South African Journal of Botany 95 (2014) 97–101

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Short communication

Antimicrobial and cytotoxic activities of the crude extracts of *Dietes bicolor* leaves, flowers and rhizomes

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ARTICLE INFO

Article history:
Received 11 July 2014
Received in revised form 25 August 2014
Accepted 27 August 2014
Available online 19 September 2014

Edited by I Vermaak

Keywords:
Dietes bicolor
Yellow wild iris
Iridaceae
Antimicrobial activity
Cytotoxicity

ABSTRACT

The crude extracts of *Dietes bicolor* leaves, flowers and rhizomes were subjected to comparative antimicrobial screening against two Gram-positive, two Gram-negative bacteria and four fungal strains using the agar well diffusion method. The minimum inhibitory concentrations (MIC) of the tested extracts were also determined. Furthermore, the cytotoxic activity was evaluated. *D. bicolor* extracts generally demonstrated notable broad spectrum antimicrobial activities (MIC values ≤ 500 μg/mL) against all tested pathogens. *D. bicolor* leaf extract showed potent broad spectrum antimicrobial activity with MIC values ranging between 0.24 and 31.25 μg/mL against all tested pathogens. Moreover, the flowers extract exhibited promising antimicrobial activities, displaying MIC values ranging between 1.95 and 125 μg/mL against the tested bacteria and fungi. However, the rhizomes extract showed moderate antimicrobial activity with MIC values ranging between 31.25 and 500 μg/mL. Despite the potent antimicrobial activity of *D. bicolor* extracts, they were ineffective as cytotoxic agents against nine tested cancer cell lines, displaying 50% inhibitory concentration (IC_{50}) values above 100 μg/mL. The reported potent antimicrobial activity along with the lack of measurable cytotoxic activity indicated that the antimicrobial activity of *D. bicolor* crude extracts is mediated through a mechanism other than cytotoxicity. These results suggest that *D. bicolor* can act as a potential source for natural antibacterial and antifungal agents with a good safety profile at a preliminary level.

1. Introduction

Infectious diseases are still a leading cause of morbidity and mortality worldwide, despite the great progress in medical technology and scientific knowledge (Moellering et al., 2007). Historically, natural products and their derivatives have been an invaluable source of therapeutic agents. *In vitro* and *in vivo* antimicrobial assays have effectively served as reliable methods to detect several classes of secondary metabolites with potent antimicrobial activity such as penicillins, tetracyclines and glycopeptides (Koehn and Carter, 2005; Silver and Bostian, 1990). However, resistance to antibiotics is intensifying, thus searching for new antimicrobial agents is an urgent unmet goal (Tekwu et al., 2012). Besides synthetic small molecules, natural products are still considered as the major sources of innovative new therapeutic entities targeting a plethora of ailments including infectious diseases (Clardy and Walsh, 2004). The screening for new antimicrobials often involves plant secondary metabolites exhibiting pharmacological activity against pathogens. Among the modern antifungals used nowadays, about 40% are of natural origin (Freiesleben and Jäger, 2014).

According to the WHO, 70–95% of the population in developing countries depend mainly on herbal medicines for their health care (WHO, 2011). Medicinal plants are freely available, less expensive, and their use is based on expertise and extensive knowledge among local communities (Mabona and Van Vuuren, 2013; Shai et al., 2008). Medicinal plants have been commonly used in traditional medicine for the treatment of infectious diseases. Antimicrobials from plant sources may exert their activity through mechanisms different from those of currently used synthetic drugs, thus they can significantly help in the treatment of resistant microbial strains. Examples of microorganisms that have gained resistance to antimicrobials include *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Salmonella enteritidis*, *Shigella dysenteriae*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Candida albicans* (Barbour et al., 2004).

Dietes is a member of family Iridaceae, subfamily Iridoideae (Goldblatt, 1981). It is closely related to both genera *Iris* and *Moraea*. The genus *Dietes* comprises of six species; five are native to South Africa and one (*Dietes robinsoniana*) is restricted to Lord Howe Island between Australia and New Zealand. The five African species include *Dietes bicolor*, *Dietes iridioides*, *Dietes flavida*, *Dietes grandiflora* and *Dietes butcheriana* (Goldblatt, 1981). *D. bicolor* (Steud.) Sweet ex Klatt, commonly known

Abbreviations: MIC, minimum inhibitory concentration; IC_{50}, 50% inhibitory concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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http://dx.doi.org/10.1016/j.sajb.2014.08.012
0254-6299/

as the Yellow wild iris, Peacock Flower or Butterfly Iris, is a rhizomatous perennial plant with sword-like leaves and Iris-like flowers that are white to creamy yellow in color and have three dark spots each surrounded by an orange outline (Pooley, 1999; Rudall, 1983). The genus name "*Dietes*" is derived from the Greek word "dis" which means twice and from the Latin "*etum*" which means a plant association for its close relationship to both *Iris* and *Moraea*. The species name "*bicolor*" means two-colored (Cunliff and Teicher, 2005).

Family Iridaceae is widely used in traditional medicine to treat flu, cold, toothache, malaria, and bruise (Lin et al., 2002). Extracts or active principles obtained from members of this family have shown a wide range of biological activities including antibacterial, antiprotozoal, antiviral, antioxidant, antinociceptive, anti-inflammatory cytotoxic, and immunomodulatory activities (Lucena et al., 2007; Rahman et al., 2003). The major secondary metabolites isolated from members of Iridaceae include isoflavonoids, flavonoids, triterpenoids, naphthoquinones, anthraquinones, naphthalene derivatives, xanthones and simple phenolics (Kukula-Koch et al., 2013; Mahabusarakam et al., 2010).

