).

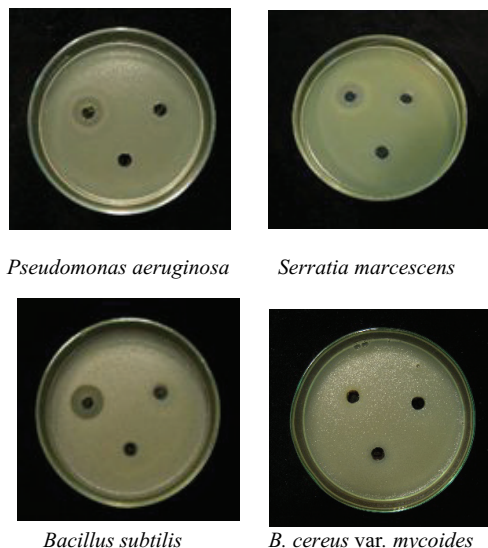


Figure 2. The zones of inhibition for the extracts of *M. charantia* at 50 mg/ml (left), 1:10 dilution (right) and reference (bottom), for *Bacillus cereus var. mycooides*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Serratia marcescens*.

The use of antibiotics has reduced the incidence of infectious diseases but their extensive uses in therapy, has led to the appearance of drug-resistant bacteria (Normanno et al., 2007), which is a major public health issue worldwide. For this purpose, numerous plant extracts were screened for antimicrobial properties that could protect people from microbial infections (Serra et al., 2008; Lou et al., 2010).

The plant extracts can also be used in combination with traditional antibiotics. In the literature, there are reports regarding the use of plant crude extracts (Aqil et al., 2005, 2006) in combination with fewer amounts of antibiotics for anti-bacterial activities, especially for antibiotic-resistant bacteria, compared to antibiotics alone (Schmidt et al., 2008).

CONCLUSIONS

Based on the present study, it can be concluded that there is a great potential in using the gemmotherapeutic principles for plant extracts in the development of more potent and efficient antimicrobial agents.

Also, the gemmotherapeutic extracts obtained from *M. charantia* young shoots, using the classic gemmotherapeutic principles, exhibit an intensive antimicrobial activity.

Further investigation is needed in order to study the synergy of fractions from *Momordica charantia*.

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