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## Studies on bactericidal efficacy of pumpkin (*Cucurbita moschata* Duchesne) peel

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### PEER REVIEW

### ABSTRACT

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#### Comments

This is a type of exploratory study and a valuable research work in which authors have successfully tried to demonstrate the antibacterial characteristic of the peel of the *C. moschata in vitro*. The antimicrobial activity was evaluated by following disc diffusion method and MIC was determined by using 96-well microtiter plates.

Details on Page

**Objective:** To explore the *in vitro* antibacterial potential of the peel of *Cucurbita moschata* Duchesne (tropical pumpkin) (*C. moschata*) against human pathogenic bacteria.

**Methods:** In the present study, dichloromethane (DCM), methanol (MEOH) and aqueous extracts of *C. moschata* peel were examined for *in vitro* antibacterial potency against eight bacterial strains *i.e.* *Bacillus cereus*, *Burkholderia cepacia*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus* using Kirby–Bauer disk diffusion susceptibility and broth micro–dilution methods.

**Results:** DCM extract of pumpkin peel exhibited the maximum zone of inhibition against *Staphylococcus aureus* (21 mm) whereas aqueous extract of pumpkin peel revealed the least zone of inhibition against *Escherichia coli* (8 mm). MEOH extract gave maximum zone of inhibition against *Pseudomonas aeruginosa* (19 mm). Broth micro–dilution method showed minimum inhibitory concentration for the DCM extract against *Burkholderia cepacia* at 6.25 mg/mL. The minimum bactericidal concentrations were also determined to know the nature of all extracts. DCM and MEOH extracts exhibited bactericidal nature to all bacterial strains except for the *Vibrio alginolyticus*. The minimum bactericidal concentrations values exhibited bactericidal nature ranging from 3.12 mg/mL to 100.00 mg/mL. The screening of antimicrobial properties of different extracts of *C. moschata* peel revealed that the DCM extract possessed good antimicrobial efficacy compared to MEOH and aqueous extracts.

**Conclusions:** Peel of *C. moschata* possesses antibacterial compounds and could be potential source for a new class of antibiotics.

#### KEYWORDS

*Cucurbita moschata*, Pumpkin peel, Antibacterial activity, Pathogenic bacteria, Foodborne diseases

## 1. Introduction

In the present scenario, the issue of the food safety has become overwhelming to the worldwide. Food agencies and food processors are troubled with the elevated number of food–borne outbreaks and diseases associated with microorganisms, especially bacteria and fungi. A study

done by Cushnie and Lamb said that bacterial infections contributed largely to general health problems and were responsible for over 50% of deaths in developing countries[1]. Moreover, in America, more than 60% of the cases involved the strains that are resistant to at least one antibacterial agent. In Malaysia, the alarming concerns about foodborne diseases have increased 100% since the year 2006. In fact,

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out of 11 226 victims of food poisoning, more than 60% were school children<sup>[2]</sup>. The major factors that lead to foodborne diseases are due to contamination during preparation, transportation or serving. Most of the reported incidences of foodborne diseases are due to invading from the microbe such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Candida albicans*. Moreover, biotechnology products like genetically modified food also contribute a big 'share' to foodborne diseases. One of the applications from genetically modified food products is the use of antimicrobial agents to prolong product shelf-life and ensure the food safety<sup>[3]</sup>. Generally, prolonging use of antimicrobial agents may cause negative effect on human health because it kills gut flora. Thus formidable threat was posed by microbial species appearing to develop drug resistance. The development and spread of resistance to currently available antibiotics is a worldwide concern. Bacterial resistance is an increasing threat to the positive treatment of infectious diseases. As bacterial resistance continues to evolve, some pathogens that were once considered routine to treat are developing, or have developed resistance to almost every antibacterial agent currently available. Numerous mechanisms have developed in microorganisms, which confer them with antimicrobial resistance. Three mechanisms particularly predominate in antimicrobial resistance: 1) enzymatic inactivation of the antimicrobial agent, 2) substitutions, amplifications or modifications of the drug target reducing the affinity of the drug to the target or 3) reducing access of the antimicrobial agents to the target by means of permeability barriers or efflux pumps. These mechanisms can either chemically modify the antibiotic, or it becomes inactive through physical removal from the cell, or modify target site so not recognized by the antibiotics. Examples include pneumococci resistant to penicillin and macrolides, methicillin-resistant staphylococci, vancomycin-resistant enterococci as well as multi-drug resistant Gram-negative organisms and fungi<sup>[4–9]</sup>. Therefore, it has become an urgent need to develop new types of highly effective and non-toxic antimicrobials from natural sources. Several scientific studies have proved that most of the plant possessed medicinal properties for their biological activities ranging from antimicrobial to antitumor. Based on long history of herbal usage, the use of herbs for the treatment of food borne pathogen is believed to be safer than the inclusion of synthetic antibiotics<sup>[10]</sup>. In India two herbs, Vetvier (*Vetiveria zizanoides*) and Tulsi (*Ocimum basilicum*) are usually used for food borne pathogen treatment<sup>[11,12]</sup>. In Southeast Malaysia and Indonesia, there are plenty of native herbs which have been used to treat food-borne pathogens<sup>[13,14]</sup>. However, there are a lack of scientific proof and validation.

