DOI:10.26524/krj.2020.18

Kong. Res. J. 7(2): 26-29, 2020
Publisher: Kongunadu Arts and Science College, Coimbatore.

https://www.krjournal.com

RESEARCH ARTICLE

EVALUATION OF ANTIOXIDANT, CYTOTOXIC AND APOPTOTIC ACTIVITIES OF METHANOLIC EXTRACT OF *MOMORDICA CYMBALARIA* HOOK F. UNRIPE FRUIT

Gunasekaran Bhuvaneswari[1], Ramaraj Thirugnanasampandan[2,*] and Madhusudhanan Gogul Ramnath[1]

[1]PG and Research Department of Biotechnology, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641 029, Tamil Nadu, India

[2]PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641029, Tamil Nadu, India

ABSTRACT

Natural products gain more attention nowadays because of their compatibility with mankind, safe and economic feasible. The main purpose of this study was to evaluate antioxidant, cytotoxic and apoptotic activities of methanolic extract of *Momordica cymbalaria* Hook F. unripe fruit (UF). A considerable DPPH, hydroxyl radical scavenging and metal ion chelating activities and significant inhibitory effect on lipid peroxidation and least activity on deoxyribose degradation was observed for methanolic UF extract. Cytotoxic activity of UF extract on HeLa cells was observed and its inhibitory concentration fifty percent was calculated as 17.69±0.01µg/mL. Apoptosis inducing ability of UF extract on HeLa cells was also observed by Propidium iodide and acridine orange/ethidium bromide (AO/EtBr) dual staining. This is the first report on antioxidant, cytotoxic and apoptotic inducing ability of methanolic UF extract of *Momordica cymbalaria* collected from Sattur, Virudhunagar district, Tamilnadu, India.

Keywords: *Momordica cymbalaria*, Antioxidant, Cervical cancer, Cytotoxicity, Apoptosis.

1. INTRODUCTION

In developing countries, cervical cancer is considered to be the most commonly found cancer in women which leads to high mortality rate (1). Currently many studies were carried out to screen active constituents from plants for treating cancer (2). Generally, apoptosis is a regulated cell death process to remove the damaged cells. However, it is important to develop the natural plant based agents which induce cytotoxicity and apoptosis in cancer cells (3).

Momordica cymbalaria Hook F. belongs to Cucurbitaceae family is well known traditional plant used to control skin diseases, rheumatism, ulcer and diarrhoea. The fruit of *M. Cymbalaria* contains high levels of calcium, potassium and vitamin C (4). Furthermore, it has a wide range of medicinal properties like cardioprotective, hepatoprotective, nephroprotective and antioxidant (5). Based on the medicinal importance of *M. cymbalaria*, the present study was designed to evaluate antioxidant, cytotoxic and apoptosis inducing activities of methanolic unripe fruit extract.

2. MATERIALS AND METHODS

2.1. Extraction

Unripe fruits (UF) *Momordica cymbalaria* Hook. F. was collected from Sattur, Virudhunagar district. The dried and powdered UF (45g) was extracted with methanol in Soxhlet apparatus at room temperature to yield crude extract.

2.2. In vitro antioxidant activities

Antioxidant activity of methanol extract UF was evaluated using various *in vitro* antioxidant assays. A stock solution was prepared by dissolving 1mg of methanol extract in 1mL of DMSO (10%) and then five different concentrations (25-125µg/mL) were prepared. The percentage of antioxidant activity was calculated using the following equation: % Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B, absorption of blank sample, A_A, absorption of test sample.

2.2.1 DPPH free radical scavenging activity

Different concentrations of test samples were made up to 1mL with DMSO and mixed individually with 500µL of 0.2mM DPPH. Reaction mixture was incubated at 37°C for 30min and then absorbance was measured at 517nm (6).

2.2.2. Metal chelation activity

Briefly, 1mL of 2mM $FeSO_4$ was added to different concentrations of test samples individually. Further, the reaction was initiated by addition of 1mL of 5mM ferrozine. The mixture was shaken vigorously and left to stand at room temperature for 10min. Absorbance was measured at 562nm after 10min (7).

