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Research Article

ASSESSMENT OF IN VITRO ANTICANDIDAL, ANTIVIRAL EFFECTS AND FREE RADICAL SCAVENGING CAPACITY OF *Cucumis Melo* L.EXTRACTS GROWING IN KERKER

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Abstract

Anticandidal, antiviral and free radical scavenging effects of aerial part and flesh extracts of *Cucumis melo L.* were investigated. The anticandidal activity was evaluated using microwell dilution method against four fungi. The antiviral activity was determined against human cytomegalovirus (HCMV) strain AD-169 (ATCC Ref. VR 538) using a cytopathic effect (CPE) reduction assay. Antiradical scavenging capacities of *Cucumis melo* extracts were tested using free radical forms of ABTS. Among tested extracts, Aerial part extracts displayed a higher anticandidal activity than flesh extracts. In addition they exhibited the highest antiviral and antiradical activities.

Keywords: *Cucumis melo L.,* Anticandidal, Antiviral, ABTS, Activity

Introduction

Plants play a significant role in maintaining human health and improving the quality of human life. They serve humans well as valuable components of food, such as seasonings and beverages as well as in cosmetics, dyes, and medicines. Many plant extracts prepared from plants have shown to exert biological activity in vitro and in vivo, which justified research on traditional medicinal plants focused on the characterization of their antimicrobial activity (Maertinez et al., 1996). Large numbers of plants have been screened as a viable source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds which are responsible for maintenance of health, to help the human body to reduce oxidative damage and to protect from coronary heart diseases and cancer (Yanga et al. 2002; Halliwell and Gutteridge, 2007) . Phytochemicals in fruits and vegetables can neutralize oxidative agents. Beneficial effects of phytochemicals are believed to be achieved through several mechanisms, such as stimulation of the immune system, modulation of gene expression and hormone metabolism, chelation of transition metals and providing antibacterial and antiviral

supports. The health benefits of vegetables in preventing cancer and cardiovascular diseases are mostly attributed to the quality and quantity of antioxidative components. The Cucurbitaceae family includes several species of cultivated plants of great economic importance, including watermelon (Citrulluslanatus L.), squash (Cucurbita maxima L.), cucumber (Cucumissativus L.) and cantaloupe (Cucumis melo L.) (Ritschel et al., 2004). Cantaloupe is one of the most consumed fruit crops worldwide due to its pleasant flavor and nutritional value. Cantaloupes are a diverse group of fresh, dessert fruits that includes the orange flesh cantaloupes, green flesh honeydew, and mixed melons. Other studies showed that cantaloupe pulp extracts possesses antioxidant and anti- inflammatory properties (Ismail et al., 2010; Vouldoukis et al., 2004).

The object of this study was to determine the anticandidal, antiviral and free radical scavenging activities of the aerial part (leaf and stem) and fruit extracts of *Cucumis melo growing* in Tunisia (Kerker).

Materials and Methods

plant material

The herb was purchased in June from a local market in Kerker (sahel Tunisia) and the plant aerial parts and fruit were authenticated and a voucher specimen was deposited in our laboratory of Faculty of Pharmacy.

Preparation of extracts

Ethanol extract

Each sample (50 g) of flesh and aerial parts (stem and leaf) was incubated with200 ml of ethanol (80%) for 3 days under magnetic stirrer. Solvent was evaporated under vacuum at 70°C to get crude extracts and it was stored at -80°C until use.

Aqueous extract

Each sample (50 g) of aerial parts and flesh of *Cucumis melo* L was extracted with water at 80°C, for 30 min under continuous shaking. The extract was filtered using a Whatman n°. 1filter paper. The water extracts were stored at − 80° C prior to experimentation.

Total phenolic contents

The total phenolic content in each extract was determined using Folin – Ciocalteus reagent according to the method of Singleton et al. (1965). Forty microliters of extract (1 mg/ml) was mixed with 200µl Folin –Ciocalteus reagent (Sigma–Aldrich, Germany) and 1160 µl of distilled water, followed by 600 µl 20% sodium carbonates (Na_2CO_3) 3 min later. The mixture was shaken for 2 h at room temperature and absorbance was measured at 765 nm. All tests were performed in triplicate. Catechin (Sigma –Aldrich, Germany) was used as a standard. The concentration of total phenolic compounds (TPC) was determined as mg Catechin Equivalent (CE) per gram extract.

Determination of antcandidal activity

fungi

The antifungal effect of the extracts was also tested against a range of pathogenic reference yeasts: *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida kreuseii* ATCC 6258 and *Candida parapsilosis* ATCC 22019.

Determination of antcandidal activity

The antimicrobial activity of the extracts was evaluated through the determination of the minimal inhibitory concentration (MIC) by the micro well dilution method (Smania et al. 2006). All extract stock solutions were prepared by dissolution in 10% dimethyl sulfox-ide (DMSO). The tested plant extract concentrations ranged from1 to 10 mg/ml. The MIC of each extract was defined as the lowest concentration which inhibited candidal growth, after incubation at 37 °C between 18 and 24h. The minimal fungicidal concentration (MFC) was determined by subculture on blood agar at 37 °C between 18 and 24 h.