Because of increasing threat of infectious diseases, the need of the hour is to find natural agents with novel mechanism of action. Fruit and vegetable peels are thrown into the environment as agro waste which can be utilized as a source of antimicrobials. It will be economic, eco-friendly

and reduce pollution. By-product recovery from the fruits wastes can improve the overall economics of processing units. Besides this, the problem of environmental pollution can also be reduced considerably. Fruits, especially tropical fruits have the capacity to produce a large number of bioactive phytochemicals<sup>[15]</sup>. The pumpkin [*Cucurbita moschata* (*C. moschata*) Duchesne] belongs to the family "Cucurbitaceae" which includes cucumbers, melons, squash and gourds. Study conducted by Tamer *et al.* revealed that pumpkin was a healthy and functional vegetable because of its rich nutrients and bioactive compounds<sup>[16]</sup>. Currently in scientific field, the study of fruits peel as an antibacterial agent has been fully established such as lemon peel<sup>[17]</sup>, pomegranate peel and many more<sup>[18]</sup>. However, for tropical pumpkin peel, there is still insufficient information that needs to be addressed and investigated meticulously. Hence, in the present study, the antibacterial potential of the tropical pumpkin (*C. moschata*) peel was screened using three different solvents *i.e.*, dichloromethane (DCM), methanol (MEOH), and distilled water (AQ) against eight types of pathogenic bacteria, three from Gram-negative and five from Gram-positive strains.

## 2. Materials and methods

### 2.1. Preparation of plant sample

Tropical pumpkin (*C. moschata* Duchesne) fruits were purchased at supermarket in Kuantan city, Pahang Darul Makmur, Malaysia in Sep 2011. The peels were taken after scrubbing off the pulpy portion from the peels and then were dried in a PROTECH laboratory air dryer (FDD-720-Malaysia) at 40 °C for 7 d and pulverized using Fritsch Universal Cutting Mill-PULVERISETTE 19-Germany. Powder was then stored in a desiccator at room temperature until further use.

### 2.2. Preparation of extracts

The extraction was conducted according to the method of Akhilesh *et al.* with some modification<sup>[19]</sup>. Using selective solvent extraction, 100 g powder of tropical pumpkin peel was macerated with non-polar solvent *i.e.*, DCM over a period of five days then extracted thrice. The whole extract was then filtered using Whatman filter paper (No. 1). Then, collected solvent was evaporated to recover the crude extract using rotary evaporator at 40 °C. Next, extract was stored in Schott's bottle (250 mL) at 2 °C and kept as aliquots until further antimicrobial evaluation. The above procedure was repeated with the same powder of tropical pumpkin peel but with polar solvent to get MEOH extract. For water extraction, 100 g of fresh peel powder was macerated with AQ for 72 h and concentrated under vacuum. All chemicals used in this study were of analytical grade, double distilled and directly purchased from the Merck.

