In Vitro Assessment of Antioxidant activity, Antimicrobial, and cytotoxicity of ultrasound-assisted acetone extracts of Plectranthus amboinicus

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Abstract: The bacteriostatic, antioxidant and cytotoxic activities of a series of Plectranthus amboinicus were determined. The bacteriostatic performance of Plectranthus amboinicus was evaluated by evaluating the bacteriostatic effect of 10 mg/mL on Gram-positive and Gram-negative bacteria and yeasts. The antioxidant activity of Plectranthus amboinicus was analyzed by DPPH method, and the results showed that it had strong ability of scavenging free radicals. In addition, the general toxicity of Plectranthus amboinicus was evaluated in the Artemia model, and the results showed that the toxicity value was not significant. In turn, through the evaluation of cell viability, all extracts were found to be non-toxic in the concentration range of 0-10 μg/mL in Vero-E6 and 786-O cells. The tested samples have encouraging antibacterial and antioxidant properties, which represents the plant's role in designing new drugs to treat infectious diseases.

Keywords: Plectranthus amboinicus; antimicrobial screening; antioxidant capacity; Vero-E6 cell; 786-O cell

1. Introduction

Plectranthus amboinicus, commonly known as country borage, is a semi-succulent perennial plant in the family Lamiaceae with a pungent oregano-like flavor and smell [1]. It is native to southern Africa and southern East Africa. It is widely cultivated and naturalized in other parts of the tropics, where it is used as a very traditional medicine, spice and ornamental plant [2]. Its leaves have a strong flavor and are used to make
stuffing for meat and poultry, beef, mutton and game. The herb is used as a substitute for oregano to mask the strong smell and taste of fish, mutton and goats. Its leaves are used to make fried foods in Telugu cuisine and are occasionally eaten as snacks [3]. In basic research, the effectiveness of essential oils with other plant essential oils for possible be used as mosquito repellents [4, 5]. Juice from its leaves is commonly used for illnesses including liver and renal conditions, to treat inflammatory diseases or swelling symptoms and cancer [6-8].

In this study, we did many assessment in Vitro of Antioxidant activity, Antimicrobial, and cytotoxicity of ultrasound-assisted acetone extracts of Plectranthus amboinicus, to provide reference for later experimental study.

2. Materials and methods

2.1 Plant material

Samples of dried leaves of Plectranthus amboinicus were provided by University of Lusofona.

2.2 Chemical

Acetone was acquired from analytic grade and purchased to Sigma-Aldrich (Steinheim, Germany). Reverse osmosis water with a resistivity of 18.2 Ω·cm at 25 °C was obtained from a Millipore system (Millipore, Burlington, MA, USA). Dimethyl sulfoxide (DMSO) and absolute ethanol were from Merck (Darmstadt, Germany). Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were from Sigma-Aldrich.

2.3 Ultrasonic extracts preparation

The dry plants are ground into small pieces and then crushed. The extract was prepared from dried leaves of 1g weight, added with anhydrous acetone of 30mL, and put into Erlenmeyer conical flask of 250mL. Ultrasonic bath operates at room temperature (25 °C) at a frequency of 35 kHz with a maximum input power of 320W for 15 minutes. In the process of ultrasonic irradiation, the temperature rise is controlled by adding ice in the water bath to reduce and maintain the temperature of the whole extraction process. The filtered extract is dried on a rotary evaporator.

2.4 TLC-DPPH analysis
For thin layer chromatography ((TLC)), ethanol was used as eluent, and 10 μL extract (1 mg/mL) was added to silica gel GF254. After drying, spray with 0.2% (v/v) DPPH methanol solution. Quercetin was used as positive control and acetone as negative control. The extract with the ability to reduce DPPH showed yellow spots under purple background.

2.5 Biologic Activities

2.5.1 Antioxidant Activity (DPPH Method)

Its free radical scavenging activity was evaluated by DPPH method. 10 μL of each extracted sample was added to 990 μL DPPH solution (0.002% in methanol). The mixture was incubated at room temperature for 30 minutes, the absorbance was measured relative to the corresponding blank at 517 nm, and the antioxidant activity (equation 1) was calculated as follows:

$$\text{AA} \% = \left( \frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}} \right) \times 100\% (\text{Equation 1})$$

Among them, AA is the antioxidant activity, and ADPPH is the absorption of blank by DPPH, such as the absorption of blank by extract or control. Quercetin was used as positive control. All the tests were carried out in triplicate.

