Journal of King Saud University – Science 34 (2022) 102216

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Phytochemical screening and characterization of the antioxidant, anti-proliferative and antibacterial effects of different extracts of *Opuntia ficus-indica* peel

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ARTICLE INFO

Article history:
Received 5 June 2022
Revised 18 June 2022
Accepted 29 June 2022
Available online 15 July 2022

Keywords:
Opuntia ficus-indica
Total phenols
Total flavonoids
MTT
Antioxidant
Antitumor
Antibacterial & Antiviral

ABSTRACT

Objectives: *Opuntia ficus-indica* peels need further investigations as they could contain multiple active molecules. Bioactive secondary metabolites are important for health maintenance and play a significant role in treating some common diseases. Hence, the present study analyzed the chemical composition and biological activities of the plant extract using in vitro models.
Methods: The antioxidant assays were estimated in terms of DPPH radical scavenging; the chemical characterization is done in terms of total polyphenol and flavonoids, as well as HPLC characterization. The SRB assay was used for anticancer analysis whereas disc diffusion and minimum inhibitory concentration was determined to assess antimicrobial activity.
Results: The content of total polyphenol (TPC) and flavonoids (TFC) differed significantly in *Opuntia ficus-indica* peel cyclohexanone extract (OFICE), *Opuntia ficus-indica* peel 70 % ethanol in water extract (OFI70EE), and *Opuntia ficus-indica* peel 70 % ethanol in water extract (OFI100EE)), with varying values of TPC and TFC. In HPLC analysis, there observed the presence of 8 phenolic and flavonoids compounds. Antioxidant analysis showed that OFICE has higher antioxidant activity. Bioassay based on MCF-7 showed that *O. ficus-indica* extracts significantly ($p < 0.05$) reduced cell viability of cancer cells at 400 µg/mL after 72 h of incubation time. Concerning the antibacterial activity, the cyclohexanone extract gave the highest antibacterial inhibition as compared to the other extracts, and the highest activity was recorded against *Listeria monocytogenes*. Also, the *Opuntia ficus-indica* peel extracts showed potential antiviral activity with the mean inhibition rates (39, 19, and 67.66 %) and (35, 16.66, and 54.66 %) against H5N1 and rotavirus respectively.
Conclusion: Overall, the study confirms the antioxidant, antimicrobial and anticancer properties of the extract and further studies in animal models are necessary to validate their pharmacological applications.

1. Introduction

Plant-derived natural chemicals play an important role in disease prevention and health benefits (Kesba and El-Beltagi, 2012; Yabalak et al., 2020b). Among, these the major ones include functional foods and nutraceutical compounds (Indu et al., 2021; Narayanankutty, 2021a). There is a high demand and need for novel antimicrobial and anticancer agents; the emerging drug resistance and chemotherapy resistance in cancer cells and microbials strains is the major reason for such increased demand for

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.102216
1018-3647/

novel drug candidates (Aslam et al., 2018; Housman et al., 2014). Various natural products and plant extracts are promising drug candidates against bacterial communities and virus (Sagaya Jansi et al., 2021; Narayanankutty et al., 2021a). Similarly, natural products are important sources of anticancer agents; several natural products including curcumin, turmeron, resveratrol, and sulforaphane (Narayanankutty, 2020). Apart from these numerous plants and extracts are emerging as drug candidates from various parts of the world.

Cactaceae plants and fruits contain glycosylated flavonols, dihydroflavonols, flavanones, and flavanols (Kuti, 1992). Many studies have found a strong connection between the phenol content of extractable polyphenol extracts from Opuntia spp. and their antibacterial and antioxidant properties (Anwar and Sallam, 2016). Furthermore, the majority of researchers have found that the extraction solvents and processing procedures utilized can alter the biological activity, yield, and phenolic component profile of the extracts examined (Abou-Elella and Ali, 2014). The prickly pear or nopal cactus Opuntia ficus-indica (L.) Mill., belongs to the dicotyledonous angiosperm Cactaceae family (Chougui et al., 2013). Opuntia fruits have a great medical potential and include several bioactive chemicals such as phenolic compounds and flavonoids, which are antioxidant compounds (Chahdoura et al., 2015). Opuntia ficus-indica (L.) has a chemical composition of (12–15 % sugars, 0.6 % protein, and 0.1 % lipids; calcium 2200 ppm, potassium 490 ppm, and magnesium 850 ppm) (Kim et al., 2006). Prickly pear cactus peels are produced in Egypt in about 58,344 tons per year (Benattia et al., 2019). We can extract a large number of bioactive chemicals from PPCP, which accounts for 36–48 % of the entire fruit and is regarded a plant waste. Furthermore, phenolic substances, flavonoids such as kaempferol and quercetin, which have antioxidant capabilities, are found in the prickly pear (Saxena et al., 2013).