### 2.3. Test organisms

Eight bacterial strains were taken into account in this study. All strains were purchased either from the American Type Culture Collection (ATCC), USA, or from the Fish Disease Laboratory, University Malaysia Terengganu (UMT), Malaysia. The test microorganisms used for the antibacterial activity screening include *Bacillus cereus* (*B. cereus*) (ATCC 11778), *Enterococcus faecalis* (*E. faecalis*) (ATCC 29212), *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 27853), *S. aureus* (ATCC 29213), *E. coli* (ATCC 20922), *Vibrio parahaemolyticus* (*V. parahaemolyticus*) (FDL-UMT-11), *Burkholderia cepacia* (*B. cepacia*) (FDL-UMT-12) and *Vibrio alginolyticus* (*V. alginolyticus*) (FDL-UMT-13).

### 2.4. Media preparation and in vitro culture

Mueller Hinton, Tryptic Soy Agar, Mueller–Hinton broth (MHB) and tryptic soy broth (TSB) were used for bacteria growth. All medium were dissolved in one liter of AQ. All solutions were sterilized by autoclave for 15 min at 121 °C. Bacteria turbidity was tested in MHB and TSB. Bacteria were then cultured onto Mueller Hinton and Tryptic Soy Agar plates to obtain colonies at 37 °C. Once a single colony of each cultured bacteria was taken out, it was sub cultured onto MHB and TSB under aerobic conditions at 37 °C to reach exponential growth. The concentration (optical density) of each bacterium broth culture was determined by using a spectrophotometer (Lambda 25 UV/VIS Spectrometer) at 625 nm. The inoculum size of each test strain was standardized by adjusting the optical density of the bacterial suspension to turbidity 0.08 to 1.00<sup>[20]</sup>.

### 2.5. Paper disc agar diffusion method

In the present study, antimicrobial susceptibility of *C. moschata* peels extracts was determined by following Kirby–Bauer disk diffusion susceptibility test<sup>[21]</sup>. This is one of the most commonly used methods of antibacterial susceptibility testing. In this test, small filter paper disks (6 mm) impregnated with a different concentrations of plant extracts were placed onto an agar plate to which bacteria had been swabbed. Sterile paper disc (6 mm) were made from Whatman's No.1 filter paper using a puncher paper. The plates were incubated overnight, and the zone of inhibition of bacterial growth was used as a measure of susceptibility. Large zones of inhibition indicated that the organism was susceptible, while very small or no zone of inhibition indicated resistance. An interpretation of intermediate was given for zones which fell between the accepted cutoffs for the other interpretations. Three different concentrations were prepared to test the variability of antibacterial activity from the initial stock concentration (500 mg/mL). Each disk was impregnated

with 10 µL, 20 µL and 40 µL of each of the extract (500 µg/µL) to give the final concentration of 5 mg, 10 mg and 20 mg per disc respectively.

### 2.6. Minimum inhibitory concentration (MIC)

Broth microdilution method recommended by Clinical and Laboratory Standards Institute, USA was used for the determination of MIC and minimum bactericidal concentration (MBC) values for each plant extract<sup>[22,23]</sup>. Sterile 96-well flat-bottomed microplates were filled with 100 µL of inoculum (MHB or TSB with bacteria) and 100 µL plant extract of different concentrations. It was then transferred to each microplate well. Serial dilutions of the extract were carried out in 0.1% dimethylsulfoxide which had no inhibitory activity against test microorganism. The final volume in the well was adjusted at 100 µL with the concentration 100 mg/mL. Serial dilutions were made to obtain concentration ranging from 0.1 mg/mL to 100 mg/mL. The control used included blank containing bacteria, tetracycline as the positive control while 0.1% dimethylsulfoxide and 1% butanol as the negative controls. The microplates were incubated at 37 °C from 18 h to 24 h. Bacterial growth was indicated by the presence of broth turbidity in the microplate wells. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used as color indicator in order to ensure the occurrence of color changes (color being changed from yellow to dark blue). The MIC values were taken as the lowest concentration of the extract in the well of the microplate that showed no turbidity after incubation<sup>[24–26]</sup>.

