HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2017.08.018

Antimicrobial activity of water and acetone extracts of some *Eucomis* taxa

Małgorzata Mizielińska[1✉], Piotr Salachna[2], Magdalena Ordon[1], Łukasz Łopusiewicz[1]

[1]*Center of Bioimmobilisation and Innovative Packaging Materials, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology Szczecin, Janickiego 35, 71-270 Szczecin, Poland*

[2]*Department of Horticulture, West Pomeranian University of Technology Szczecin, Papieża Pawła VI 3, 71-459 Szczecin, Poland*

ARTICLE INFO

Article history:
Received 15 Jun 2017
Received in revised form 16 Jul 2017
Accepted 18 Aug 2017
Available online 14 Sep 2017

Keywords:
Antibacterial
Antifungal
Medicinal plants
Pineapple lily

ABSTRACT

Objective: To evaluate the antibacterial and antifungal properties of acetone and water extracts of selected *Eucomis* taxa.

Methods: The bulbs of *Eucomis bicolor*, *Eucomis comosa* (*E. comosa*) and *E. comosa* cv. were dried and examples from each experimental treatment were ground separately into powder. Each sample was divided into two groups with equal amounts of powder, and respectively extracted with water and a 70% solution of aqueous acetone. The crude water extracts were then filtered through a 0.2 μm filter. The 70% aqueous acetone extracts were next concentrated at 40 °C. After the evaporation of the acetone, the samples were additionally filtered through a 0.2 μm filter. The antibacterial and antifungal activities of the extracts against chosen microorganisms were then determined.

Results: The results of the study demonstrated that the water and acetone extracts of *Eucomis* bulbs have an influence on the viability of *Staphylococcus aureus* and *Bacillus atrophaeus* strains. In the case of mediums containing *E. comosa* and *E. comosa* cv. extracts, a decrease in the number of gram-positive bacteria was dependent on the extract concentration. The best results were obtained in the case of the 25% extracts. The water and acetone extracts of *Eucomis* bulbs did not cause a decrease in the number of *Escherichia coli* cells. Additionally, antifungal activity against *Aspergillus niger*, *Botrytis cinerea*, *Stachybotrys chartarum*, *Mucor circinelloides* and *Rhizopus oryzae* cells were also not observed.

Conclusions: The tested gram-negative and fungi microorganisms show resistance towards acetone and water extracts of *Eucomis* bulbs. The highest activity is found in the case of *Aspergillus clavatus*, *Staphylococcus aureus* and *Bacillus atrophaeus* strains, which shows water and acetone extracts of 25%.

1. Introduction

Eucomis (L.) L'Hér (Asparagaceae, formerly Hyacinthaceae) is a small genus consisting of bulbous geophytes extensively used in southern African traditional medicine [1]. Different parts of the plant (bulbs, roots, stems and leaves) have been used mainly against various ailments including respiratory, venereal diseases, rheumatism, nausea, kidney and bladder problems, abdominal distension, urinary diseases, pain and fever [2–4]. The major phytochemically compounds of *Eucomis* plants are homoisoflavanoids, as well as spirocyclic nortriterpenoids, benzopyranones, saponin glycoside and chromanone that possess a wide array of biological activities [1,5]. Apart from the pharmacological value, *Eucomis* plants are very attractive ornamental crops with great potential for use in garden, as cut flower and as potted plants [6].

With a great number of antibiotics having been found to be ineffective, the use of medicinal plants (*e.g. Eucomis*) as a potential source of novelle ads in the treatments of microbial infections has increased [3,4]. There is an urgent need to identify novel, active chemotypes as leads for new drug development [5]. Different plant parts and extract solvents of various *Eucomis* species have been tested for *in vitro* and *in vivo*

✉First and corresponding author: Małgorzata Mizielińska, PhD, Center of Bioimmobilisation and Innovative Packaging Materials, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology Szczecin, Janickiego 35, 71-270 Szczecin, Poland.
Tel: +48 91 4496132
Fax: +48 91 4496590
E-mail: cbimo@zutedu.pl
Peer review under responsibility of Hainan Medical University.

antimicrobial screening and antioxidant activity [2,7,8]. It has been shown *Eucomis* extracts inhibited *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) [7,9] as well as fungal cells, such as *Candida albicans* (*C. albicans*) [10]. The genus *Eucomis* includes 10 species and many cultivars, which have become increasingly popular [6,7]. However, the knowledge on pharmacological properties of some *Eucomis* species and cultivars is very limited.