Hence, the present study analyzed the phytochemical composition of *Opuntia ficus-indica* using different chromatographic techniques and the antioxidant, antimicrobial, anticancer, and antiviral activities of different extracts from the plant is also analyzed.

2. Materials and methods

2.1. Plant materials

Prickly pear fruits were obtained from the Department of Tropical Fruits, Agricultural Research Center, Giza, Egypt, in May 2019, Fig. 1. Peels were separated, washed, and dried at room temperature and ground into fine powder for extraction.

2.2. Preparation of Opuntia ficus-indica extracts

The peels of ripe prickly pear fruits were chopped into little pieces and dried at 80 °C overnight. The dry pieces were milled as a mechanical preparation and stored in the freezer until they were needed. The dried materials were extracted progressively with 500 ml of cyclohexanone, 70 %, and 100 % ethanol. At 45 °C, each fraction was evaporated separately using a rotating vacuum evaporator. The extracts were stored at −25 °C in light-protected containers until they were used.

2.3. Phytochemical analysis

2.3.1. Estimation of total polyphenol content (TPC)

The total polyphenol content of the extract was determined using the reaction with Folin-Ciocalteu reagent using the gallic acid as internal standard. The TPC of samples were expressed in mg gallic acid equivalence (GAE) according to the standard methods described previously (Kim et al., 2022).

2.3.2. Estimation of total flavonoid content (TFC)

The flavonoid content was quantitatively estimated in terms of the rutin equivalent and expressed as mg rutin equivalent (RE) (Anusmitha et al., 2021). The reaction was based on the spectrophotometric changes induced by the $AlCl_3$ with flavonoids in the extract.

2.4. Analysis of phenols by HPLC

The phytochemical composition of the extracts was analyzed using HPLC method using appropriate standards. The column, solvent system and chromatographic conditions were set according to the methods described by Skendi et al. (2017). Individual compound identities were validated by comparing the retention times of corresponding standards to the peaks in *Opuntia ficus-indica* extracts.

2.5. Antioxidant activity: DPPH radical scavenging activity assay

The antioxidant activity was measured by DPPH radical scavenging activity (Anusmitha et al., 2021; Yabalak et al., 2020a). The stock solution was prepared using 10 mg / 1 ml DMSO. Serial dilutions for each extract were prepared. Readings were recorded at λ = 540 nm. The percentage of DPPH inhibition = [1-(A sample -A background)/ (A DMSO -A background)] X 100.A calibration curve was constructed using the inhibition rate values of the standard Trolox solution.

2.6. In vitro antitumor activity: Analysis of cell viability by SRB assay

Antitumor activity was performed by SRB assay is described as the preferred method according to quantitative estimation of the number of cell viability in a culture protocol (Kim et al., 2022; Koottasseri et al., 2021). MCF-7 was purchased from the NCI, Cairo, Egypt.

Fig. 1. Prickly pear (*Opuntia ficus-indica*).

2.7. Antimicrobial activity assay

2.7.1. Test bacteria

Clinical isolates of Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus,* and *Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli, Salmonella typhi,* and *Shigella boydii*) were cultured in nutritional broth at 37 °C for 24 h before being tested for antibacterial activity.

The diameters of the inhibition zones were measured in millimeters for determining the antibacterial activity of the investigated substances using a modified Kirby-Bauer disc diffusion method (Narayanankutty et al., 2021a; Narayanankutty et al., 2021b). Filter discs impregnated with 10 > l of solvent (cyclohexanone, 100 % ethanol in water, and 70 % ethanol in water) served as a negative control for antimicrobial activity, while standard discs of Gentamycin (antibacterial agent) served as a positive control.