### 2.7. MBC

The incubation of bacteria after treating with samples from the MIC studies which did not show any growth of bacteria after incubation period were sub cultured on the surface of the fresh agar plates and incubated at 37 °C for 24 h. The MBC values were recorded as the lowest concentration of samples that did not permit any visible bacteria colony growth on the agar plate after the period of incubation<sup>[27]</sup>.

### 2.8. Statistical analysis

Values represent the mean from three independent experiments.

## 3. Results

In the present study, antibacterial susceptibility of *C. moschata* peel extracts was determined by using Kirby Bauer disk diffusion test. Total eight types of bacteria *i.e.*,

Gram-positive bacteria: *B. cereus*, *E. faecalis* and *S. aureus* and Gram-negative bacteria: *B. cepacia*, *P. aeruginosa*, *V. alginolyticus*, *V. parahaemolyticus* and *E. coli* were taken into consideration. Three different solvents based on their degree of polarity (DCM, MEOH and AQ) were used to evaluate and compare the antibacterial activity for various parameters. The antibacterial activity of each extract was expressed in the term of average diameter of zone of inhibition. The results of the present study are encouraging as all the tested extracts revealed antibacterial activity, though the inhibitory activity was not concentration dependent and strain specific. Table 1 shows the diameter of inhibition zone at different concentration 5, 10 and 20 mg per disc exhibited by each extract towards the selective bacteria. DCM and MEOH extracts showed good antibacterial activity for all tested microbes except for the aqueous extract.

**Table 1**

Means of inhibition growth diameter obtained by disc diffusion method using different concentrations of non-polar and polar extracts of the peel of *C. moschata* against selected bacteria.

Microbial strains	Solvent	Extracts concentration (mg/disc)			Positive control (30 µg/disc)	Tetracycline	Chloramphenicol	Negative control
		5 mg	10 mg	20 mg				
<i>Bacillus cereus</i> (ATCC 11778)	DCM	NA	NA	NA	15.0±0.5	11.0±0.7	NA	
	MEOH	NA	18.0±1.4	19.0±0.7	19.0±0.7	17.0±1.4	NA	
	AQ	NA	NA	NA	16.0±0.0	13.0±0.7	NA	
<i>Burkholderia cepacia</i> (FDL-UMT-12)	DCM	11.0±0.7	9.0±0.7	10.0±0.7	34.0±2.1	33.0±2.1	NA	
	MEOH	NA	NA	NA	23.0±0.7	29.0±0.7	NA	
	AQ	NA	NA	NA	29.0±1.4	33.0±2.1	NA	
<i>Enterococcus faecalis</i> (ATCC 29212)	DCM	9.0±1.4	10.0±0.7	10.0±0.0	16.0±0.0	25.0±0.7	NA	
	MEOH	NA	NA	NA	18.0±0.0	29.0±0.7	NA	
	AQ	NA	NA	NA	9.0±0.0	30.0±0.7	NA	
<i>Escherichia coli</i> (ATCC 20922)	DCM	NA	NA	NA	17.0±0.0	16.0±0.7	NA	
	MEOH	NA	12.0±2.8	17.0±1.4	15.0±0.7	17.0±0.7	NA	
	AQ	NA	8.0±0.0	10.0±0.7	18.0±0.7	17.0±0.7	NA	
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	DCM	14.0±1.4	15.0±0.7	19.0±1.4	20.0±0.0	15.0±0.0	NA	
	MEOH	10.0±0.7	11.0±0.7	19.0±1.4	25.0±0.0	10.0±0.0	NA	
	AQ	NA	13.0±0.7	14.0±0.7	6.0±0.0	10.0±1.4	NA	
<i>Staphylococcus aureus</i> (ATCC 29213)	DCM	18.0±3.5	21.0±0.7	19.0±1.4	23.0±1.4	23.0±1.4	NA	
	MEOH	NA	11.0±0.7	15.0±0.0	21.0±2.1	22.0±0.7	NA	
	AQ	NA	NA	NA	22.0±0.0	22.0±0.0	NA	
<i>Vibrio alginolyticus</i> (FDL-UMT-13)	DCM	NA	9.0±0.0	11.0±0.7	10.0±0.7	10.0±0.0	NA	
	MEOH	13.0±0.0	8.0±0.7	8.0±0.7	11.0±0.7	31.0±1.4	NA	
	AQ	NA	NA	NA	22.0±0.0	32.0±0.0	NA	
<i>Vibrio parahaemolyticus</i> (FDL-UMT-11)	DCM	9.0±0.0	11.0±0.0	7.0±0.0	24.0±0.7	14.0±0.7	NA	
	MEOH	NA	10.0±0.7	12.0±0.7	25.0±0.0	22.0±0.7	NA	
	AQ	NA	NA	NA	26.0±0.0	24.0±0.0	NA	