2.5.2 Brine Shrimp Lethality Bioassay (General Toxicity)

Through the lethal test on Artemia (brine shrimp), the general toxicity of P. amboinicus was evaluated. The detection concentration of each sample is 10 mg/mL. The number of dead larvae was recorded 24 hours later, and the lethal concentration (%) was calculated according to formula (2):

$$\text{Lethal concentration} = \frac{\text{Total}_{A.\text{salina}} - \text{Alive}_{A.\text{salina}}}{\text{Total}_{A.\text{salina}}} \times 100\% (a)$$

2.5.3 Antimicrobial Activity

The in vitro antibacterial activity of Plectranthus amboinicus extract against
gram-positive bacteria Staphylococcus aureus, gram-negative bacteria Escherichia coli and Saccharomyces cerevisiae was determined. The antibacterial activity was screened by well diffusion assay. Briefly, 100 µL of microorganism suspension, concentrated at 0.5 in McFarland scale, was inoculated in a petri dish containing Miller Hinton (bacteria) or Sabrus Agar (yeast). Dig a well in Agar with a sterile Pasteur straw. 50 μL (10 mg/mL), negative control or positive control (vancomycin for gram-positive bacteria, nystatin for Saccharomyces cerevisiae, ofloxacin for gram-negative bacteria, amphotericin B for yeast) were added to each well. After incubation at 37 °C for 24 h, the growth inhibition zone around the hole was measured and the results were expressed as millimeter (Mm) as the median of at least 3 repeats.

2.5.4 In Vitro Cell Viability by MTT Assay

Monkey kidney cells (Vero-E6) and monkey kidney clear cell carcinoma (786-O) cells were cultured in the medium of DMEM with 10% fetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin and 50 µg/mL gentamicin. The viability of cells was detected by crystal violet staining. The inoculation of Brie microwell plate with 96-hole microplate is about 30000/well, incubated for 24 hours, adding different concentrations (2, 10, 50, 100 µg/mL). After 48 hours, the culture medium was discarded, the cells were washed with phosphate buffer (PBS, washed with 96% ethanol and stained with crystal violet. The absorbance was measured at 595 nm, and the cytotoxicity of the sample was expressed by absorbance fraction. At least two independent experiments were conducted, each using four repetitive cultures.

3. Results

3.1 The extraction yield of Plectranthus amboinicus

In our study, after we repeated the operation for 3 times, we got the product of 77.1 mg. That’s means the extraction yield of Plectranthus amboinicus was 7.71%.

3.2 DPPH analysis

3.2.1 TLC-DPPH（Qualitative test）

As can be seen in the figure 1, the positive control group is yellow, indicating that the component has antioxidant capacity; similarly, the sample spot is yellow too, suggesting that the sample has antioxidant capacity, but the color of the sample itself
is darker, so the next step needs to be verified by quantitative experiments.

3.2.2 DPPH Method (Quantitative test)

The antioxidant activity of the sample was evaluated using a DPPH assay, which evaluates the potential of test samples to quench DPPH radicals via hydrogen-donating ability. Herein (Figure 2), it was observed that PA derivatives possessed a high free radical scavenging ability.

3.3 General Toxicity

The mortality rate of the sample group was lower than that of the positive control group, but higher than that of the blank group, which means that the sample had some toxicity to the Artemia salina.
3.4 Antimicrobial Activity

Only one group has well diffusion, which was SA. We measured it’s diameter of sample and Positive control group, 2.4cm, and 2.2cm respectively, which maybe means that the sample has great properties to antimicrobial activity.

3.5 MTT Assay

All extracts at a concentration of 2µg/mL and 10µg/mL showed low cytotoxicity towards Vero-E6 and 786-O. The most cytotoxic concentration was 100µg/mL, followed by 50µg/mL, all of them compared with blank group with significant differences.
4. Discussion and Conclusion

The antimicrobial, antioxidant, and cytotoxic activities of Plectranthus amboinicus was addressed throughout this investigation.

The antimicrobial activity of Plectranthus amboinicus was evaluated against a series of Gram-positive and Gram-negative bacteria and yeast strains. The sample has an antibacterial effect for Sa, but not for EC and SC. Moreover, it was observed that my sample exhibited low toxicity in the Artemia salina.

In addition, the antioxidant activity of Plectranthus amboinicus (calculated by using the DPPH method) was lower little than Positive group, which was 77.93% and 87.0% respectively.

Finally, we demonstrated that Plectranthus amboinicus did not show in vitro cytotoxic effects on Vero-E6 and 786-O under the concentration of 10µg/mL. Even though the results aren't perfect, such as the Artemia salina experiment, our experiment provides a basis for the next study and lays the foundation for future experiments.

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare they have no competing interests.

Funding

This study was supported by National Key R&D Program-Special Topics for Modernization of Traditional Chinese Medicine (No. 2018YFC1707200, No. 2018YFC1707206).

Acknowledgements

Special thanks to Jiangxi University of traditional Chinese medicine and University of Lusofona for its support of this research.
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