2.7.2. Determination of MICs

In antimicrobial susceptibility testing, the *Opuntia ficus-indica* extracts demonstrated substantial antibacterial activity. The condensed extract was used to make a stock solution of 10 mg/mL using dimethyl sulfoxide (DMSO) as solvent. This was serially diluted to obtain various concentrations ranging from 3.12 mg/mL to 50 mg/mL.

2.8. Antiviral assay

2.8.1. Virus stocks

Both characterized Avian Influenza Virus and Rotavirus were kindly provided by the Center of Excellence for Influenza viruses, National Research Centre, Egypt.

2.8.2. Determination of cytotoxicity of Opuntia ficus-indica extracts by MTT assay

The *O. ficus-indica* extracts were tenfold serially diluted with Dulbecco's modified eagle medium (DMEM). The cytotoxicity effect of the three *O. ficus-indica* extracts was determined in MDCK and African Rhesus monkey kidney MA-104 cell line for H5N1 and Rotavirus respectively, (using MTT assay), according to the previous methods (Anusmitha et al., 2021). The percentage of cytotoxicity compared to the untreated control cells was determined using the following equation:

Cytotoxicity (%) = (Absorbance of cells without treatment – Absorbance of cells with treatment) × 100/ Absorbance of cells without treatment. % Cytotoxicity versus sample concentration was used to estimate the concentration which exhibited 50 % cytotoxicity (IC_{50}).

2.8.3. Plaque titration assay for viruses

The Plaque titration assay was implemented for counting the plaque-forming units (PFUs), as previously described (Tobita, 1975). The virus titer was calculated through the following equation according to:

Plaque forming unit (PFU)/ml = No. of plaques × Reciprocal virus dilution × multiplicand number to complete the volume of the inoculums to 1 ml.

2.8.4. Antiviral activity for O. ficus-indica extracts

The plaque reduction assay was done according to (Hayden et al., 1980) in a six-well plate, where MDCK cells and MA-104 cell line for H5N1 and Rotavirus respectively with 10^5 cells/ mL. The plaques were counted and the reduction percentage in plaques formation in contrast to the control wells was recorded as the following equation:

$$\%\,\text{Inhibition} = \frac{\text{Viral count (untreated)} - \text{Viral count (treated)}}{\text{Viral count (untreated}} x10$$

2.9. Statistical analysis

All experiments and analyses were carried out in triplicate, with mean values and standard deviations calculated accordingly. The differences between them and values of multiple groups were analyzed by IBM® SPSS® Statistics software, significance was defined at $P \leq 0.05$ (IBM® SPSS®, 2011).

3. Results

3.1. Total phenolic acid and flavonoid contents

The amount of total phenolic acids and flavonoids in *Opuntia ficus-indica* peel cyclohexanone extract (OFICE), *Opuntia ficus-indica* peel 70 % ethanol extract (OFI70EE), and *Opuntia ficus-indica* peel 100 % ethanol (OFI100EE) differed significantly (Table 1). The amounts of phenolic acids in the dry weight of plant material was 51.11, 40.49, and 28.27 mg GAE/100 g, respectively as measured by the Folin-Ciocalteau method. The aluminium chloride ($AlCl_3$) method was used to determine the flavonoid concentration in the extracts, and the levels were 2.24, 0.10, and 1.30 mg RE/100 g dry weight.

3.2. HPLC analysis

Data in Figs. 2 and 3 showed that OFICE, OFI70EE, and OFI100EE contain 8 phenolic and flavonoids compounds. From the results obtained, it is proved that the best solvent for extraction is OFICE, OFI70EE, and OFI100EE, respectively. The main constituent is catechin followed by gallic acid, caffeic acid, chlorogenic acid, and ellagic acid (phenolic acids). In addition, *O. ficus-indica* extracts contain many flavonoids compounds such as quercetin, kaempferol, and rutin. The *O. ficus-indica* extracts contain high concentrations of quercetin. Peel the prickly pear extract contains the main constituent being ferulic acid (34.8 %), caffeic acid (2.6 %) and vanillic acid occurring in small quantities (0.1 %).

