MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES
Published by The Malaysian Analytical Sciences Society
ISSN 1394 - 2506

BIOLOGICAL PROPERTIES OF CUCUMBER (*Cucumis sativus* L.) EXTRACTS

(Sifat-Sifat Biologi Ekstrak Timun (*Cucumis sativus* L.))

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Received: 9 December 2014; Accepted: 16 October 2015

Abstract

Peel and pulp of *Cucumis sativus L.* fruit were extracted from aqueous (water) and phosphate buffered solution at incubated temperature of 37°C similar to normal human physiological temperature to investigate the potential of these extracts as antibacterial and cytotoxic agents using antibacterial susceptibility assay against six pathogenic bacteria of gram positive and negative and cytotoxic assay against human non-small cell lung carcinoma cell line (H1299) and human breast adenocarcinoma cell line (MCF-7). The phytochemical contents of all extracts were determined to correlate with their biological properties.

Keywords: *Cucumis sativus* L., biological activities, anticancer, aqueous cucumber extract

Abstrak

Kulit dan pulpa buah *Cucumis sativus* L. telah diekstrak dengan menggunakan akueus (air) and larutan penimbal fosfat yang sedang dieram pada suhu 37°C sama dengan suhu normal fisiologi manusia untuk mengkaji potensi pelbagai ekstrak tersebut sebagai agen antibakteria and agen sitotoksik dengan menggunakan asai kelemahan antibakteria terhadap enam bakteria patogenik gram positif dan negatif dan asai sitotoksik terhadap sel karsinoma paru-paru bukan kecil manusia (H1299) dan sel adenokarsinoma payudara manusia (MCF-7). Kandungan fitokimia dalam semua ekstrak telah ditentukan supaya berhubung kait dengan sifat-sifat biologi.

Kata kunci: *Cucumis sativus* L., aktiviti biologi; anti-kanser; ekstrak akueus timun

Introduction

Cucumis sativus L. (*C. sativus*) are usually served as appetizers or deserts. They are also associated with cooling, healing, soothing and emollient effects. Most importantly, they are found to exhibit a wide spectrum of activity including antioxidant [1] and amylolytic [2]. However, all the reported studies involved extracts from either exothermic condition (high temperature soxhlet extraction) or using volatile organic solvent (e.g. dichloromethane, ethanol, and methanol). Previous studies found that the methanolic fruit pulp and ethanolic leaves and stems of *C. sativus* extracts possessed slightly different phytochemicals content. However, both extracts do contained similar phytochemicals e.g. alkaloid, saponin, glycoside and tannin [3, 4]. The ethanolic leaves and stem showed weak antifungal activities against *Aspergillus niger*, *Blastomyces dermatittidis*, *Candida albicans*, *Pityrosporum ovale*, *Trichophyton spp.*, *Microsporum spp*. and moderate cytotoxic effect on the brine shrimp nauplii [4]. To date, no

extraction has been done fresh and at 37°C, similar to human physiological temperature. This study was initiated due to the fact that cucumbers are commonly eaten fresh. The present study describes the potential of fresh *C. sativus* extracts in phosphate buffered solution and aqueous extracted at 37°C as cytotoxic and antibacterial agents.

Materials and Methods

Extraction of *Cucumis sativus* L.

Three unripe cucumbers (in the range of 220 g – 320 g each) were randomly selected from the local farm in Kuantan. They were thoroughly washed and scrubbed, then left to dry. The peel and pulp were separated with their masses recorded. The peel and pulp were separately blended with phosphate buffered saline solution in a ratio of 1:3 (1 g of either portion of peel or pulp in 3 mL of PBS). The solution was incubated in water bath at 37°C for 8 hours. The slurry was filtered using gauze cloth and then centrifuged at 6500 rpm for 15 minutes in 4°C to obtain the supernatants. Dry PBS extract powder was obtained after spray-dried at an inlet temperature of 155°C with 5% maltodextrin in accordance to previous method [5]. Dry aqueous extract powder was obtained following the above mentioned method with the substitution of PBS with deionized water.