Antiviral activity

Cell toxicity assay

The evaluation is based on the reduction of MTT (3-[4,5-dimethylthiazol-2- yl]-2,5-diphenyltetrazolium bromide). The MTT colorimetric assay was performed in 96-well plates (Polydoro et al. 2004). Human diploid embryonic lung fibroblasts (MRC -5) cells

were seeded in 96-well plates at a concentration of 105 cells per well and incubated for 24 h at 37 °C in a 5% CO2 enriched atmosphere. After treatment with various concentrations of each extract, cells were incubated for an additional48 h at 37C. After that, medium was removed, the cells in each well were incubated with 200 mL of MTT solution (5 mg mL^{-1}) for 2 h at 37° C. MTT solution was then discarded and 200 mL insoluble formazan crystal was added. The optical density (OD) was measured at 540 nm. Data were obtained from triplicate wells. The cytotoxic concentration of the compound was expressed as IC_{50}, the concentration of the tested material required to kill the cells by 50%.

Test viruses

Human cytomegalovirus (HCMV) strain AD -169 (ATCC Ref. VR 538) was grown on MRC-5 cells in MEM medium until complete cytopathic effect (CPE). The titer viral was used at a final concentration of $100TCID_{50}$ (50% Tissue Culture-Infective Dose) which were determined by the method of Reed and Muench (1938).

Antiviral activity assay

A CPE reduction assay for screening the antiviral activities of the plant extracts was employed. In brief, 100 $TCID_{50}$ (50% tissue culture-infective dose) virus suspension and serial two-fold dilutions of crude extracts were added simultaneously to confluent cell monolayers in a 96-well plate. The dilution medium without samples and with virus suspension were respectively added, to the cell cultures to serve as cell control and virus control. The plates were incubated at 37° C in a humidified CO_2 atmosphere for 3–5 days. The concentration that reduced 50% of CPE compared to the virus control was estimated from the plots of data and was defined as the 50% inhibitory concentration (IC_{50}). The selective index (SI) was calculated from the ratio CC_{50}/IC_{50} (Kujumgiev et al., 1999).

Radical scavenging activity

Radical cation $ABTS^{+}$• scavenging activity

The standard method described by Dorman and Hiltunen (2004) was adopted with minor modifications. This assay assesses the total radical scavenging capacity based on the ability of a compound or an extract to scavenge the stable ABTS radical $ABTS^{+}$•The blue-green ABTS radical form was produced through the reaction between ABTS and potassium persulfate in water. A concentrated $ABTS^{+}$• stock solution was diluted with phosphate buffered saline (PBS) at pH 7.4 to a final absorbance of 0.7 ± 0.02 with a wavelength of 734 nm and at a temperature of 25 °C. Solutions with different diluted concentrations of our samples (extracts and natural products) were prepared in ethanol. Ten microliters of an antioxidant-containing solution were added to 990 ml of $ABTS^{+}$• solution and the absorbance was measured at 734 nm. Sample Absorbance was compared to a blank where 10 µl of the solvent were added to 990 ml of the ABTS+• solution. Absorbance was measured at 20 minutes after addition of the antioxidant. All measureents were performed in triplicate. Results were expressed as percentage inhibition.

Results and Discussion

Total phenolic content

Phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to antioxidant and antibacterial activities. The TPC was expressed in mg catechin equivalent per gram of extract (mg CE/g of extract). The results of the total phenolic content of *Cucumis melo* extracts were given in Table 1. The total phenolic content varied from 10.15 to 75.34 mg CE/g of extracts. The results indicate that the ethanolic extract of aerial parts of *C. melo* had the highest total phenolic content (75.34 mg CE/g of extracts) whereas the lowest content was measured in the aqueous flesh extract (10.15 mg CE/g of extracts). The result presented in Table1 illustrates the efficiency of ethanol for the extraction of total phenolic compounds. Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups (Peter and Wong, 2006). This finding is in agreement with some previous studies which reported that the total phenolic content of leaf extract is higher than in other parts of the plant for Beta vulgaris, Petroselinum crispum and Coriandrum sativum (Pyo et al., 2004; Wong et al., 2006). This suggests that leaf might be the part that is rich in phenolic compounds in many plants.

Table 1 Polyphenolic content of *Cucumis melo* L. extracts

Extracts	Total phenolic content mg CE/g extracts	
Aerial part	Aqueous	25.21±1.2
	Ethanol	75.34±2.2
flesh	Aqueous	10.15±1.5
	Ethanol	15.58±0.15

Values are given as means ± SD; total phenolic content (mg CE/g) is given in mg catechin equivalent/g extract

Anticandidal activity

All the extracts tested from Tunisian *Cucumis melo* showed anticandidal activity against all tested fungi. MIC ranged from 0.256 to 2.5 mg/ml and MFC ranged from 2.5 to 5 mg/ml (See Table 2). The strongest inhibitions were obtained with ethanolic extract of aerial parts with MIC of 0.256 mg/ml and MFC of 2.5 mg/ml. The aqueous extract of aerial parts of *C. melo* showed also anticandidal activity with MIC of 0.512 mg/ml. Moderate anticandidal activity was also observed with flesh extracts. The anticandidal activity might also be attributed to the high quantity of polyphenols, which are known to possess efficient antimicrobial activity (Ediziri et al., 2011). In other works phenolic compounds have been reported to be responsible for antimicrobial properties (Penna et al., 2001).