Values are expressed as mean value standard deviation ( $n=3$ ) in millimeters.

NA=Non Active, DCM=dichloromethane, MEOH=methanol, AQ=distilled water. Numbers indicate the mean diameters of inhibition of triplicate experiments  $\pm$  SD in millimeters.

Some of these extracts showed no inhibitory activity against some strains of bacteria during test. Antibacterial activity of DCM extract was found to be the highest against *S. aureus* and the antibacterial activity of MEOH extract was found to be the maximum against *B. cereus* whereas aqueous extract demonstrated the highest antibacterial activity against *P. aeruginosa*. As shown in Table 1, the extracts displayed relative antibacterial activity against most of the tested bacteria with diameter of inhibition zone ranging

between (7.0 $\pm$ 0.0) mm to (21.0 $\pm$ 0.7) mm. Certain bacteria *i.e.*, *P. aeruginosa*, *E. coli*, *E. faecalis*, and *B. cereus* shared the same trend that the diameter of zone of inhibition was found to be parallel with the increasing concentrations. However, there was exception for *S. aureus*. The zones of inhibitions were not proportional to the concentration of extracts. For instance, against *S. aureus*, the diameter of zone of inhibition at the lowest concentration of 5 mg was (18.0 $\pm$ 3.5) mm but for the second and third concentration, 10 mg and 20 mg respectively, the diameter of zone of inhibitions were (21.0 $\pm$ 0.7) mm and (19.0 $\pm$ 1.4) mm, respectively. Similar observations were noticed against *B. cepacia*, *V. parahaemolyticus*, and *V. alginolyticus*.

The MIC values of all extracts were detected following the addition of 20  $\mu$ L MTT. The MIC results ranged from 6.25 to 100.00 mg/mL are illustrated in Table 2. The lowest MIC value (6.25–12.50 mg/mL) was observed with DCM extract on Gram-negative bacteria *i.e.*, *B. cepacia* and *V. parahaemolyticus*. The MEOH extract showed the most potent antibacterial activity against *B. cepacia* and *V. parahaemolyticus* at 25.00 mg/mL. The highest MIC value was found to be 100 mg/mL exerted by aqueous extract against all bacteria tested.

**Table 2**

MIC values of non-polar and polar extracts of the peel of *C. moschata*.

Bacterial strain	DCM extract	MEOH extract	AQ extract
<i>Bacillus cereus</i>	50.00	100.00	100.00
<i>Burkholderia cepacia</i>	6.25	25.00	100.00
<i>Enterococcus faecalis</i>	50.00	50.00	100.00
<i>Escherichia coli</i>	25.00	100.00	100.00
<i>Pseudomonas aeruginosa</i>	50.00	50.00	100.00
<i>Staphylococcus aureus</i>	25.00	50.00	100.00
<i>Vibrio alginolyticus</i>	100.00	100.00	100.00
<i>Vibrio parahaemolyticus</i>	12.50	25.00	100.00

DCM: Dichloromethane, MEOH: Methanol, AQ: Aqueous, Positive control: tetracycline+test bacteria: no growth was observed, Negative control: Butanol and dimethylsulfoxide+test bacteria: growth was observed. Values are means of triplicate determinants. Numbers indicate the mean values of inhibition of triplicate experiments  $\pm$ SD at mg/mL.