3.3. Antioxidant activity

Antioxidant activity for OFICE, OFI70EE and OFI100EE was evaluated at two doses 10 and 100 μg/ml. The results showed that OFICE has higher antioxidant activity > 50 % inhibition at both doses than OFI70EE, and OFI100EE, which presented a high positive correlation ($p < 0.05$) of DPPH and IC_{50} was 843.2 ± 11.37 μg/ml. Data obtained for antioxidant activities of OFI70EE and OFI100EE reveals that both extracts have approximately the same value for antioxidant activity < 50 % inhibition at two doses 10 and

Table 1
The total polyphenol content (TPC) and total flavonoid content (TFC) of different extracts of *Opuntia ficus-indica* peel.

O. ficus-indica extracts	TPC (mg/g)	TFC (mg/g)
OFICE	51.11 ± 2.73[a,b]	2.23 ± 0.260 [a,b,c]
OFI70EE	28.27 ± 0.85	0.10 ± 0.062
OFI100EE	40.49 ± 2.50 [a]	1.30 ± 0.23 [a]

*TPC expressed in mg GAE/100 g dry weight of extract; TFC expressed in mg RE/100 g dry weight of extract; Each value is the mean ± SD of triplicate measurements. The data are presented as the mean ± SD of technical replicates (n = 9). a- indicate significant difference with OFI70EE, b-indicate significant difference between OFI100EE.

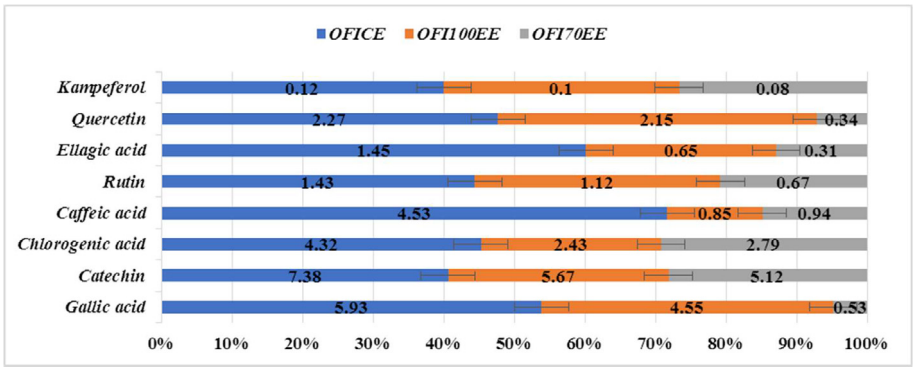

Fig. 2. The Phenolic acids and flavonoids in *O. ficus-indica* extracts by HPLC analysis.