In this study, the authors aimed to investigate the antimicrobial activity of water and acetone extracts of three *Eucomis* taxa: *Eucomis bicolor* (*E. bicolor*) Baker, *Eucomis comosa* (*E. comosa*) Hort. ex Wehrh. and *E. comosa* cv. This represents the first report on the antimicrobial activity of the extracts of *E. comosa* cv.

2. Materials and methods

2.1. Test plants

The tests were made up of bulbs from three *Eucomis* taxa: *E. bicolor* Baker, *E. comosa* Hort. ex Wehrh. and *E. comosa* cv. in this study. The bulbs were collected in September 2014 after the flowering period of plants grown in a greenhouse of the Experimental Station of Department of Horticulture at West Pomeranian University of Technology in Szczecin, Poland (53°25′N, 14°32′E).

2.2. Chemicals/mediums

Acetone (Sigma, Aldrich) was used to extract the active substance from the selected *Eucomis* bulbs. TSB, TSA and Sabouraud mediums (Merck, Germany) were used to verify the antimicrobial properties of any extracts. All mediums were prepared according to the Merck protocol.

2.3. Test organisms/bacterial organisms

The test microorganisms used in this study were obtained from a collection of the Leibniz Instytut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The strains were supplied from the Czech Collection of Microorganisms (CCM). The organisms used in this study were: *S. aureus* strain DSMZ 346, *Bacillus atrophaeus* (*B. atrophaeus*) DSM 675 IZT and *E. coli* DSMZ 498, *Aspergillus niger* (*A. niger*) CCM 8189, *Aspergillus clavatus* (*A. clavatus*) CCM F-660, *Botrytis cinerea* (*B. cinerea*) CCM F-16, *Stachybotrys chartatum* (*S. chartatum*) CCM F-237, *Mucor circinelloides* (*M. circinelloides*) CCM 8328 and *Rhizopus oryzae* (*R. oryzae*) CCM 8076.

2.4. Extraction

The bulbs were dried before subjecting them to microbiological tests. The dried bulbs from each experimental treatment were ground separately into powder. Each of three kinds bulbs was divided into two groups with equal amounts of powder, and respectively extracted with water and a 70% solution of aqueous acetone. The samples extract with water containing *Eucomis* bulb powder were kept separately from the shaker (IKA® 4000) for 2 h at 70 °C. The aqueous acetone solutions containing the bulb powder samples were kept in a sonication bath for 1 h, at a constant 15 °C by adding ice to the water. The crude water extracts were filtered through a 0.2 μm filter. The 70% aqueous acetone extracts were concentrated at 40 °C. After acetone evaporation, the samples were filtered through a 0.2 μm filter and used in the subsequent experiments.

2.5. Antibacterial activity

To verify the antimicrobial properties of *Eucomis*, TSB and TSA extract mediums were prepared. At the initial step of the experiments, the bacterial cells of *E. coli* and *S. aureus* were pre-grown on TSA for 24 h at 30 °C. The cell concentration was expressed as colony-forming units (CFU) per mL and determined with serial decimal dilutions and plating on TSA. After incubation, the biomass was suspended in a sterile 0.85% NaCl solution to obtain 1.5×10^8 CFU/mL. The TSB medium was then prepared. The next step was to prepare 5%, 13%, 25% and 50% solutions of water and acetone extracts in 10 mL of TSB. The decimal dilutions of the samples were prepared. The suspended biomass was added to sterile flasks together, which contained TSB with extracts at a ratio of 1:10, and further was mixed with a magnetic stirrer (Dragon Lab, China) for 15 min. The medium containing biomass that did not contain any extracts was the control sample. After stirring, 100 μL of each sample was introduced into the mediums and incubated at 30 °C for 24 h. The results were presented as an average of three samples with standard deviation.

2.6. Antifungal activity

A determination of the antifungal properties of the extracts was carried out using Sabouraud agar and Sabouraud bullion mediums. The method used to test antimicrobial susceptibility was based on extract diffusion. In first step of the experiments, fungal cells of *A. niger*, *A. clavatus*, *B. cinerea*, *S. chartatum*, *M. circinelloides* and *R. oryzae* were pre-grown in Sabouraud bullion for 48 h at 25 °C. After incubation, the biomass was homogenized and plated on Petri dishes of Sabouraud agar. The samples were then incubated for 2 h at 4 °C. After the incubation of 3 holes (three duplicates of each extract), each 0.5 cm diameter medium was drilled. About 150 μL of each extract (separately) was introduced into one the medium hole. The Petri dishes were wrapped with parafilm and incubated at 25 °C for 48 h. After incubation, inhibition zones could then be observed.