Many plants are used in traditional medicine in the form of crude extracts or infusions to treat various infections without scientific evidence (Noumi and Yomi, 2001). Scientific evidence for medicinal claims represents the most important limiting factor for indigenous herbal products development (Van Wyk, 2011). Infusions of the inner part of *D. iridioides* rhizomes are used by traditional healers in South Africa for the treatment of diarrhea and dysentery (Pujol, 1990). Despite the traditional use of *Dietes* for the treatment of diarrhea and dysentery, there are no scientific reports studying this effect. We could not even find any data in literature regarding the phytochemical or pharmacological properties of this genus. The scarcity of scientific information on the therapeutic activity of *D. bicolor* hinders its full utilization in evidence based complementary medicine and as a source of anti-infective agents. Aiming to tackle this problem, we evaluated the *in vitro* antimicrobial activity of *D. bicolor* crude extracts obtained from various parts including leaves, flowers and rhizomes. Moreover, the cytotoxic activity of these extracts on nine different cell lines was also evaluated.

2. Materials and methods

2.1. Plant material

D. bicolor leaves, flowers and rhizomes were collected in April, 2011 from a botanical garden on Cairo–Alexandria road and were kindly identified and authenticated by Mrs Therease Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and Director of Orman Botanical Garden, Giza, Egypt. Voucher specimens were deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University (voucher specimen number IA-10411).

2.2. Preparation of D. bicolor extracts

Plant material (leaves, flowers and rhizomes) were air-dried and ground into coarse powder. Fifty grams from each powder were extracted with 80% methanol (Kuete et al., 2012) for 3 days at room temperature. Extracts were filtered, evaporated under vacuum at 45 °C, then lyophilized and kept in the refrigerator for further biological studies.

2.3. Chemicals and reagents

Solvents used in the study were of analytical grade. All microorganisms and culture media for the antibacterial and antifungal screening assays were supplied by the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo, Egypt. Clotrimazole, the antifungal standard drug, was obtained from Bayer Health Care Pharmaceuticals, Germany. Gentamicin, the antibacterial standard drug, was obtained from Memphis Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt. For the assessment of cytotoxic activity, cell culture reagents were obtained from Lonza (Basel, Switzerland). Cancer cell lines including HeLa (cervical cancer cell line), DLD-1 (colorectal adenocarcinoma cell line), HCT 116 (colorectal carcinoma), T47D (human ductal breast epithelial tumor cell line), MCF-7 (breast cancer cell line), MDA-231 (breast cancer cell line), K562 (human erythromyeloblastoid leukemia cell line) and Molt4 (human acute lymphoblastic leukemia cell line) were obtained from the Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan. Mucosal mast cell-derived rat basophilic leukemia (RBL-2H3) cell line was purchased from the American Type Culture Collection line (Bioresource Collection and Research Centre, Hsin-Chu, Taiwan). Dulbecco's modified Eagle's minimal essential medium (DMEM) powder and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Antimicrobial activity

2.4.1. Microorganisms used in the antimicrobial assays

The antimicrobial activity of the tested extracts was assessed against standard Gram-positive bacteria including *Staphylococcus aureus* (RCMB 000108) and *Bacillus subtilis* (RCMB 000102) and Gram-negative bacteria including *Pseudomonas aeruginosa* (RCMB 000104) and *Escherichia coli* (RCMB 000107). The tested fungi included *Aspergillus fumigatus* (RCMB 002007), *Geotrichum candidum* (RCMB 052009), *Candida albicans* (RCMB 005008) and *Syncephalastrum racemosum* (RCMB 005005). All microorganism cultures were obtained from the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo, Egypt.

2.4.2. Antibacterial activity

The antibacterial activity was evaluated *in vitro* using the agar well diffusion method. Centrifuged pellets of bacteria from a 24 h old culture containing approximately 10^4–10^6 CFU (colony forming unit) per mL were spread on the surface of the nutrient agar (NaCl 0.5%, tryptone 1%, yeast extract 0.5%, agar 1%, 1000 mL of distilled water, pH 7.0) which was autoclaved at 121 °C for at least 20 min then cooled down to 45 °C, poured into Petri dishes and allowed to settle. After solidification, wells of 6 mm diameter were cut in the medium with the help of a sterile metallic borer. Extracts were prepared at a concentration of 10 mg/mL in dimethyl sulfoxide (DMSO). The tested samples (100 μL) were loaded into the wells. DMSO was loaded as the negative control. All plates were observed for a zone of inhibition at 37 °C for 24 h. The activity was determined by measuring the diameter of the inhibition zone (in mm). Each inhibition zone was measured three times by caliper to get an average value. The test was carried out three times for each bacterium culture (Rahman et al., 2001). Gentamicin was used as the positive control at a concentration of 30 μg/mL.

2.4.3. Antifungal activity

The tested samples were separately screened for their antifungal activity in vitro against *A. fumigatus*, *G. candidum*, *C. albicans* and *S. racemosum* using agar well diffusion method on Sabouraud dextrose agar plates (Rathore et al., 2000). The fungi culture was purified by a single spore isolation technique (Choi et al., 1999). Sabouraud dextrose agar plates were prepared using a homogeneous mixture of glucose–peptone–agar (40:10:15) sterilized by autoclaving at 121 °C for 20 min. The sterilized solution (25 mL) was poured in each sterilized Petri dish in the laminar flow and left for 20 min to form the solidified Sabouraud dextrose agar plate. These plates were inverted and kept at 30 °C in the incubator to remove moisture and to check for any contamination.

2.4.4. Antifungal assay

Fungal strain was grown in 5 mL Sabouraud dextrose broth (glucose: peptone; 40:10) for 3 to 4 days to achieve 10^5 CFU/mL cells. The fungal culture (0.1 mL) was spread out uniformly on the Sabouraud dextrose