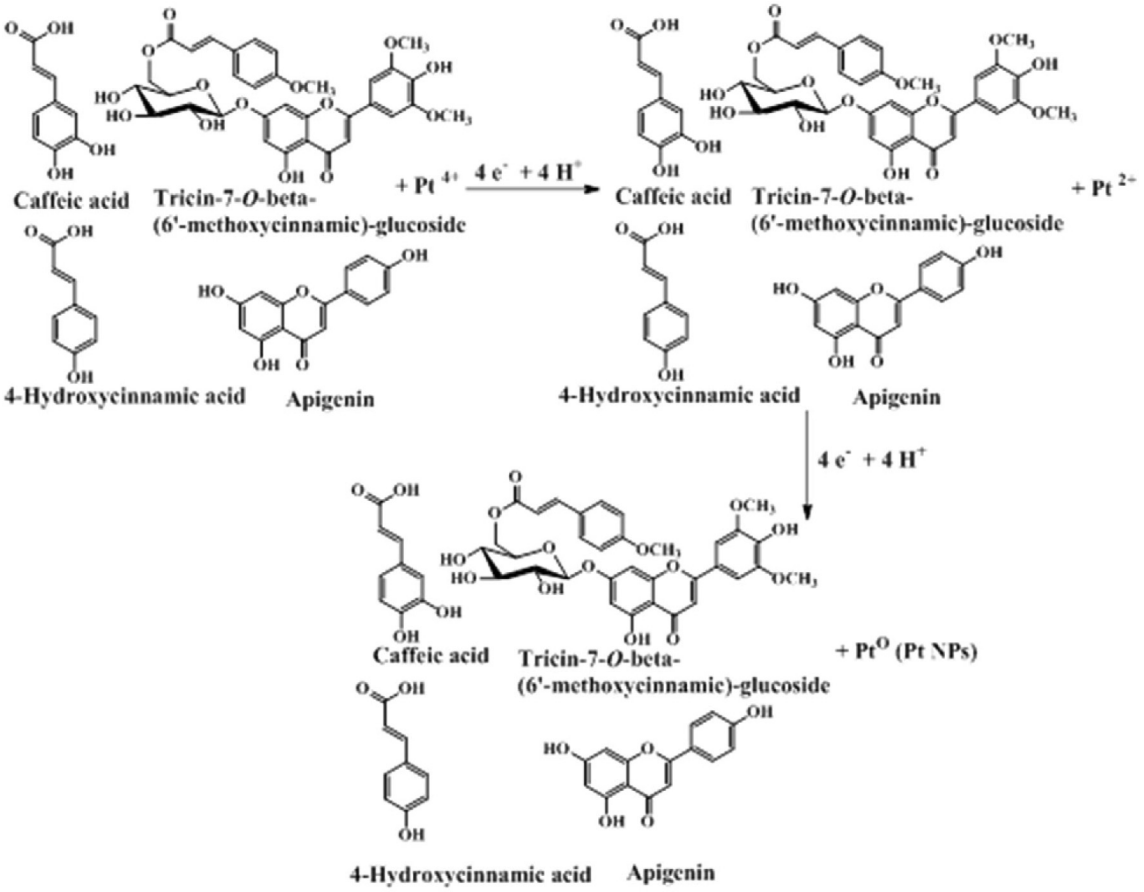

Fig. 3. Bioactive compounds of OFIPs involve simultaneously chemical reduction of Pt NPs and the antioxidant role for these compounds (Ishak et al., 2020).

100 μg/ml. On the other hand, IC_{50} were 346.9 ± 8.97 and 551.7 ± 14.66 μg/ml for OFI70EE and OFI100EE, respectively.

3.4. In vitro antitumor activity by SRB assay

The assay is based on mammalian cells involving antioxidant and upregulation of some cellular self-defense mechanisms, which are related to prevention as well as treatment of cancer. The cytotoxicity of different concentrations (1, 10, 100, 200, 300, 400 μg/ml) for OFICE, OFI70EE, and OFI100EE of OFIPs, against MCF-7 cell line detected by SRB assay. Examination of the cultures under a phase-contrast inverted microscope showed significant effects on cell viability depending on concentrations of *O. ficus-indica* extracts.

A high dose of OFICE, OFI70EE, and OFI100EE highly significantly ($p < 0.05$) reduced cell viability of MCF-7 cancer cells at concentration 400 μg/ml after 72 h. incubation time. Thus, a high dose was the best compared to other concentrations, where it's the percent of cell viability were 47 %, 66 %, and 73. Moreover, the IC_{50} was 1506 μg/mL, 1972.7 μg/mL, and 2236 μg/mL for OFICE, OFI70EE, and OFI100EE, respectively. In other words, an increasing concentration decreased cell viability for cancer cells. Showing all treated tumor cells were inhibited with all tested concentrations beginning from 1 to 400 μg/ml. Respecting the effect of three *O. ficus-indica* extracts on cells exhibits dose-dependent toxicity. At low dose 1 μg/ml OFICE, OFI70EE and, OFI100EE, the cells viability of cancer cell was 91 %, 97 % & 99 %, respectively. Moreover, the treated cancer cell at 10, 100, 200 and 300 μg/ml of OFICE gives 87 %, 72 %, 68 %,

53 %, respectively, whereas, OFI70EE and OFI100EE give 93 %, 96 %, 84 %, 90 %, 79 %, 85 %, 71 % and 78 % viability, respectively.

As over the above results of in vitro cytotoxicity of three *O. ficus-indica* extracts for biocompatibility evaluations on MCF-7 cell line after 72 h incubation, with different concentrations, ranging from 1 to 400 μg/ml, OFICE at different concentrations showed significantly reduced cell viability than the ethanol extracts and the former has a greater effect as antitumor.