*Correspondence: Ramaraj Thirugnanasampandan, PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India E.mail: thirugnanasampandanr_bo@kongunaducollege.ac.in

2.2.3. Hydroxyl radical scavenging activity

Reaction mixture includes 100µL of 7.5mM $FeSO_4$, 100µL of 7.5mM 1,10-phenanthroline, 500µL of 0.2M phosphate buffer (pH 7.8) and test samples at different concentrations individually. The reaction was started by adding 50µL of 30mM H_2O_2. After incubation at room temperature for 5min, the absorbance of the mixture was read at 536nm (8).

2.2.4. Inhibition of linoleic acid peroxidation

Briefly, 500µL of linoleic acid (20mM), 300µL of TrisHCl (100mM, pH 7.5), 100µL of ascorbic acid (5mM) were mixed individually with the test samples and linoleic acid peroxidation was initiated by the addition of 100µL of $FeSO_4$ (4mM), incubated for 60min at 37°C and terminated by the addition of 2mL of ice cold TCA (10%). Further, 1mL of TBA (1% in 50mM NaOH) was added to 1mL of the reaction mixture, followed by heating at 95°C for 60min. The reaction sample was read at 532nm (9).

2.2.5. Prevention of deoxyribose degradation activity

Different concentrations of the test samples were mixed individually with 500µL of 20mM deoxyribose, 500µL of 0.1M $NaPO_4$, 500µL of 20mM H_2O_2 and 500µL of 50mM $FeSO_4$. The reaction mixture was incubated for 60min at 37°C and terminated by the addition of 2mL of 10% ice cold TCA. 1mL aliquot of the reaction mixture was added with 1mL of 1% TBA. The TBA/sample mixture was heated in a water bath at 95°C for another 60min. The absorbance was read at 532nm (10).

2.3. In vitro cytotoxicity study

2.3.1. Cell culture

HeLa cell line purchased from National Centre for Cell Science (NCCS), Pune was routinely maintained in DMEM supplemented with 10% fetal bovine serum, L-glutamine (1%), streptomycin and penicillin (1%) at 37°C in a humidified incubator (Nectarnova, Canada) containing 5% CO_2.

2.3.2. MTT assay

1x10^4 cells/100µL medium was added in each well of 96 well plate and incubated for 24h. Then, the cells were treated with various concentrations of UF extract and further incubated for 48 h. About 20 µl of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and the plate was incubated at 37°C for 4h. The medium was removed and 100µL of DMSO was added to each well. After 10 min of incubation at 37°C, the plate was read at 570 nm using a microplate reader (11). % of cell viability = ([AB – AA]/AB)x100, where AB, absorption of blank sample, AA, absorption of test sample.

2.3.3. Propidium iodide staining

HeLa cell was cultured in 24 well plate and treated with different concentrations of UF extract (25, 50 and 75 µg/mL). After 24h of treatment, the cells were washed with 500µL of PBS and fixed in 500µL of 70% ethanol for 30 mins. The cells were washed twice with 500µL of PBS and incubated for an hour with 200µL of Propidium iodide (500µM) and visualized under fluorescent microscopy (12).

2.3.4. Acridine orange/ethidium bromide (AO/EtBr) dual staining

Morphological analysis of apoptosis by acridine orange/ethidium bromide dual staining was performed (13). Briefly, 2x10^4 cells per well was seeded in a 24 well plate and treated with different concentrations of UF extract (25-75 µg/mL) for 24 h. After incubation, 10 µL of 1 mg/mL AO and EtBr mixture was added to each well. Apoptotic nuclei were visualized and photographed under Olympus CKX42 fluorescent microscope.

3. RESULTS AND DISCUSSION

3.1. Extraction of unripe fruit

The unripe fruit of *Momordica cymbalaria* was shade dried at room temperature and ground into a fine powder. Further the powder (45 g) was extracted with methanol using Soxhlet apparatus which yielded 475 mg of crude extract. Preliminary phytochemical screening showed methanolic UF extract had high content of total phenols and it was believed that those bioactive constituents are more important in promoting several medicinal properties (14).

3.2. In vitro antioxidant activity

Scavenging of free radicals is considered to be a significant approach in preventing the damage of biologically important macromolecules such as lipids and proteins and get rid of oxidative stress mediated diseases like neurodegenerative diseases, cancer and premature aging (15). More number of research studies have been carried out to screen a potential and innocuous antioxidant from plant origin.