Phytochemical Screening

Phytochemical screening was done using a small portion of the dry extract of the aqueous and PBS extracts of both *C. sativus* pulp and peel to determine the presence of tannins, flavonoids, alkaloids, saponins and steroids following some modified methods [6,7].

Dragendroff's test (Alkaloid)

Dry powder extracts were dissolved in 5 mL of 1% hydrochloric acid on steam bath with addition of a few drops of Dragendroff reagent. Positive result of alkaloid was observed with the presence of turbidity or yellow precipitation.

Steroid test

Dry powder extracts were dissolved in 3 mL of chloroform and layered with concentrated sulfuric acid. Observation of the color reddish brown at the interface showed the presence of steroid.

Froth test (Saponin)

Dry powder extracts were dissolved in 2 mL distilled water and shook vigorously for few minutes. Formation of stable foam showed the presence of saponin.

Shinoda test (Flavonoid)

Dry powder extracts were added with few pieces of magnesium ribbon and then dropwised with concentrated hydrochloric acid. Positive result of flavonoids was observed with the presence of the color pink scarlet.

Ferric (III) chloride (Tannin)

Dry powder extracts were added with few drops of ferric (III) chloride solution. A change of color of blue-green to black showed the presence of tannins.

Antibacterial Susceptibility Assay

Six bacteria of gram positive [*Bacillus cereus* (ATCC 117788), *Staphylococcus aureus* (ATCC 29213) and *Staphylococcus epidermidis* (IMRS 384/1052)] and gram negative [*Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (IMR K 41/09A)] were obtained from the microbe stock culture maintained on nutrient agar media at Kulliyyah of Science, IIUM. The antibacterial activity was determined in accordance to the disc diffusion method [8]. The disc was impregnated with 100 mg of extract per disc and inoculated on a dish. The plates were incubated overnight (24 hours) at 37°C. Zones of inhibition formed were measured to obtain the mean diameter. Streptomycin (10 μg/disc) was used as positive control and sterilized blank discs as negative control. The assay was done in triplicates.

Cytotoxic Assay

Cytotoxic assay was done at the Animal Cell and Tissue Culture Laboratory, Kulliyyah of Science, International Islamic University Malaysia using human non-small cell lung carcinoma cell line (H1299) and human breast

adenocarcinoma cell line (MCF-7) that were kindly provided by Prof. Dr. Masa Aki Ikeda, Section of Molecular Craniofacial Embryology, Tokyo Medical and Dental University, Japan to Dr. Solachuddin Jauhari Arief Ichwan, Kulliyyah of Dentistry, International Islamic University Malaysia. Both Cell lines were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin 100 I.U./mL at 37°C under humidified air, with 5% CO_2 atmosphere. Cytotoxicity was determined using the microtitration of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) following Mosmann's method [9] using cisplatin and 5-fluorouracil as standard. Cytotoxicity was expressed as IC_{50}, i.e. the concentration that reduced the absorbance of treated cells by 50% with reference to the control (untreated cells).

Results and Discussion

The yield of the aqueous pulp and peel extracts was calculated to be 10.0% and 13.5% respectively, where as the PBS pulp and peel extract yielded 8.9% and 13.0%, respectively. Low percentage yields of extracts were obtained because cucumber composed mainly of water (~95%).

Phytochemicals Screening

The phytochemical result tabulated in Table 1 was found to be screened positive within 5 mins for all the extracts. The result showed that all extracts contained appreciable amount of flavonoids, saponins, and steroids, with the exception that phosphate buffered saline (PBS) extracts contained alkaloids, which was absent in all aqueous extracts. It was found that PBS peel extract contained more saponins with foam formation of more than 1 cm, followed by the aqueous peel extract (foam formation of 1 cm), showing that peels contained more saponins than pulp extracts. All extracts do not have tannins, which was present in macerated ethanolic cucumber extracts [4]. It is worthy to note that variations in solvent and method extraction greatly affect the presence and composition of the secondary metabolite [7].