3.5. Antibacterial activity

The intensive use of antibiotics is usually followed by the presence of resistant strains of microorganisms. The sight of the resistance of bacteria to drugs, the search for natural compounds having antibacterial activity is an urgent demand to deal with the harmful effects of those pathogenic microorganisms. Accordingly, during this study-three *O. ficus-indica* extracts i.e. OFICE, OFI70EE, and OFI100EE were tested against different microorganism's Gram-positive *B. cereus, L. monocytogenes* and *S. aureus*, and Gram-negative bacteria *E. coli, S. boydii,* and *S. typhi.*

The OFICE displayed that the highest antibacterial activity by preventing the growth of the tested isolates compared with the other extracts (OFI70EE and OFI100EE). The strongest antibacterial activity of the OFICE has recorded against *L. monocytogenes* strains with inhibition zone 34 mm, whereas the lower activities were against the *S. boydii* and *S. typhi* strains with inhibition zone 17 mm.

The antibacterial data are recorded (Table 2, 3) and illustrated in Fig. 4. The inhibitory effect of OFICE started at 6.25 mg/ml with inhibition zones of 6.4 mm against B. cereus, while extract concentration at 12.5 mg/ml suppressed *L. monocytogenes, S. aureus, E. coli, S. boydii,* and *S. typhi* growth at 12.5 mg/ml with inhibition zones of 8, 9, 9, 5 and 9 mm, respectively.

3.6. Cytotoxicity assays

In the obtained data the MDCK and MA-104 cell lines were treated with different concentrations of the three *O. ficus-indica* extracts for 24 h, and then the MTT compound was added on the next day to determine cell viability. Data revealed that all three *Opuntia ficus-indica* extracts showed no cytotoxicity on the tested cell lines on both H5N1 and Rotavirus. At least three different concentrations of each tested peel extract were prepared in triplicates. The calculated IC_{50} for each peel extract is presented in Table 1. The *Opuntia ficus-indica* extracts have a wide range of 50 % toxicity to the cell lines that ranged from 450 μg/μl to > 1000 μg/ μl and from 367 μg/μl to > 800 μg/ μl for H5N1 and Rotavirus respectively (Table 4).

3.7. Inhibitory potential of the Opuntia ficus-indica extracts acts against viruses

The antiviral activity of the three *Opuntia ficus-indica* extracts against rotavirus and H5N1 in vitro was investigated in this study.

Table 3
The inhibition zone (mm) of the different *Opuntia ficus-indica* extracts against different pathogenic bacteria.

Microorganisms	Cyclohexanone	Ethanol 90 %	Ethanol 70 %
Bacillus cereus	25	8	6
Listeria monocytogenes	34	6	6
Staphylococcus aureus	19	6	6
E. coli	24	6	6
Shigella boydii	17	7	6
Salmonella typhi	17	9	6

These findings (Table 5) demonstrated that all of the *Opuntia ficus-indica* extracts tested had potential action against rotavirus and H5N1 and should be favored for further research. Few investigations on the antiviral properties of our tested *Opuntia ficus-indica* extracts on animal and human viruses have been published, with the majority of studies focusing on plant viruses. The antiviral activity of cyclohexanone peel plant extract against rotaviruses and H5N1 has never been reported before. The plaque infectivity titer of H5N1 and Rotavirus was initially computed and proved to be 2107 and 2106 PFUs/ml for H5N1 and Rotavirus, respectively, according to the collected data. *Opuntia ficus-indica* extracts in various concentrations of 25 g/l, 50 g/l, and 100 g/l were produced and tested. The percentages of H5N1 and Rotavirus inhibition for each peel extract are shown on the graph (Table 5).

OFI70EE suppressed the H5N1 virus by 23, 41, and 53 %, respectively. For concentrations of 25 g/L, 50 g/L, and 100 g/L, respectively, OFI100EE inhibited the H5N1 virus by 7, 23, and 27 %, while OFICE inhibited the H5N1 virus by 53, 72, and 78 %. OFI70EE, on the other hand, inhibited rotavirus by 17, 39, and 49 %, respectively. For concentrations of 25 g/l, 50 g/l, and 100 g/l, OFI100EE inhibited rotavirus by 8, 21, and 21 %, whereas OFICE inhibited rotavirus by 47, 51, and 67 %. For H5N1 and rotavirus, the mean inhibition rates were 39, 19, 67.66 % and 35, 16.66, 54.66 %, respectively. The results showed that OFICE, at a concentration of 100 g/L, had the greatest inhibitory impact on H5N1 and rotavirus, followed by OFI70EE.