3. Results

The results of the study clearly demonstrated that *E. comosa* water extracts had a marked influence on the viability of the *S. aureus* strain. The medium containing the *E. comosa* water extract caused an average 2 log decreases in the number of bacterial cells. In the case of mediums containing *E. comosa* or *E. comosa* cv. extracts, a decrease in the number of *S. aureus* was dependent on the extract concentration. The highest results were obtained in 25% extracts. In comparing the antibacterial activity of water extracts from three species of *Eucomis*, the most effective results were obtained from the *E. comosa* species (Table 1). This was also highlighted below in the *E. comosa* acetone extracts, and that also showed antibacterial activity against the selected microorganism. It was demonstrated that water and acetone extracts of *Eucomis* sp. did not have an influence on a decrease in the number of *E. coli* cells (Table 1).

Table 1
The influence of *Eucomis* taxa extracts on the viability of *S. aureus*, *E. coli* and *B. atrophaeus* [concentration of bacterial cells (10^6 CFU/mL)].

Taxa	Concentration of extract (%)	*S. aureus*		*E. coli*		*B. atrophaeus*	
		Water extract	Acetone extract	Water extract	Acetone extract	Water extract	Acetone extract
E. comosa	50	8.96 ± 6.60	29.20 ± 1.39	7.13 ± 0.57	32.00 ± 5.00	5.67 ± 0.24	12.70 ± 0.57
	25	0.80 ± 0.26	34.33 ± 1.09	6.93 ± 0.11	39.50 ± 5.07	2.70 ± 0.24	5.07 ± 0.78
	5	3.60 ± 0.45	32.25 ± 1.05	2.77 ± 0.40	39.90 ± 5.12	3.90 ± 0.53	6.07 ± 0.42
E. comosa cv.	50	148.17 ± 13.08	41.17 ± 1.10	4.53 ± 0.45	34.00 ± 5.13	20.30 ± 0.30	1.26 ± 0.06
	25	0.43 ± 0.02	3.67 ± 0.58	5.63 ± 0.25	34.70 ± 5.13	3.97 ± 0.70	0.60 ± 0.07
	5	24.40 ± 3.17	24.47 ± 2.37	4.87 ± 0.90	68.80 ± 0.50	17.10 ± 0.15	0.70 ± 0.04
E. bicolor	50	168.33 ± 13.09	87.77 ± 5.93	7.10 ± 0.70	16.00 ± 3.61	72.70 ± 5.51	10.20 ± 1.00
	25	49.33 ± 8.73	13.67 ± 5.03	5.13 ± 0.46	69.33 ± 3.00	34.13 ± 0.27	5.17 ± 0.84
	5	17.67 ± 3.51	23.33 ± 0.55	3.03 ± 0.38	68.70 ± 1.53	34.20 ± 0.25	2.87 ± 0.38
Control	–	179.30 ± 47.61	199.0 ± 91.65	3.60 ± 0.46	46.30 ± 2.52	48.70 ± 6.51	199.00 ± 9.17

While *E. comosa* water extracts triggered a decrease in the viability of the *B. atrophaeus* strain. The medium containing *E. comosa* and E. *comosa* cv. water extracts caused a 1 log decrease in the number of bacterial cells, on average. The highest results were obtained for mediums containing water or acetone extracts at 25% (Table 1). The higher activity against *B. atrophaeus* cells was obtained in the case of acetone *Eucomis* sp. extracts, rather than those from water. The range from a 2 log to a 3 log reduction in the number of *B. atrophaeus* cells was also observed.

The results of the study demonstrated that *E. bicolor*, *E. comosa* and *E. comosa* cv. extracts were not effective against *A. niger*, *B. cinerea*, *S. chartatum*, *M. circinelloides* and *R. oryzae* cells. As was emphasized below, the water and acetone extracts of *E. comosa* and *E. comosa* cv. bulbs only showed antifungal properties against *A. clavatus* cells. The highest inhibition zones were observed in the case of *E. comosa* bulb acetone extracts with an average diameter of 18 mm.