Table 1. Phytochemical contents and the IC_{50} value for the various cucumber extracts

Cucumber Extracts	Phytochemical contents	IC_{50} value (mg/mL)	
		MCF-7	H1299
PBS Peel	Flavonoids, Saponins, Alkaloid, and Steroids	290.0	52.0
PBS Pulp	Flavonoids, Saponins, Alkaloid, and Steroids	125.0	42.0
Aqueous Peel	Flavonoids, Saponins, and Steroids	-	-
Aqueous Pulp	Flavonoids, Saponins, and Steroids	-	-

Antibacterial Susceptibility Assay

The antibacterial assay showed that the aqueous extracts were inactive against all the microorganisms tested. These results are well predicted as the extracts from organic solvents exhibit more significant antimicrobial activities compared to the aqueous extracts [7]. The PBS peel extract of *C. sativus* were only active against *Staphylococcus aureus* (inhibition zone of 7.0±0 mm). This emphasized on the presence of saponins, which contributed higher degree of antibacterial activities [10]. It is interesting to note that the PBS pulp extract showed activity against gram-negative bacteria, *Klebsiella pneumoniae* (7.0 ±0 mm), which have shown resistancy against various antibiotics, cefuroxime (30 µg), cephalothin (30 µg) and oxacillin (1 g) [11]. This selective antibacterial activity of PBS extracts would be contributed by the flavonoids content as evident in previous study [12].

Cytotoxic Assay

Both PBS extracts were active against human non-small cell lung carcinoma cell line (H1299) and human breast adenocarcinoma cell line (MCF-7) compared to the inactive aqueous extracts (as shown in Table 1). It is interesting to note that the PBS extracts are more active towards the p53 deficient human cell line H1299 than the estrogen dependent MCF-7 cell lines. These two carcinoma cell lines, H1299 and MCF-7 existed in an opposite nature, one through signaling pathway and the other by hormonal influential, respectively. The PBS pulp extract was active against H1299 (IC_{50} = 42.0 mg/mL) and against MCF-7 (IC_{50} = 125.0 mg/mL) when compared to the PBS peel

extract against H1299 (IC_{50} = 52.0 mg/mL) and against MCF-7 (IC_{50} = 290.0 mg/mL). This pattern of activity suggested that the content of alkaloids and saponins played an important role as chemotherapeutic agents to stimulate apoptotic process in tumor cells as they are only present in the PBS extracts [13]. Another contributing property of alkaloids involved the free radical scavenging effects. These dietary flavonoids from cucumber are required to synergize with antioxidants for optimal cellular functions. It can be deducted that antioxidants and radical scavenging components of *C. sativus* fruit extract can easily cross the cell membrane and cope with the intracellular ROS formation [14].

Conclusion

Generally, *C. sativus* phosphate buffer solution (PBS) extracts exhibited more significant antibacterial activity and cytotoxic activity compared to the aqueous extracts. The PBS extracts showed selective activity when assayed against two distinctive cancer cell lines, the p53 deficient human non-small cell lung carcinoma cell line (H1299) and the estrogen dependent human breast adenocarcinoma cell line (MCF-7). It was found that the PBS pulp and peel were significantly active against H1299 with IC_{50} of 42.0 and 52.0 mg/mL, respectively. The peels and pulp of PBS extracts also demonstrated antibacterial activity specifically against gram positive, *S. aureus* and gram negative, *K. pneumonia*, respectively. It is worthy to note that the powder form of *C. sativus* extracts has been successfully attained from the spray dry method possessed phytochemicals that displayed potential cytotoxic effects on selected human cancer cell lines. The biological properties exhibited by the extracts were further supported by the presence of the phytochemicals such as flavonoids, saponins, alkaloids and steroids.

Acknowledgement

The authors would like to acknowledge Kulliyyah of Science, IIUM and Kulliyyah of Allied Health Science, IIUM for financial and facilities support.

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