ANTIBACTERIAL SCREENING OF MOMODRICA CHARANTIA GEMMOTHERAPIC EXTRACT

Ivan PAULIUC [1]*
[1]Banat's University of Agricultural Sciences and Veterinary Medicine, Department of Exact Sciences, Timisoara, Calea Aradului No. 119, Romania
Corresponding author: 0729.13.12.78, ivan.pauliuc@gmail.com.

Abstract
In this paper is presented for the first time, a new approach in screening of Momordica, in which gemmotherapic extracts made from collecting and extracting the young buds from young plants were used, instead of testing the leaves or fruits of mature plants. The tests were made using the hole in plate assay, on two gram negative bacteria: *Pseudomonas aeruginosa* and *Serratia marcescens* and on three positive bacteria: *Bacillus subtilis, Bacillus cereus var. mycoides* and *Streptococus faecalis*. Young buds from *M. charantia* were extracted with 30% alcohol and tested on the bacteria. The crude extracts showed significant inhibitory effects on Bacillus subtilis but little or no effect on the other bacteria. A reference solution without the extract was used as a negative control.

Keywords: antibacterial activity, gemmotherapy, Momordica charantia, hole in plate

Introduction

Gemmotherapy extracts have been used long time ago, being known for their higher content in active compounds (Rozencwajg 2008). This method has not being used in developing antimicrobials. Today, more than ever, there is an increasing pressure and need for new, more powerful compounds. 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. The antimicrobial properties of many plant compounds from a wide variety of plant species have been assessed (Benoit-Vical et al.,2001). Plant-produced compounds have been incorporated into a wide range of medical applications, (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 (Serra et al., 2008; Lou et al., 2010).

The antimicrobial activity of *Momordica charantia* is well documented, the plant possessing high inhibitory effects on many species of bacteria and fungi.(Abalaka et all. 2011), anti-inflammatory activities (Kobori et al., 2008), (Lii et al., 2009) and antioxidant (Wu and Ng, 2008). Several species of *Momordica* have been screened and the compounds have been identified and tested separately but the gemmotherapic method has not yet been used on this plant.

In this study, we tested a new and different method of extraction of active compounds from young buds of *M. charantia* based on the assumption that gemmotherapic extracts must have a more intense inhibitory activity compared with the traditional extracts tested so far, (Braca 2008), (Grover 2004).

Momordica charantia, Figure 3, a climber belonging to family Cucurbitaceae, is commonly known as bitter gourd or bitter melon in English and karela in Bengali. Momordica means, to bite (referring to the jagged edges of the leaf, which appear as if they have been bitten). All parts of the plant, including the fruit have bitter taste. The fruit is oblong and resembles a small cucumber; young fruit is emerald green that turns to orange-yellow when ripe. It is an herb, grows in tropical areas of Asia, Amazon, East Africa, and the Caribbean and cultivated throughout the world for its use as vegetable as well as folk medicine.

Materials and Methods

Plant material collection

The plants of *M. charantia* were cultivated in special greenhouse at the University of Agricultural Sciences in Timisoara and they were identified by specialists. Early buds were collected from very young plants and put immediately in alcohol of 96% concentration.

Preparation of gemmotherapic extracts

The solutions were made with equal thirds of alcohol, glycerol and distilled water and a concentration of 50 mg/ml of extract. The fresh buds were collected, 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 10oC. The extract was then filtered, rediluted for the tests and filtered through a sterile membrane filter for sterilization. Two concentrations were tested: one of 50 mg/ml and the second, a 1:10 dilution factor solution.

Microorganisms

The bacteria used in this experiment are two gram negative bacteria: *Pseudomonas aeruginosa* and *Serratia marcescens* and three positive bacteria: *Bacillus subtilis, Bacillus cereus var. mycoides* and *Streptococus faecalis*. The bacterial cultures were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck).

The hole in plate assay and antibacterial activity

The antibacterial activity was determined using the hole-in-plate bio assay procedure (Hugo et al., 1983; Vlientick et al., 1995). All bacterial cultures were maintained on nutrient agar slants at temperature of 4ºC and sub cultured onto nutrient agar broth for 24 hours prior to testing. The bacteria were counted using a Burker chamber and the optical density was measured at 600 nm using a spectrophotometer. The values are shown in Table 1. 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 cork borer was sterilized with alcohol and flame. 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 37º C for 24 hours. After the incubation period, the zones of inhibition were measured using a digital caliper. Studies were performed in triplicates and the mean value was calculated. The mean zones of inhibitions were compared by one way analysis of variance. A solution of only alcohol, glycerol and water in equal ratios was used as a negative reference.

Statistical analysis

All values were expressed as means ± standard error means. The data for each microorganism were analyzed by using one way analysis of variance (ANOVA) technique and means were compared by using LSD at 5% (0.05) probability level.

Results

From the measurements obtained, it is clear that the inhibition activity is significantly higher at the same concentration in gemmotherapic extracts than in traditional extraction

procedures. 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.