Table 2 Anticandidal activity of *Cucumis melo* L. extracts using microwell dilution method

	Extracts	***Candida glabrata***		***Candida albicans***		***Candida kreussei***		***Candida parapsilosis***	
		MIC	**MFC**	**MIC**	**MF C**	**MIC**	**MFC**	**MIC**	**MFC**
Aerial parts	Et	0.256	2.5	0.256	2.5	0.256	2.5	0.256	2.5
	Aq	0.512	2.5	0.512	2.5	0.512	2.5	0.512	2.5
Flesh	Et	2	5	2.5	5	2.5	5	2.5	5
	Aq	2	5	2.5	5	2.5	5	2.5	5

Antiviral activity

The antiviral activity was estimated on the basis of the cytopathic effect (CPE) of the virus-infected confluent monolayer of MRC 5 cells. The mean IC_{50}, CC_{50} and SI values are given in Table 3. All extracts were not toxic against MRC5 cells (CC_{50}>300 µg mL^{-1}). The most active extracts were ethanol and aqueous extracts of aerial parts of *C. melo*, which inhibited HCMV virus replication at 100 and 150µg/ml without showing cytotoxic effects and with a selective index higher than 3 for the ethanolic extract. Good antiviral activity was also found with flesh extracts. The observed antiviral activity may be due to the higher amount of phenolic compounds particularly flavonoids and tannins known to possess good antiviral activities (Namba et al., 1998). Aruoma et al. (1996) reported that extracts from rosemary and provencial herbs showed potential antioxidant and anti - HIV activities.

Table 3 Antiviral activity of *Cucumis melo* L. extracts

	Extracts	Anti-HCMV		
		IC_{50} (µg mL^{-1})[a]	CC_{50} (µg mL^{-1})[b]	SI[c]
Aerial parts	Aqueous	150	>300	>2
	ethanolic	100	>300	>3
Flesh	Aqueous	250	>300	>1,2
	ethanolic	250	>300	>1,2
Ganciclovire[d]		0,8	>200	>250

[a]IC_{50} is the concentration of the sample required to inhibit 50% virus- induced CPE. [b]CC_{50} is the concentration of the 50% cytotoxic effect. [c]SI (selective index) is the ratio CC_{50}/IC_{50}. Ganciclovir, which are clinically used anti-HCMV drugs was used as positive control in the antiviral activity.

Radical scavenging activity

Compared with Trolox, the maximal inhibition percentage values calculated after 20 minutes of reaction showed different antiradical activities for all four tested extracts. The ethanol aerial parts extract shows the best ABTS inhibition with IC50 of 8.16 mg/ml. The aqueous aerial parts extract also showed good antiradical activity with IC50 of 10.12 mg/ml. Aqueous flesh extract showed a moderate inhibition with a IC50 value of 20.21 mg/ml. The observed antioxidant activity may be explained by the total phenolic content in the active extracts. A high correlation between total phenolic content and antioxidant activity was reported in different studies (Edzir et al., 2011; Mahjoub et al., 2009)

Table 4 ABTS inhibition percentage of *Cucumis melo L.* extracts

	Extracts	IC_{50}(mg/ml)
Aerial parts	Aqueous	10.12±2.5
	Ethanol	8.16±1.32
Flesh	Aqueous	20.21±0.56
	Ethanol	17.56±1.2
Trolox		0.122±0.02

IC_{50} (mg/ml) concentration scavenging 50% of ABTS free radicals.

Anticandidal, antiviral and free radical scavenging effects of aerial part and flesh extracts of *Cucumis melo L.* were investigated. The anticandidal activity was evaluated using microwell dilution method against four fungi. The antiviral activity was determined against human cytomegalovirus (HCMV) strain AD-169 (ATCC Ref. VR 538) using a cytopathic effect (CPE) reduction assay. Antiradical scavenging capacities of *Cucumis melo* extracts were tested using free radical forms of ABTS. Among tested extracts, Aerial parts extracts displayed a higher anticandidal activity than flesh extracts. In addition they exhibited the highest antiviral and antiradical activities.

Conclusion

The present study shows that ethanolic and aqueous extracts of aerial parts of *Cucumis melo L*. had the highest total phenolic content. They also showed the best anticandidal activity. Furthermore, the ethanolic aerial parts extract showed strong radical scavenging activity against ABTS• radical and an important antiviral activity against HCMV. Thus, these extracts can be considered as potential new sources of natural antioxidants for food and neutraceutical products. At present it is not yet established what components are responsible for the observed activities, Further work should therefore be performed on the isolation and identification of the active compounds.

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