4. Discussion

Medicinal plants are important sources of various bioactive compounds and also the nutrient molecules for the well-being of various organisms (Narayanankutty, 2021b; Narayanankutty et al., 2021c). Plants are rich suppliers of various chemicals, including phenols, flavonoids, alkaloids, phenolic acids, tannins, saponins, anthocyanins, lignans, carbohydrates, carotenoids, and isoflavones, according to a previous study (Rasoulpour et al., 2020). Opuntia ficus-indica is one among the widely utilized plants in Arabian subcontinent. The present study analyzed the antioxidant, antimicrobial, and anticancer activities and phytochemical composition analysis of the plant. There observed high polyphenol content in the plant; corroborating with our results, previous studies also reported the total phenolic content of Opuntia ficus-indica

Table 2
Effect of *O. ficus-indica* extracts on the diameter IZ (mm) of pathogenic bacteria MICs.

Microorganisms	Concentrations (mg\mL)					
	100	50	25	12.5	6.25	3.12
0	*Bacillus cereus*	25	20	15	9	6.4
0	*Listeria monocytogenes*	35	30	20	8	0
0	*Staphylococcus aureus*	23	19	12	9	0
0	*E. coli*	24	19	17	9	0
0	*Shigella boydii*	17	9	6	5	0
0	*Salmonella typhi*	17	15	11	9	0

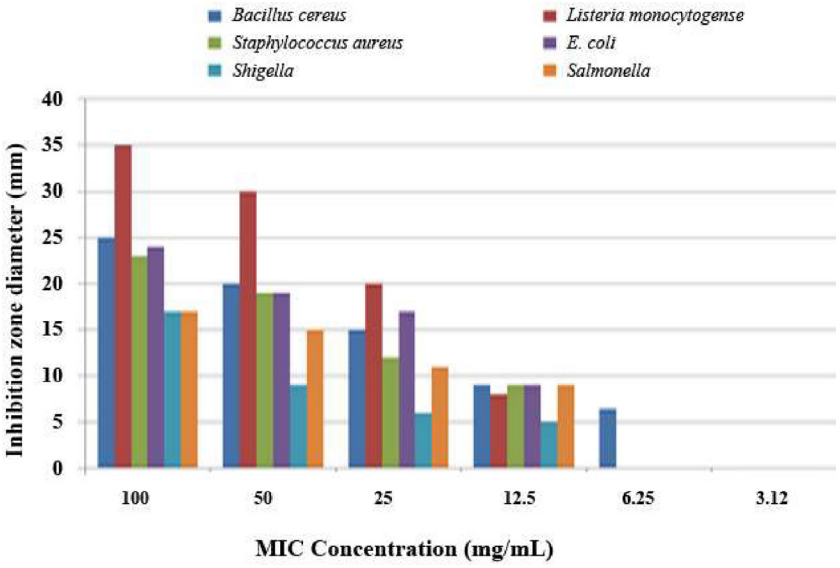

Fig. 4. Effective MICs of OFICE against different pathogenic bacteria.

Table 4
Cytotoxicity assay of *O. ficus-indica* extracts on the cell lines.

No.	Extracts	IC_{50} (µg/ µl) for H5N1	IC_{50} (µg/ µl) for Rotavirus
1	OFI70EE	630	700
2	OFI100EE	>1000	>800
3	OFICE	450	367

*IC_{50}: The concentration of *O. ficus-indica* extracts, which exhibited 50% cytotoxicity.

extracts varied from 221.3 to 1501.7 g GAE/100 g dry weight, while the phenolic compounds values of water extracts were 612.10 g GAE/ 100 g (Abou-Elella and Ali, 2014). In addition, the identified compounds such as quercetin, kaempferol, and rutin are reported to possess antiproliferative, anticarcinogenic, and antioxidant activities (Chougui et al., 2013; Illam et al., 2017; Job et al., 2021; Parathodi Illam et al., 2019).

Studies from different authors attributed the inhibitory effect of plant extracts against microbial pathogens to their phenolic composition (Fiad et al., 2020). The inhibitory effect of those phenolics might be explained by adsorption to cell membranes, interaction with enzymes, or deprivation of substrate and metal ions (Fiad et al., 2020; Illam et al., 2017). A great variation in MIC of OFICE as demonstrated in several investigations could be because of the variation in their method of extraction (Moosazadeh et al., 2014). The study observed significant antimicrobial properties of the plant against *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, E. coli, Shigella boydii,* and *Salmonella typhi*; it is therefore possible that the plant could offer strong defense against various bacterial diseases.

