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Research Article

IN VITRO ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF *CUCUMIS SATIVUS* L. PEEL EXTRACTS

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ABSTRACT

The present study evaluated the phytochemicals, antioxidant and antimicrobial potential; and thin layer chromatographic studies of *Cucumis sativus* L. peel extracts. The total flavonoid content was assessed using Aluminium Chloride reagent method. The antioxidant potential was evaluated using *in vitro* assays *viz* DPPH radical scavenging assay, FRAP and Phosphomolybdenum assay. The antimicrobial activity was determined against *Shigella flexneri, E coli, Staphylococcus aureus* and *Klebsiella pneumonia* using Agar Well Diffusion method. Qualitative phytochemical analysis confirmed the presence of alkaloids, saponins, diterpenes, steroids and flavonoids. Total Flavonoid content in the *Cucumis sativus* L. peel was 3.50 µg/mg QE. *Cucumis sativus* L. peel showed highest radical scavenging activity of 71% at the concentration of 600 µg/mL. In determination of antioxidant activity via FRAP and Phosphomolybdenum assay cucumber peel extract showed highest absorbance of 0.80 and 0.94 respectively at the concentration of 300 µg/mL. The peel extracts inhibited the growth of all the tested pathogens by forming inhibition zones ranging from 11-21 mm. Thin layer chromatographic studies of the cucumber peel extracts constituted different coloured phytochemical compounds with different R_f values. This study highlights the pharmacological properties of *Cucumis sativus* L. peel.

Keywords: *Cucumis sativus* L. peel, flavonoids, Antimicrobial, Antioxidant

INTRODUCTION

Cucumber (*Cucumis sativus* L.) belongs to Cucurbitaceae family such as melon, watermelon, pumpkin and zucchini. It is widely consumed fresh in salads or fermented (pickles) or as a cooked vegetable[1]. They are widely used for various skin problems including swelling under the eyes and sunburn. It is believed that they promote refreshing, cooling, healing, soothing, emollient and anti-itching effect to irritated skin[2]. The nutrient profile of *Cucumis sativus* L. includes water (96.4%), protein (0.4%), fat (0.1%), carbohydrate (2.8%), mineral (0.3%), calcium (0.01%), phosphorus (0.03%), iron (1.5 mg/100 g) and vitamin B (30 IU/100 g). Ascorbic acid and Enzyme such as crepsin, proteolytic enzyme, oxidase, succinic, malic dehydrogenase have also been reported in the fruits[3]. The bioactive compounds isolated from cucumber includes cucurbitacins, cucumegastigmanes I and II, cucumerin A and B, vitexin, orientin, isoscoparin 2″-O-(6‴-(E)-p-coumaroyl) glucoside, apigenin 7-O-(6″-O-p-coumaroylglucoside)[1,5].

Cucumber exhibits wide range of *in vitro* and *in vivo* pharmacological effects. Cucumber extract showed antioxidant activities against various assays including DPPH, reduction assay, total oxyradical scavenging capacity (TOSC) assay, trolox equivalent antioxidant capacity (TEAC), total radical-trapping antioxidant parameter (TRAP) or ferric reducing-antioxidant power (FRAP) assays[6,7,8,9]. *Cucumis sativus* L. fruit and seed extracts are reported to have antibacterial and antifungal activity[10, 11]. *Cucumis satvis* L. showed cytotoxic activity against human cancer cell lines[12]. The fruit and peel of cucumber are shown to have antidiabetic and hypocholesterolemic activity[13, 14]. Dixit and Kar[15] studied the glucose regulating role of *Cucurbita pepo, Cucumis sativus* and *Praecitrullus fistulosus* peel extracts in mice. The study reported that all the three peel extracts nearly reversed most of the changes induced by alloxan suggesting their possible role in ameliorating diabetes mellitus and related changes in serum lipids. Gill et al[16] reported that the cucumber seed possess significant antiulcer potential owing to its antioxidant activity.

