Food and Chemical Toxicology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Pancratium triflorum Roxb. (Amaryllidaceae) and *Molineria trichocarpa* (Wight) N.P. Balakr (Hypoxidaceae): Cytotoxic and antioxidant activities

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ARTICLE INFO

Keywords:
Antioxidant
Cytotoxic activity
Molineria trichocarpa
Pancratium triflorum
DPPH
Total phenolics
Total flavonoids

ABSTRACT

The present investigation was carried out to evaluate the antioxidant and cytotoxic activities of *Pancratium triflorum* Roxb and *Molineria trichocarpa* were collected from South Vagaikulam, Tirunelveli district, Tamil Nadu, India. The antioxidant activities of *P. triflorum* extracts were as follows with the IC_{50} values methanol (228.13 µg/mL) > chloroform (311.33 µg/mL) > acetone (398.08 µg/mL) > petroleum ether (410.16 µg/mL). The antioxidant activities of *P. triflorum* and *M. trichocarpa* extracts were as follows with the IC_{50} values methanol (80.93 µg/mL) > acetone (98.02 µg/mL) > chloroform (186.84 µg/mL) > petroleum ether (209.64 µg/mL). Among the various extracts of *P. triflorum,* methanolic extracts showed the strongest phosphomolybdenum reduction (140.56 g AA/100 g). Among the tested extracts, acetone extracts of *M. trichocarpa* showed maximum inhibition with 71.36 ± 5.86%. In *P. triflorum*, chloroform extracts showed maximum inhibition (69.51%). The petroleum ether extract of *M. trichocarpa* was found to be most effective at which 50% mortality (LC_{50}) and 90% mortality (LC_{90}) of brine shrimp nauplii were found to be 29.22 and 184.82 mg/mL. This study results revealed the antioxidant and cytotoxic properties of *P. triflorum* and *M. trichocarpa.* Further investigations are needed to isolate and validate the active principles of the extract responsible various pharmacological properties.

1. Introduction

Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive. In recent years much attention has been devoted to natural antioxidant and their association with health benefits (Talukdar et al., 2011; Butkutė et al., 2018; Rajput et al., 2017; Bala Murugan et al., 2018; Chand et al., 2018). The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades in hope of finding an efficient remedy for several diseases (Halliwell, 2008). Brine shrimp bioassay is very simple, rapid and inexpensive method and has successfully been used as prescreening of bioactive compounds having antitumor activity (McLaughlin et al., 1993). This assay has been established as a safe, practical and economical method for the determination of the bioactivity of synthetic compounds (Almeida et al., 2002), mycotoxins of fungal pathogens (Schmidt et al., 1995), marine products (Ara et al., 1999) as well as higher plant products (Nino et al., 2006; Stefanello et al., 2006). National Cancer Institute (NCI), USA has found a significant correlation between the brine shrimp assay and *in vitro* growth inhibition of human solid tumor cell lines (Silva et al., 2007). The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts (Mclughilin and Rogers, 1991; Sam, 1993).

Plants of the Amaryllidaceae family are well-known for their ornamental value but also for the alkaloids they produce. Some species of this family contain galanthamine, an acetyl-cholinesterase inhibitor approved for the treatment of Alzheimer's disease (Hostettmann and Marston, 1995), as well as other alkaloids with interesting pharmacological activities: antimalarial, antiviral and antiproliferative (Campbell et al., 1988; Hohmann et al., 2002; Szlávik et al., 2004). Recently, flavonoid constituents of *Pancratium maritimum* L. have been reported (Youssef et al., 1998; Ramadan et al., 2000). The members of Hypoxidaceae are used by different ethnic groups to treat various disorders viz., abdominal pains, impotency and internal tumours (Hutchings et al., 1996). Plant derived secondary metabolites such as alkaloids, glycosides, flavonoids, phenolics, saponins, tannins and terpenoids received considerable attention in recent years due to their vast

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https://doi.org/10.1016/j.fct.2018.02.055
Received 14 December 2017; Received in revised form 22 February 2018; Accepted 24 February 2018

pharmacological properties including antimicrobial, antioxidant, cytotoxicity, anticancer and chemopreventive effects. In the available literature, there is no report on the antioxidant and cytotoxicity studies on *Pancratium triflorum* Roxb. (Amaryllidaceae) and *Molineria trichocarpa* (Wight) N.P. Balakr (Hypoxidaceae). With this knowledge the present study was aimed to evaluate the antioxidant and cytotoxic activities of *P. triflorum* and *M. trichocarpa* extracts.