Bacterial strains which displayed considerably good sensitivity to pumpkin peel extracts were selected to determine the bactericidal/bacteriostatic nature of extracts by the MBC test. The MBC test was carried out for those wells that did not show any growth of bacteria. Table 3 shows the results of MBC assay for DCM, MEOH and AQ extracts of pumpkin peels. DCM and MEOH extracts exhibited bactericidal nature to all bacterial strains except for the *V. alginolyticus*. The MBC values exhibited bactericidal nature ranging from 3.12 mg/mL to 100.00 mg/mL. Aqueous extract did not inhibit the growth of bacteria tested except for *E. coli* and *V. parahaemolyticus* at the concentration 100.00 mg/mL. From the above result, it can be concluded that most of the bacteria tested gave positive results that lead to bactericidal activity except for not-determined are

stated in Table 3 which indicate that those bacteria are bacteriostatic in nature.

**Table 3**

MBC values of non-polar and polar extracts of the peel of *C. moschata*.

Bacterial strain	DCM extract	MEOH extract	AQ extract
<i>Bacillus cereus</i>	50.00	50.00	ND*
<i>Burkholderia cepacia</i>	3.12	12.50	ND*
<i>Enterococcus faecalis</i>	50.00	50.00	ND*
<i>Escherichia coli</i>	25.00	50.00	100.00
<i>Pseudomonas aeruginosa</i>	50.00	100.00	ND*
<i>Staphylococcus aureus</i>	25.00	100.00	ND*
<i>Vibrio alginolyticus</i>	ND*	ND*	ND*
<i>Vibrio parahaemolyticus</i>	6.25	25.00	100.00

\*ND: Not determined. Values are means of triplicate determinants. Numbers indicate the mean values of inhibition of triplicate experiments  $\pm$ SD at mg/mL.

#### 4. Discussion

Recently, pharmaceutical and scientific communities have been focusing on medicinal plants and the therapeutic values of natural compounds are reported in many publications to validate the claims of their biological activity. Due to the challenge of emerging incidences of drug-resistant pathogens, attention has been drawn to the antimicrobial activity of plants and their metabolites[28]. In the present study, the antibacterial activity of DCM, MEOH and AQ extracts of *C. moschata* peels were evaluated against eight food borne pathogens consisting of three Gram-positive and five Gram-negative bacteria. The method involved were disc diffusion to obtain average zones of inhibition and broth micro-dilution to find MIC.

The results obtained from this investigation revealed that all three extracts taken into account exhibited significant antibacterial activity against certain bacteria. The difference in activity was due to the type of solvents used to extract the plant material. Ncube *et al.* stated that methanol, ethanol and water are the most frequently used solvents to study antimicrobial activity in plants[28]. They also pointed out that the types of solvents used play important role to determine the biologically active compounds present in plant material. Therefore, the selection of solvents should depend on the targeted compounds from the extract. In this study, DCM, methanol and water were used for the preparation of different extracts of *C. moschata* peel. The effect of the different types of solvents used during extraction was clearly observed. The data clearly justified that DCM extract of *C. moschata* inhibited almost all the bacteria tested except for *E. coli* while MEOH extract managed to inhibit six of bacteria studied except for *B. cepacia* and *E. faecalis*. Aqueous extract of pumpkin peels inhibited only *E. coli* and *P. aeruginosa*. With respect to different solvents used in this study, DCM extract of pumpkin peel showed the better antibacterial activity followed by MEOH extract and finally aqueous extract. In short, the trend of antibacterial

susceptibility was found to be the dependent on the polarity of the solvents. Water is universal solvent, used to extract plant product with antimicrobial activity. Parekh *et al.* mentioned that plants extracts from organic solvent have been found to give more consistent antimicrobial activity compared to water extract[29]. Recently, many research studies have been done to discover the presence of various bioactive compounds like flavonoids, tannins, phenol in fruits peels like mangosteen, pomegranate, citrus, and grape[30–34]. However, there are no reports available on the antibacterial activities of the tropical pumpkin. It could be presumed that the antimicrobial properties of the pumpkin peel extracts are due to the presence of non-polar substances present in greater quantity in DCM extract.