The pharmacological properties of *Cucumis sativus* L. fruit and seed have been extensively studied, but comparatively very few studies have been reported on the therapeutic potential of *Cucumis sativus* L. peel. Moreover, the plethora of waste generated from food industry; recent interest in reaping bioactive compounds from the waste generated from fruit and vegetable; and consumers increasing demand for natural health benefitting products are the rationale behind the present study. Therefore, the present study aimed to identify the phytochemicals; and to evaluate the antioxidant and antimicrobial activity of *Cucumis sativus* L. peel.

MATERIALS AND METHOD

Preparation of peel extracts

Fresh mature cucumbers were purchased from the local market. The cucumbers were washed thoroughly under running tap water and were manually peeled using a sterilized peeler. The peels were then shade dried at room temperature for 5 days. The shade dried cucumber peels were powdered in a laboratory blender and was kept in airtight bottles until further use. The powdered cucumber peel was soaked in methanol and chloroform for 72 h by maceration technique. The supernatant was filtered through

Whatman No.1 filter paper and concentrated using rotary evaporator and dry residue was preserved at 5°C in airtight bottles until further use.

Qualitative phytochemical screening

The qualitative phytochemical tests were performed to screen the presence of bioactive components in methanol and chloroform extract of *Cucumis sativus* L. peel. The screening was performed for triterpenes/steroids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and phenolic acids according to standard method[17-21].

Estimation of total Flavonoid content by $AlCl_3$ reagent method

The total flavonoid content of peel extracts of *Cucumis sativus* L. was determined by the $AlCl_3$ reagent method[22]. The extract (500µg/mL) was mixed with 0.5 mL of 5% $NaNO_2$ solution and allowed to stand for 5 mins. Then 0.3 mL of 10% $AlCl_3$ solution was added and the mixture was allowed to stand for further 5 min. Finally, 1 mL of 1 M NaOH solution was added, and the final volume of the mixture was brought to 5 mL with distilled water. The mixture was incubated for 15 mins at room temperature and absorbance was measured at 510 nm. The total flavonoid content was expressed as quercetin equivalent (µg/mg of extract), which is a common reference standard.

Antioxidant activity

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay

The DPPH radical scavenging activity was carried out according to the method described by Blois[23]. One mL of the peel extracts was taken in of various concentrations and mixed with 1 mL of 0.1 mM of DPPH solution in methanol. The reaction mixture was kept at room temperature for 30 min. Absorbance was read at 517 nm in spectrophotometer. The percentage of the radical scavenging activity was calculated as follows.

$$\% \text{ of inhibition} = \text{Control} - \text{Sample} / \text{Control} \times 100$$

FRAP (Ferric Reducing Antioxidant Power) assay

The Fe^{3+}reducing power assay was done according to the method described by Yen and Chen[24]. 10 mg of the *Cucumis sativus* L. peel (methanol and chloroform extract) was taken in different concentrations and were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide (1%).The mixture was incubated at 50ºC for 20 min. 1 mL of 10% trichloroacetic acid was added to the mixture. Then 1 mL of 0.1% of freshly prepared ferric chloride was added and the absorbance of the resultant solution was measured at 700 nm.

Phosphomolybdenum reduction assay

The antioxidant activity was evaluated by reduction assay method by the formation of green phosphomolybdenum complex[25]. 1 mL of various concentrations of the *Cucumis sativus* L. peel extracts (methanol and chloroform extract) were combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. The samples were cooled to room temperature and the absorbance of the mixture was measured at 695 nm against blank.

Antibacterial activity

The antibacterial activity of the *Cucumis sativus* L. peel methanol and chloroform extract was analysed by well diffusion method[26]. Muller Hinton agar was prepared according to the standard procedure and 25 mL was poured into the plates and was allowed to solidify. The standard inoculum suspension was streaked over the surface of the media using sterile cotton swab to ensure the confluent growth of the organism and the plates were allowed to dry for 5 minutes. After drying, the different concentrations (50, 75 and 100 µg/mL) of the methanol and acetone extract of cucumber peel were poured into the wells. Tetracycline was used as a standard (1 µg/mL) and methanol was used as control. Finally, the inoculated plates were incubated for 24 hours at 37ºC for bacteria. The zone of inhibition was measured and noted.