2. Materials and methods

2.1. Collection of materials

Healthy, disease free plant samples of *Pancratium triflorum* Roxb. (Amaryllidaceae) and *Molineria trichocarpa* (Wight) N.P. Balakr (Hypoxidaceae) were harvested from South Vagaikulam, Tirunelveli district, Tamil Nadu, India. The collected plants samples were brought to the laboratory and washed well with running tap water for 10 min to remove the soil particles and other debris. Then the samples were washed thoroughly with distilled water. For drying, washed plant samples were blotted on the blotting paper and spread out at room temperature under shade for a period of fifteen days. The shade dried samples were ground to fine powder using tissue blender. The powdered samples were then stored in refrigerator at 4 °C for further use.

2.2. Preparation of extracts

30 g of dried and powder whole plant materials (includes the aerials parts and underground parts) were extracted successively with 180 mL of petroleum ether, chloroform, acetone and methanol by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extract was prepared by directly boiling the powder with distilled water for 8 h. All extracts were frozen and freeze-dried. The powder was stored in an amber bottle and stored at 4 °C in a refrigerator for later antioxidant tests. The crude extracts were screened for the occurrence or absence metabolites by the standard method.

For quantitative analysis and antioxidant assays, the extracts were dissolved in DMSO (w/v) (5 mg of crude petroleum ether, chloroform, acetone and methanolic extracts of *P. triflorum* and *M. trichocarpa* were dissolved in 5 mL of DMSO (mg/ml)).

2.3. Antioxidant assays

2.3.1. Total phenolics content

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003). The petroleum ether, chloroform, acetone and methanolic extracts of *P. triflorum* and *M. trichocarpa* were taken in the test tubes and made up to the volume of 1 mL with distilled water 0.5 mL of Folin-Ciocalteu reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. The reaction mixtures were vortexed and placed in dark for 40 min, and the absorbance was recorded at 725 nm against blank. The analysis was performed in triplicates and the results were expressed as GAE.

2.3.2. Total flavonoids content

The flavonoid contents of *P. triflorum* and *M. trichocarpa* were estimated as per the method described by Zhishen et al. (1999). Initially, 500 µL of petroleum ether, chloroform, acetone and methanolic extracts of *P. triflorum* and *M. trichocarpa* were taken in different test tubes. To the petroleum ether, chloroform, acetone and methanolic extracts of *P. triflorum* and *M. trichocarpa,* 2 mL of distilled water was added, Then 150 µL of 5% $NaNO_2$ was added to all the test tubes incubated at room temperature for 6 min. After incubation, 150 µL of $AlCl_3$ (10%) was added to all the test tubes including the blank. Once again, all the test tubes were incubated at room temperature for 6 min. Then 2 mL of 8% NaOH was added, which was made up to 5 mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 min at room temperature. The pink color developed was read spectrophotometrically at 510 nm. The amount of flavonoids was calculated in GAE.

2.3.3. Phosphomolybdenum assay

The antioxidant activity was evaluated by the green phosphomolybdenum complex formation according to the method described by Prieto et al. (1999). An aliquot of 100 µL petroleum ether, chloroform, acetone and methanolic extracts of *P. triflorum* and *M. trichocarpa* (in 1 mM dimethyl sulfoxide) were combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 mL vial. The vials were capped and incubated in a water bath at 95 °C for 90 min. After the samples were cooled down to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results are mean values expressed as g of ascorbic acid (AA) equivalents/100 g extract.

2.3.4. DPPH radical scavenging activity

The antioxidant activity of *P. triflorum* and *M. trichocarpa* were determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Braca et al. (2001). Different extracts (petroleum ether, chloroform, acetone and methanolic extracts) of *P. triflorum* and *M. trichocarpa* with various concentrations were taken and the volume was adjusted to 100 µL with methanol. About 5 mL of a 0.1 mM methanolic solution of DPPH was added to the aliquots of sample and shaken vigorously. Negative control was prepared by added 100 µL of methanol in 5 mL of 0.1 mM methanolic solution DPPH. The tubes were allowed to strand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm against the blank (methanol). Radical scavenging activity of the sample was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH concentration.

2.3.5. Scavenging of hydrogen peroxide

The ability of *P. triflorum* and *M. trichocarpa* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined from absorption spectra at 230 nm with molar absorbtivity 81 (mol/L)/cm. The petroleum ether, chloroform, acetone and methanolic extracts of *P. triflorum* and *M. trichocarpa* (100–200 mg/mL) were added to hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage inhibition activity was calculated using the formula

$$\% \text{ scavenging activity} = [(\text{Control OD} - \text{Sample OD})/\text{Control OD}] \times 100$$

2.4. Cytotoxic activity

2.4.1. Hatching of brine shrimp

Artificial sea water (38 g NaCl/1000 mL tap water) was taken in small tank and shrimp eggs were added to one side of the divided tank and the side was covered. The shrimps were allowed for 4 h to hatch and mature as nauplii. During this period constant oxygen supply, temperature (around 37 °C) and light supply was maintained. The hatched shrimps were taken for bioassay.

2.4.2. Application of test sample to the test tube containing brine shrimp nauplii

55 clean test tubes were taken and separated by 10 mL in each test