Table 1: The Burker chamber count data and the optical density

Bacterium	Density cells/ml	OD at 600 nm
Pseudomonas aeruginosa	$1.6x10^8$	0.228
Serratia marcescens	$1.7x10^8$	0.511
Bacillus subtilis	$8x10^7$	1.193
Bacillus cereus var. mycoides	$15x10^5$	0.186
Streptococus faecalis	$2.2x10^8$	0.494

Table 2: Antimicrobial activity of Momordica charantia by well diffusion method after 18 hours

Bacteria/Zone of inhibition in mm*	Pseudomonas aeruginosa	Serratia marcescens	Bacillus subtilis	Bacillus cereus var. mycoides	Streptococus faecalis
50mg/ml	6.2±0.2	3.2±0.5	7.9±0.2	5.2±0.4	4.3±0.2
1:10 dilution	4.3±0.3	2.6±0.4	3.2±0.4	1.3±0.2	3.2±0.3
Reference	1.6±0.4	2.1±0.2	1.4±0.3	0.4±0.4	0.6±0.2

*Values were expressed as the mean ± SD. (N=3)

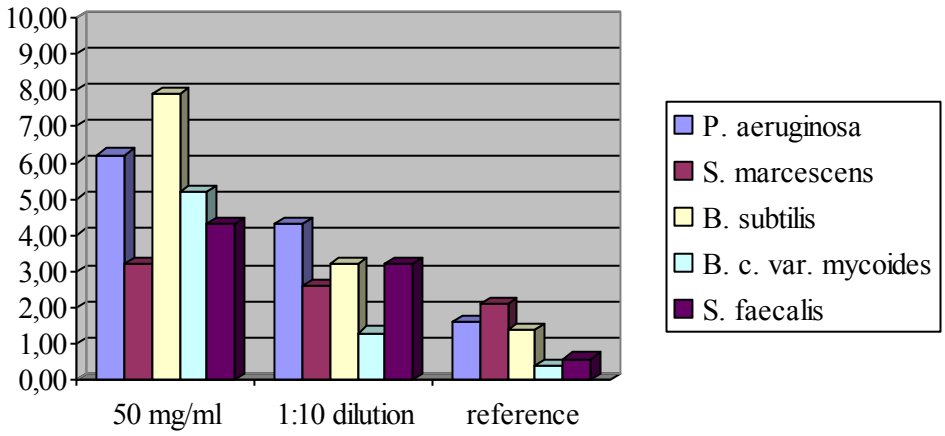

Figure 1. Antimicrobial activity of *Momordica charantia* solutions and the reference, zone of inhibition in mm

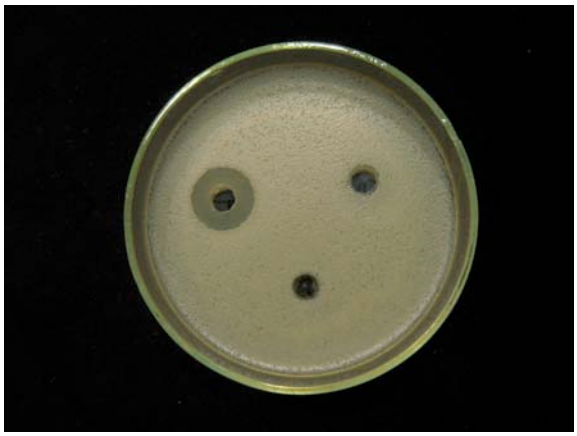

Figure 2. The zone of inhibition at 50 mg/ml (left), 1:10 dilution (right) and reference (bottom) for Bacillus cereus var. mycoides

Figure 3. Momordica charantia

Discussion

Based on the present investigations, it was concluded that there is a great potential in using the gemmotherapic extracts in the development of more potent and efficient antimicrobial agents. The development of new methods for obtaining antimicrobials will continue to perfect.

It is true that the use of antibiotics has reduced the incidence of infectious diseases but their extensive uses in therapy and in animal food has led to the appearance of drug-resistant bacteria (Normanno et al., 2007), which is a major public health issue worldwide. Consumer awareness over the potential risks of synthetic food additives to human health has renewed the interest in using naturally occurring alternatives. Therefore, MCA extracts were screened for antimicrobial properties that could serve to protect consumers from microbial infection (Serra et al., 2008; Lou et al., 2010).

Plant based antimicrobials have huge potential as they can serve the purpose without any side effect that are often associated with synthetic antimicrobials. So it can be concluded that leaves and twigs of the selected plant can be regarded as good natural antibiotics with considerable degree of antimicrobial activity.

Following this study, we will continue to test other gemmotherapic based extracts from other species and we will try to perfect the method and to search new innovative approach in developing efficient antimicrobials.

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). Further investigation is needed into the synergy of fractions or purified natural compounds from *Momordica species* and antibiotics.

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