Thin Layer Chromatography

The thin layer chromatography method was performed for the detection of active compounds, based on the results (Retention factor) R_f value of the separated compounds can be calculated easier. Thin layer chromatography (TLC) was performed on pre-coated 20cm × 20cm and 0.25mm thickness. Aliquots of the extract were spotted on the TLC plates which was 0.2 mm above from the bottom with the help of a capillary tube. Then the sample spotted sheets were placed in the respective solvent system adopted as mobile phase. The solvent system used for methanol extract was toluene: ethyl acetate: methanol in the ratio 1:0.8:0.2. The solvent system used for chloroform extract was toluene: ethyl acetate in the ratio 1.5:0.5. The spots in the chromatogram were well observed.

RESULTS

Phytochemical Screening and Flavonoid estimation

The result of the phytochemical analysis showed that both the *Cucumis sativus* L. peel extracts contained alkaloids, saponins, diterpenes, and flavonoids. While steroids were present only in methanol extract; glycoside was present only in chloroform extract. The flavonoid content of *Cucumis sativus* L. peel methanol extract was higher, i.e. 3.50µg/mg QE than chloroform extract (0.88 µg/mg QE).

Antioxidant Activity

The antioxidant activity of the *Cucumis sativus* L. peel extracts were investigated using *in vitro* assays namely DPPH Assay, FRAP Assay and Phosphomolybdenum Assay.

DPPH Assay

DPPH is a stable free radical, which has been widely used in phytomedicine for the assessment of scavenging activities of bioactive fractions. The results of DPPH assay for *Cucumis sativus* L. peel is presented in Table 1 and 2. The *Cucumis sativus* L. peel chloroform extract showed better radical scavenging activity than methanol extract. IC_{50} value with 381.6 µg/mL and 329 µg/mL were observed in methanol and chloroform extract respectively.

The Rf value was calculated using the following formula
Rf = Distance travelled by the solute / Distance travelled by the solvent

FRAP Assay

Ferric ion reducing power assay measures the electron donating capacity of an antioxidant. The result of FRAP assay is presented in Table.3. The absorbance at 300 µg/mL concentration was 0.80 and 0.30 for *Cucumis sativus* L. peel methanol and chloroform extract respectively. An increased absorbance is indicative of higher reducing power; therefore, it is clear that methanol extract had higher reducing power than chloroform extract.

Phosphomolybdenum assay

The result of Phosphomolybdenum assay is presented in Table 4. From the Table, it can be inferred that chloroform extract had greater activity (0.94) for phosphomolybdenum assay than the Methanol extract of *Cucumis sativus* L. peel. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH[25].

Antimicrobial activity

The result of antimicrobial activity of *Cucumis sativus* L. peel extracts are presented in table.5. Both the extracts had good inhibitory activity against all the tested organisms *viz Shigella flexneri, E coli, Staphylococcus aureus* and *Klebsiella pneumonia*. The methanol extract showed inhibited diameter zones (IDZ) ranging from 11-21 mm, with highest zone of inhibition exhibited by *Staphylococcus aureus*. IDZ for chloroform extract were in the range 12-17 mm.

Thin Layer Chromatography

The number of spots observed and their corresponding R_f values are presented in table 6 and 7. *Cucumis sativus* L. peel Methanol extract yielded four spots, while nine spots were identified for chloroform extracts. Data obtained from TLC analysis helps in selection of appropriate solvent system for separation of pure compounds by column chromatography[27].

