EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

G

ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 13 No. 2 (2021)

Citation: *Egypt. Acad. J. Biolog. Sci. (G.Microbiolog) Vol.13 (2) pp.79- 90 (2021)*
DOI: 10.21608/EAJBSG.2021.211278

Egypt. Acad. J. Biolog. Sci., 13(2):79-90(2021)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Antimicrobial Activity of Capsaicin and Its Derivatives against *Klebsiella pneumoniae*

Mervat G. Hassan[1], Ghada Abdulrazik[1]*, Mohamed E. El Awady[2], Ahmed A. Hamed[2], and Mohamed O. Abdel-Monem[1]

1-Botany and Microbiology Department, Faculty of Science, Benha University, Egypt.
2-Microbial Biotechnology Department, National Research Centre, El-Buhouth St. 33, Dokki-Cairo 12622, Egypt.

*E. Mail: **ghadaabdelrazik@yahoo.com**

ARTICLE INFO

Article History
Received: 30/11/2021
Accepted:18/12//2021
Available:20/12/2021

***Keywords*:**
Hot green pepper, potato peel, ammi visnaga, Antimicrobial *klebsilia pneumoniae*, Capsaicin

ABSTRACT

Antibiotic resistance is a global threat to public health, causing continual anxiety of almost intractable bacterial diseases. It is critical to investigate new antibiotic-free strategies to combat bacterial infections like "Klebsiella pneumonia". As a result, the phytochemicals produced by the plant as natural products with antimicrobial capabilities have gotten a lot of interest in the pharmaceutical sciences. Three different plant materials (hot green pepper, potato peel, and ammi visnaga) were collected and extracted by different solvents and screened as antibacterial activity against ten bacterial isolates (S1 to S10). These isolates were identified with Cultural, morphological and biochemical characteristics, then by GNB card and VITEK 2 system. These isolates were identified as *Acintobacter baumannii, Klebsilia pneumoniae, Proteus mirabilis and pseudomonas aeruginosa*. The hot green Pepper has a strong antibacterial activity against *Klebsiella pneumoniae* (S3) strain with a minimum inhibition concentration (MIC) of 12 mg/ml and minimum bactericidal concentration (MBC) of 18 mg/ml. The total phenolic content of ethanolic extract of hot green pepper was determined as 8.4±0.9 mg/g. Capsaicin and its derivatives were determined by High-performance liquid chromatography (HPLC) from ethanolic extracts of spicy green pepper. Capsaicin concentration measured 0.66 mg/g, and DihydroCapsaicin measured 0.48 mg/g but Nor DihydroCapsaicin measured 0.10 mg/g.

INTRODUCTION

Multidrug-resistant organisms (MDROs) are classified as such because they are resistant to more than one antimicrobial agent in vitro. MDRO infections can result in insufficient or delayed antibiotic treatment, as well as inferior patient outcomes (Anderson *et al.*, 2006; Ibrahim *et al.*, 2000). Gram-negative bacteria with high resistance to antibiotics (e.g. multidrug-resistant carbapenemase-producing Klebsiella pneumoniae) deserve special attention among MDROs; these microorganisms Antimicrobial agents can be resistant to all currently available antimicrobial agents, or they might be vulnerable exclusively to older, possibly more lethal antimicrobial agents like polymyxins, leaving only limited and unsatisfactory treatment options (McGowan, 2006; Pitout & Laupland, 2008).

When you consider the small number of novel antimicrobial drugs under development, the problem of increasing antimicrobial resistance becomes much more dangerous (Boucher *et al.,* 2009).

The term "medicinal plant" refers to 250,000 identified higher plant species; research on these plants used as remedies in traditional folk medicine can lead to the identification of various physiologically active compounds (Mojarad *et al.,* 2005). Throughout generations, medicinal herbs have been prescribed and utilized based on a strong conviction in their power to treat diseases (Okigbo & Ogbogu, 2008). Medicinal plants are thoroughly vetted and evaluated for a variety of uses, including pharmacology, pharmaceutical botany, medicinal and clinical microbiology, phytopathology, food preservation, and food preservation are some of the topics covered in pharmacology (Kianbakht & Jahaniani, 2003). Phytochemicals can be found in a variety of forms in plants and herbs (Dorman & Deans, 2000). Terpenoids, alkaloids, lectins, polypeptides, quinones, phenolics, flavonoids, coumarins, and other phytochemicals are examples (Cowan, 1999).

Plants constitute a promising unexplored source with tremendous potential for producing antimicrobial drugs, according to indigenous and local knowledge (Anago *et al.,* 2011). Plant compounds that may either suppress or kill microbes are frequently studied for generating novel medications to treat a variety of infectious diseases (Cowan, 1999).

Capsicum, a hot green pepper from the Solanaceae family, belongs to the genus Capsicum (Dutta & Dutta, 2001). It is grown and consumed all across the world, but especially in Africa and Asia. Vitamins A, B, and E are abundant in the five long peppers. They're also high in important minerals including magnesium, zinc, iron, phosphorus, and potassium (Otunola *et al.,* 2010). They're recognized for their preservation and therapeutic properties, in addition to enhancing flavor (Onu & Aliyu, 1995). Antimicrobial activities of extracts from Capsicum annuum fruit have been researched to a considerable extent, with conflicting results. Bacillus, Clostridium, Pseudomonas, Listeria, Salmonella, Staphylococcus, and Streptococcus were all inhibited by crude tissue extracts from many distinct C. annuum varieties (Bacon *et al.,* 2017). Streptococcus pyogenes, Listeria monocytogenes, Clostridium sporogenes, and Clostridium tetani were all suppressed by the extract from jalapeño fruit (Cichewicz & Thorpe, 1996).