4. Discussion

Through the work of the presented study, it is clear that the water and acetone extracts of *E. bicolor*, *E. comosa* and *E. comosa* cv. bulbs possess promising antibacterial properties against the *B. atrophaeus* and *S. aureus* strains. The results obtained from a broth dilution method support the general indication that the gram-positive organisms are more sensitive to extracts than the gram-negative bacteria. Similar observations were also made by Masondo *et al*. [7] and Bisi-Johnson *et al*. [9]. *B. subtilis* and *S. aureus* were found to be sensitive towards *Eucomis* sp. bulb extracts. In contrast to the results obtained in this study, the authors confirmed that the extracts displayed activity against *E. coli*. Similar results were reported by Masondo *et al*. [7] who noted that *Eucomis* extracts only inhibited a small number of bacterial strains, such as *B. subtilis*, *E. coli* and *S. aureus*. As mentioned earlier by the authors, ethyl acetate extracts of *Eucomis autumnalis* (*E. autumnalis*) showed remarkable activity against *E. coli*, *B. subtilis* and *S. aureus*. It was also noted that crude extracts of *E. comosa* and *Eucomis schijffii* showed significant antibacterial action against *S. aureus*. The ethanol and ethyl acetate *E. autumnalis* bulb extracts were additionally found to be active against *C. albicans* [10]. Ndhlala *et al*. [11] had compared acetone and water extracts of *E. autumnalis* bulbs, which showed that 70% acetone extracts demonstrated higher antimicrobial activity against *B. subtilis*, *E. coli*, *Klebsiella pneumoniae*, *S. aureus* and *C. albicans*, than water extracts. Our research confirmed that acetone was a superior extractant to water. As mentioned by Dzoyem *et al*. [12], acetone was the best extractant for antimicrobial activity in several plant species, with the authors reporting that *E. comosa*, *E. autumnalis* and *Eucomis schijffii* also have antimicrobial properties. The authors' present work offers clear evidence that *E. bicolor* and *E. comosa* cv. bulbs extracts are also active against a number of chosen microorganisms, such as *A. clavatus*, *B. atrophaeus* and *S. aureus* strains.

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

[1] Koorbanally C, Crouch NR, Mulholland DA. The phytochemistry and ethnobotany of the southern African genus *Eucomis* (Hyacinthaceae: Hyacinthoideae). In: Imperato F, editor. *Phytochemistry: advances in research*. Trivandrum, Kerala, India: Research Signpost; 2006, p. 69-85.
[2] Taylor JLS, Van Staden J. The effect of age, season and growth conditions on anti-inflammatory activity in *Eucomis autumnalis* (Mill) Chitt. plant extracts. *Plant Growth Reg* 2001; **34**(1): 39-47.
[3] Fennell CW, Light ME, Sparg SG, Stafford GI, van Staden J. Assessing African medicinal plants for efficacy and safety: agricultural and storage practices. *J Ethnopharmacol* 2004; **95**(2–3): 113-121.
[4] Louw CA, Regnier TJ, Korsten L. Medicinal bulbous plants of South Africa and their traditional relevance in the control of infectious diseases. *J Ethnopharmacol* 2002; **82**(2–3): 147-154.
[5] Du Toit K, Elgorashi EE, Malan SF, Mulholland DA, Drewes SE, van Staden J. Antibacterial activity and QSAR of homoisoflavanones isolated from six Hyacinthaceae species. *S Afr J Bot* 2007; **73**(2): 236-241.
[6] Salachna P, Zawadzińska A. Effect of daminozide and flurprimidol on growth, flowering and bulb yield of *Eucomis autumnalis* (Mill.) Chitt. *Folia Hort* 2017; **29**(1): 33-38.
[7] Masondo NA, Finnie JF, van Staden J. Pharmacological potential and conservation prospect of the genus *Eucomis* (Hyacinthaceae) endemic to southern Africa. *J Ethnopharmacol* 2014; **151**(1): 44-53.
[8] Salachna P, Grzeszczuk M, Wilas J. Total phenolic content, phenolic content, photosynthetic pigment concentration and antioxidant activity of leaves and bulbs of selected *Eucomis* L'Hér. taxa. *Fresen Environ Bull* 2015; **24**(11): 4220-4225.
[9] Bisi-Johnson MA, Obi CL, Hattori T, Oshima Y, Li S, Kambizi L, et al. Evaluation of the antibacterial and anticancer activities of some South African medicinal plants. *BMC Complement Altern Med* 2011; **11**: 14.

[10] Motsei ML, Lindsey KL, van Staden J, Jäger AK. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. *J Ethnopharmacol* 2003; **86**(2–3): 235-241.

[11] Ndhlala AR, Mulaudzi RB, Kulkarni MG, van Staden J. Effect of environmental factors on seedling growth, bulb development and pharmacological properties of medicinal *Eucomis autumnalis*. *S Afr J Bot* 2012; **79**: 1-8.

[12] Dzoyem JP, Aro AO, McGaw LJ, Eloff JN. Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments. *S Afr J Bot* 2016; **102**: 70-74.