Table 1: DPPH activity of Methanolic extract of *Cucumis sativus* L. peel

Sl.No	Concentration (µg/mL)	% of Inhibition
1	50	13.43
2	100	19.65
3	150	21.14
4	200	23.7
5	250	28.1
6	300	39.3

Table 2: DPPH activity of chloroform Extract of *Cucumis sativus* L. peel

Sl.No	Concentration (µg/mL)	% of Inhibition
1	100	21.32
2	200	33.19
3	300	45.49
4	400	62.95
5	500	67.8
6	600	71.3

Table 3: FRAP assay of Methanolic and Chloroform extract of *Cucumis sativus* L. peel

Sl.No	Concentration (µg/mL)	Absorbance at 700 nm	
		Methanol	Chloroform
1	50	0.67	0.02
2	100	0.72	0.10
3	150	0.73	0.14
4	200	0.75	0.25
5	250	0.76	0.26
6	300	0.80	0.30

Table 4: Phosphomolybdenum assay of Methanol and Chloroform extract of *Cucumis sativus* L. peel

Sl. No	Concentration (µg/mL)	Absorbance at 695 nm	
		Methanol	Chloroform
1	50	0.69	0.51
2	100	0.73	0.86
3	150	0.83	0.88
4	200	0.85	0.92
5	250	0.88	0.93
6	300	0.91	0.94

Table 5: Antimicrobial activity of Methanol and Chloroform extract of *Cucumis sativus* L. peel

S.No	*Cucumis sativus* L. peel Extract	Organisms	Zone of Inhibition (mm)			
			Standard	50 µL	75 µL	100 µL
1	Methanol	*Shigella flexneri*	27	12	13	15
2		*E coli*	29	11	16	18
3		*Staphylococcus aureus*	28	18	19	21
4		*Klebsiella pneumonia*	26	16	18	19
1	Chloroform	*Shigella flexneri*	28	12	13	16
2		*E coli*	29	14	15	16
3		*Staphylococcus aureus*	28	13	16	17
4		*Klebsiella pneumonia*	27	12	13	15

Table 6: R_f value of *Cucumis sativus* L. peel methanol extract

Compounds observed under UV @ 235nm	R_f value
1	0.76
2	0.63
3	0.52
4	0.47

Table 7: R_f value of *Cucumis sativus* L. peel chloroform extract

Compounds observed under UV @ 235nm	R_f value
1	3.7
2	3.3
3	2.7
4	2.2
5	1.6
6	1.1
7	0.8
8	0.4
9	0.3

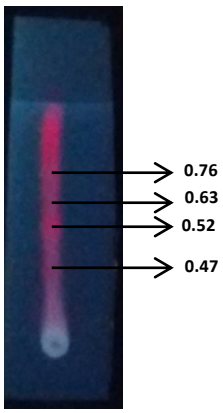

Figure 1: TLC of methanol extract of *Cucumis sativus* L. peel

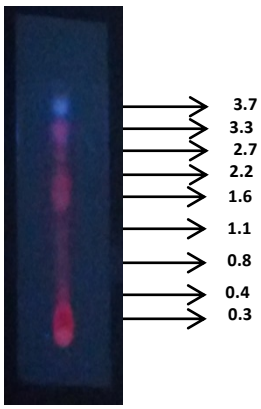

Figure 2: TLC of chloroform extract of *Cucumis sativus* L. peel

DISCUSSION

The qualitative phytochemical analysis revealed the presence of bioactive compounds in cucumber peel. In a study conducted by Foong et al.,[12] on cucumber peel phosphate buffer saline extracts the presence of alkaloids, saponins, steroid and flavonoids was confirmed, with the exception of diterpenes and glycoside shown in the present study. This difference in result could be due to the different solvent used. The cucumber peel has considerable amount of flavonoids and can be considered as a cheap source of flavonoids. Flavonoids have been reported to exert multiple biological property including antimicrobial, antioxidant, cytotoxicity, anti-inflammatory as well as antitumor activities[28]. Although cucumber peel extracts exhibited poor free radical scavenging potential, the peel had good reducing power. The reductive capacity of a compound depends on the presence of reductones, which exhibit antioxidative potential by breaking the free radical chain and donating a hydrogen atom[29]. The results obtained for FRAP assay confirms the presence of antioxidant reductones in cucumber peel. The result obtained for antimicrobial activity in the present study was contrary to the study conducted by Foong et al[12]., 2015, where phosphate buffer saline cucumber peel extracts was only active against *Staphylococcus aureus* (inhibition zone of 7.0±0 mm). Therefore, from the present study it can be concluded that cucumber peel possesses bioactive compounds; and has good antioxidant and antimicrobial activity.

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