The concentration needed to scavenge fifty percent of free radicals by UF extract was calculated as 127.47±0.01 µg/mL and 155.82±0.01 µg/mL against DPPH and hydroxy radicals respectively. Metal chelating activity was calculated as 112.30±0.02 µg/mL. The UF extract showed maximum antioxidant activity with an IC_{50} value of 13.98±0.01 µg/mL to inhibit the linolenic acid peroxidation and least activity was observed against prevention of deoxyribvose degradation with IC_{50} value of 175.00±0.02 µg/mL. To support our present findings, earlier literature stated that methanolic extract of *M. Cymbalaria* seed possessed DPPH radical scavenging ability. It is

believed that presence of phenols maybe responsible for the observed antioxidant activity simply by donating hydrogen and metal conjugating properties (14, 16, 17).

3.3. Cytotoxic activity and apoptotic induction

Plant based medicines have been used for long time in several Asian countries and many *in vitro* studies have been reported their anticancer activity (18, 19).The methanol extract of UF induced the cytotoxicity in HeLa cells after 24h exposure and inhibitory concentration fifty percent was calculated as 17.69±0.01µg/mL. Apoptosis inducing potential was studied against HeLa cells using acridine orange/ethidium bromide dual staining ((Fig.1) and propidium iodide staining (Fig. 2). Apoptosis induction was observed at of 75µg concentration which showed morphological changes and late apoptosis with membrane damage and nuclear condensation.

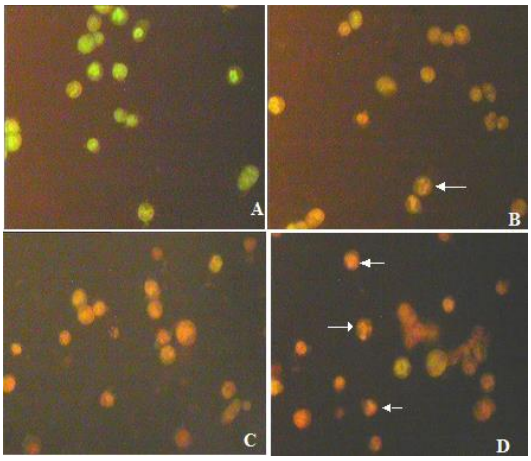

Fig. 1. Acridine orange/ethidium bromide (AO/EtBr) dual staining of HeLa cells treated with *M. cymbalaria* unripe fruit extract. A, Control; B, 25 µg; C, 50 µg; D, 75 µg. Green colour indicates live cells; Orange colour indicates apoptotic bodies; White arrow indicates chromatin condensation

Ethanol fruit extract (FE) of *Momordica charantia* resulted a cytotoxicity against K562, A549, MCF-7 and Jurkat cell lines and caused accumulation of A549 and MCF-7 cells in the S phase (20). Methanol extract of *M. charantia* fruit induced apoptosis on A549 human lung cancer cells using various stains and caspase-3 and p53 activity (21). In agreement with these literature reports, it is assumed that *M. cymbalaria* consists of effective anticancer compounds which might be responsible for the observed cytotoxicity and apoptotic activities.

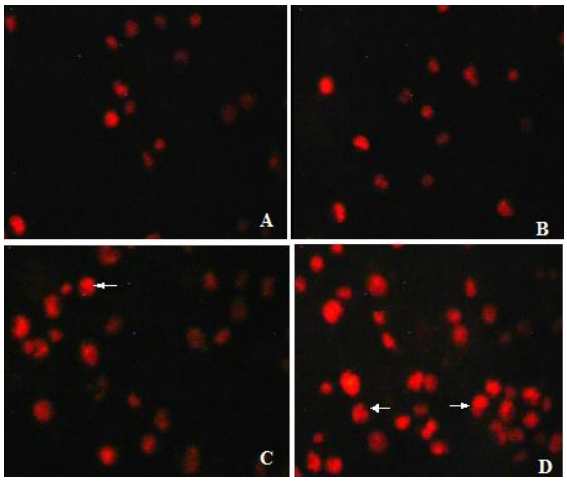

Fig. 2. Propidium iodide staining of HeLa cells treated with *M. cymbalaria* unripe fruit extract. A, Control; B, 25 µg; C, 50 µg; D, 75 µg. White arrow indicates apoptotic bodies

4. CONCLUSION

The methanol extract of *Momordica cymbalaria* UF could be considered as a good plant based antioxidant supplement. In addition, the UF could be used as an effective ingredient in preparing herbal medicine.

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