Flavonoids are also the most abundant phenolic chemicals in nature, with a wide range of actions, including antiviral activity (Ozçelik et al., 2011). The possible mechanism of antiviral effect could either the interference with viral adsorption or by the inhibition of viral replication. Human rotavirus is a potentially fatal virus that causes severe diarrhea in children under the age of five years, primarily in impoverished countries (Knipping et al., 2012). Influenza virus, particularly H5N1, is also regarded as a major global public health issue. The use of vaccines to combat rotavirus and H5N1 has some drawbacks because they are costly and may be out of reach for developing countries (Cecílio et al., 2012). As a result, new chemicals must be found to be used as alternative strategies to control H5N1 and rotavirus infection. In comparison to natural substances, either as pure compounds or as extracts obtained from plants utilized in conventional medicine, artificial antiviral agents were not superior. Many plants have a diverse range of biological activity, including antiviral properties (Palombo, 2006). To study the antiviral effect of three Opuntia ficus-indica extracts, the cytotoxicity of the tested Opuntia ficus-indica extracts should be determined.

Numerous plant extracts against rotavirus are reported as viral inhibitors (Chingwaru et al., 2011). By limiting viral adsorption, pre-treatment of the cells may provide protective benefits against some viruses (Schnitzler et al., 2008). It's also worth noting that flavonoids can prevent viruses from infecting cells by inhibiting a variety of pathways, similar to how DASM inhibits cell fusion.

Table 5
Inhibitory activity of *Opuntia ficus-indica* extracts against the H5N1 virus.

No.	Treatments	Conc. (µg/ µl)	(%) Viral inhibition		% Mean value of viral inhibition	
			H5N1	Rotavirus	H5N1	Rotavirus
1.	OFI70EE	32	23	17		
		8	41	39		
		4	53	49	39	35
2.	OFI100EE	32	7	8		
		8	23	21		
		4	27	21	19	16.66
3.	OFICE	32	53	47		
		8	72	51		
		4	78	66	67.66	54.66

Meanwhile, flavonoids have been shown to impede virus replication by blocking enzymes like reverse transcriptase and cellular DNA or RNA polymerase (Tang et al., 2012). Evan though many publications have focused on the identification of bioactive compounds, it is important to remember that plants are complex, and a single compound may not be responsible for the observed activity, but rather a combination of minor and major compounds interacting in a preservative or synergistic manner (van Vuuren, 2008). To summarize, *Opuntia ficus-indica* peel extracts s may have an inhibitory effect on H5N1 and rotavirus, but more research is needed to identify the most potent compounds, their mechanisms of action in animal models, and their application in vivo investigations.

Results of the anticancer activity were consistent with some studies, that flavonoids of OFI possessed many notable biological activities; such as inhibition of lipid peroxidation, oxidation of low-density lipoproteins, anti-carcinogenic activities (Park et al., 2007). The antioxidant effects are due to the major flavonoids encountered in OFI (Kuti, 1992) and flavonoids are more efficient antioxidants than vitamins and OFI have been shown to induce hyperpolarization of the plasma membrane and to raise the intracellular pool of calcium in human Jurkat *T*-cell lines (Aires et al., 2004), also, the highest anticancer activity of extract (Wu et al., 2004).

Overall, the present study confirms the antimicrobial, antioxidant and anticancer potential of *Opuntia ficus-indica*. However, further studies using animal models and clinical trials are necessary to validate the application of the plant and bioactive compounds in medicine.

5. Conclusions

The bioactive compounds were extracted from the prickly pear samples by using three solvents: cyclohexanone, 70 % ethanol and100% ethanol, and these compounds were identified by HPLC. These extracts showed antimicrobial effect against pathogenic virus and bacteria, which indicates the possible application as a antimicrobial agent. There also observed significant selective cytotoxicity towards the cancer cells; however, more studies using different cell lines are necessary to use it as an anticancer agent. Optimal exploitation of the prickly pear peel may thus yield various bioactive compounds which can exhibit antioxidant, antimicrobial and antiviral activity. However, further studies using other in vitro and in vivo model experiments are necessary to validate the results of the study and it is also recommended to conduct a thorough biosafety analysis using animal models.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors acknowledge the support from Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R15), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

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