Certain bacteria *i.e.*, *P. aeruginosa*, *E. coli*, *E. faecalis*, and *B. cereus* shared the same trend that the diameter of zone of inhibitions was found to be parallel with the increasing concentrations. However, there was exception for *S. aureus*. The zones of inhibitions were not proportional to the concentration of extracts. For instance, against *S. aureus*, the diameter of zone of inhibition at the lowest concentration of 5 mg was (18.0 $\pm$ 3.5) mm but for the second and third concentrations, 10 mg and 20 mg respectively, the diameter of zone of inhibitions were (21.0 $\pm$ 0.7) mm and (19.0 $\pm$ 1.4) mm, respectively. Similar observations were noticed against *B. cepacia*, *V. parahaemolyticus*, and *V. alginolyticus*. The reasons of these results are still unclear and undefined[35]. On the other hand, from the observation, these results might be due to the development of bacterial resistance. Taking into account, the fundamental behavior of bacteria as living thing, higher pressure from the external factors could make them more stress. Related to this study, higher concentration of plant extracts would increase their tendency to create more resistance against antibacterial agents from plant extracts. According to Jalal *et al.* bacteria are single-celled organisms that have small numbers of genes[36], in which even a single random genetic mutation can greatly affect their ability to cause disease. Moreover, too high concentrated plant extract per disc could also be one of the reasons behind the observed finding. Generally, in other antibacterial studies the maximum concentration being used against microbes is 5 mg per disc, which was the minimum concentration, used in the present study. The reason for using high concentration is at low concentration the antibacterial activity was not prominent through disc diffusion test. This might be due to the fact that the plant extracts do not have enough biological active constituents to elicit their antibacterial effects at lower concentrations. Thus, when the concentration was increased, significant antibacterial activity was manifested by pumpkin peel extracts. Furthermore, presence of the impurities in the crude extracts might have diminished the antibacterial activity at low concentration. Since the extract's active pharmaceutical ingredients were neither isolated nor identified, the presence of more than one

active pharmaceutical ingredient with either antagonistic pharmacological property could be ascribed to this observation<sup>[37]</sup>.

Generally, in most studies, plant extracts showed that Gram-positive strains are considered to be more susceptible to antibacterial agents as compared to Gram-negative due to different cell wall structures<sup>[38]</sup>. Gram-negative strains possess an outer phospholipid membrane carrying the structural components of lipopolysaccharide. Thus, it makes the cell wall impermeable to antibacterial chemical substances. Compared to Gram-positive strain, it contains outer peptidoglycan layer which is not an effective permeability barrier<sup>[39]</sup>. However in this study, there was no clear trend observed for Gram-positive and Gram-negative strains. This finding is in agreement with the finding observed by Nurmahani *et al.*<sup>[40]</sup>, where no clear trends were found on the different types of bacteria on the antibacterial activity of dragon fruit peel extracts. Negi and Jayaprakasha also found the same trend by using pomegranate peel extracts<sup>[41]</sup>.

Aqueous extract of pumpkin peels showed the least antimicrobial activity. This result is supported by the study conducted by Yamaji *et al.* that water soluble compounds have no antimicrobial significance and water soluble phenolic compounds generally demonstrate significant antioxidant properties<sup>[42]</sup>. Moreover, in another study, among the 20 different solvents evaluated, chloroform was found to be the best solvent for the extraction of non-polar biological active compounds<sup>[43]</sup>. From the polarity index, the position of dichloromethane is above than chloroform. This study is in line with Tian *et al.* on *Gala chinensis* which revealed that the different solvents polarity does affect the bioactivity like antioxidant and antimicrobial activities<sup>[44]</sup>. In the same study, increase polarity of one solvent was found to decrease the antimicrobial activity.

MIC test is considered the gold standard for determining the susceptibility of organisms to antimicrobials and therefore is frequently used to judge the performance of all other methods of susceptibility testing. MIC test is used in diagnostic laboratories to confirm unusual resistance, to give a definitive answer when a borderline result is obtained by other methods of testing, or when disc diffusion methods are not appropriate<sup>[45]</sup>. It has been clearly observed in many studies that the micro-dilution method is more sensitive and reliable than the disc diffusion method with respect to evaluate antimicrobial potential of any antimicrobial substance<sup>[46,47]</sup>. The MIC of samples was detected following the addition of 20  $\mu$ L MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). MIC test indicates the lowest sample concentration that had prevented this change. In the present investigation, there was no trend observed on the inhibition of different types of bacteria. The MIC results varied range from 6.25 mg/mL to 100.00 mg/mL.

The MBC value exhibited bactericidal nature ranged

from 3.12 mg/mL to 100.00 mg/mL. These results are in line with other findings mentioning that for the bactericidal properties higher concentration is usually required to kill all bacterial cells<sup>[48]</sup>. Bactericidal nature is determined by lower concentration of extracts that kills all tested bacteria which is guided by MIC test. For bacteriostatic nature certain concentration only prevents the growth of bacterial<sup>[49]</sup>. At high concentration, bacteriostatic agents are often bactericidal against some susceptible organism. Nevertheless, aqueous extracts of pumpkin peels did not inhibit the growth of bacteria tested except for *E. coli* and *V. parahaemolyticus* at the concentration 100.00 mg/mL. From the above result, it can be concluded that most of the bacteria tested gave positive results that lead to bactericidal activity except for not-determined which indicates that those bacteria are bacteriostatic in nature.

In a nutshell, the results of disc diffusion method, MIC and MBC of *C. moschata* against *B. cereus*, *E. faecalis*, *B. cepacia*, *P. aeruginosa*, *S. aureus*, *V. alginolyticus*, *V. parahaemolyticus*, and *E. coli* indicated that *C. moschata* antimicrobial effect is independent of acquisition of resistance by the bacteria against antibiotics and thus could be a potential and safe antimicrobial agent in future. The present study revealed that all the microbes tested were sensitive to different extracts from pumpkin peel. However, dichloromethane extract of pumpkin peel showed remarkable antimicrobial activity against most of the bacterial strains and could be classified as a good source for potent natural antimicrobial agent against microbes taken into account in this study.

### Conflict of interest statement

Authors have declared that no competing interests exist.

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### Comments

#### Background

Undoubtedly, the development of bacterial resistance to currently available antibiotics is a worldwide concern and responsible for the high number of fatalities among patients. Bacterial resistance is an increasing threat

to the positive treatment of infectious diseases. Fruit and vegetable peels are thrown into the environment as agro waste that can be effectively utilized as a source of antibacterial agents. It will be economic, eco-friendly and could reduce pollution associated with them. By-product recovery from the antibacterial fruits wastes can improve the overall economy of processing units. Besides this, the problem of environmental pollution can also be reduced considerably.

### Research frontiers

The present research work describes antibacterial activity of non-polar and polar extracts of pumpkin (*C. moschata*) peel against Gram-positive and Gram-negative bacteria and assessed by estimating Kirby-Bauer disk diffusion susceptibility and broth micro-dilution methods.

### Related reports

The antimicrobial test was done using the Kirby-Bauer disk diffusion susceptibility and broth micro-dilution methods. Minimum inhibitory concentration of each extract was also determined which is considered crucial about the authenticity of antimicrobial agents. Many studies have evidenced about the superiority of these methods to explore antimicrobial potential of plant extracts as well as individual constituents *in vitro*.

### Innovations and breakthroughs

In the present work, authors have explored the antibacterial potential of the *C. moschata* peel *in vitro*. It is a good innovative work. Fruit and vegetable peels are thrown into the environment as agro waste which can be utilized as a source of antimicrobial agents in future. It will be economic, eco-friendly and could reduce pollution related to them. By-product recovery from the fruits wastes can improve the overall economy of processing units. Besides this, the problem of environmental pollution can also be reduced considerably.

### Applications

In future studies, antibacterial potential of this plant could further be evaluated in order to isolate individual constituents responsible for antimicrobial effect. This scientific study further supports and suggests the use of this plant as an adjuvant along with commonly used antimicrobial agents.

### Peer review

This is a type of exploratory study and a valuable research work in which authors have successfully tried to demonstrate the antibacterial characteristic of the peel of the *C. moschata* *in vitro*. The antimicrobial activity was evaluated by following disc diffusion method and MIC was determined by using 96-well microtiter plates.

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