Bacteria such as Enterococcus faecium, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species bacteria, which cause a high rate of antibiotic resistance each year (Rice, 2010). Klebsiella pneumoniae (K. pneumoniae), a gram-negative, aerobic, rod-shaped bacterium, is usually hospital-acquired and occurs primarily in patients with impaired immune defenses (Calfee, 2017).

MATERIALS AND METHODS

Plant Material:

Three distinct plant materials were obtained from the local market (hot green pepper, potato peel, and ammi visnaga) and dried at room temperature. The samples were crushed and transported to a glass container, where they were kept until the extraction operation was completed in the lab.

Preparation of Extracts:

Fresh, pristine plant materials were cleaned and oven-dried for 48 h. at 60 °C, then kept at room temperature before being crushed to a fine powder. The aqueous extract was made by boiling 10 gm of powder in 100 ml of boiling distilled water for 15 minutes. The mixture was filtered before being dried in the oven at 50 °C. To reach a final concentration of 100 mg/ml of extract. Different solvent extracts of plant materials were made by mixing 10 g of dry powder with 60 ml of various solvents (acetone, di-ethyl acetate, ethyl acetate, methanol and ethanol). The mixture was shaken for 15 minutes before being allowed to cool to room temperature. The supernatant was taken away. The filtrate

was combined with 60 mL solvents, agitated, and the supernatant extracted once again. This extraction procedure was carried out three times in total. After that, the extract was filtered. The filtrate was placed in a vial and dried in the oven at 50°C until totally dry. The dried extract was re-suspended for a final concentration of 100 mg/ml extract (N'guessan *et al.,* 2007).

Sampling Isolation and Cultivation of Bacteria:

At Benha University Hospital's Microbiology and Immunology Department, ten bacterial samples were gathered. Inoculated samples were placed on MacConkey and blood agar plates and incubated aerobically at 37°C.

Cultural, Morphological and Biochemical Characteristics of Test Organisms:

Ten bacterial isolates were collected and subjected to cultural, morphological, shape, gram stain and motility characteristics. Then, biochemical tests were done as catalase, citrate, coagulase, indol, methyl red, voges proskauer, oxidase, urease, triple sugar iron agar, H_2S production, nitrate reduction, utilization of different sugars as glucose, lactose, sucrose, mannose.

Identification -GNB Card and VITEK 2 System:

The bacterial isolates were identified by VITEK 2 system card for gram-negative bacilli (ID-GNB card) is a 64-well plastic card with 41 fluorescent biochemical assays, 18 of which are enzymatic tests. There were 101 distinct taxa of gram-negative rods in the ID-GNB database. Four strains were employed as quality controls. *Acintobacter baumannii* (2410692403232212), *Klebsilia pneumoniae* (2607730653164010), *Proteus mirabilis* (0013000341442210) and *Pseudomonas aeruginosa* (0003451103500252) were among the strains tested. To facilitate the identification of the test strains, all four quality control strains have to be accurately identified (Funke *et al.,* 1998).

Antibacterial Activity Screening by Disk Diffusion Method:

Bacterial cultures were cultivated overnight in nutrient broth before being diluted with sterilised Muller-Hinton broth to make 0.5 mL (Marc-Farland). The diluted cultures were inoculated into Muller-Hinton agar and dried for 10 minutes at 22°C±2°C. Moisture was eliminated in excess. By soaking sterilised blotting paper discs in extracts, test discs were created. Blotting paper discs were dipped in various solvents as a negative control. Tetracycline paper discs with a concentration of 0.25 mg mL were utilised as a positive control. Inoculated with each test culture, discs were placed to the surface of nutrient agar and incubated at 37°C for 18 to 24 hours. The inhibition surrounding each disc was evaluated after the incubation time (Taylor *et al.,* 1995).

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Extract:

This experiment was carried out in nutritional broth. To assess the MIC, serial dilutions of the highest active extract (ethanolic extract from hot green pepper). Two hundred liters of each concentration were homogenized in 1800 litres of Mueller-Hinton broth infected with Klebsiella pneumoniae (0.5 McFarland). For 18 to 24 hours, the tubes were incubated at 37 °C. The presence of turbidity suggested bacterial proliferation with a wavelength of 620 nm Antibacterial activity was understood as the absence of bacterial growth. The MIC was determined by determining the lowest concentration of extract in a broth tube that showed no apparent bacterial growth (Committee *et al.,* 2003; Hammer *et al.,* 1999). Sub-culturing from plates with concentrations equal to or above the MIC on new nutrient broth yielded the minimal bactericidal concentration (MBC). At 37 °C, the tubes were incubated for 18 to 24 hours. Individual tubes were streaked on fresh nutrient agar plates after incubation and incubated for an additional 18 to 24 hours at 37°C to detect visible colonies on the agar.

Determination of the Total Phenolic Content:

Folin-spectrophotometric Ciocalteu's technique was used to determine the total phenolic content of the ethanolic extract of

hot green pepper (Slinkard & Singleton, 1977). Gallic acid was used as a standard.

Capsaicin Content:

HPLC was used to identify and quantify capsaicin, and the procedure outlined was followed (Zhuang *et al.,* 2012) with some modifications. For sample preparation, the dry materials were crushed finely in a blender after being dried to a uniform weight in an oven at 55 °C. In 20 mL of 95 percent ethanol, 1 g of dry materials was extracted. The extract was filtered through a 0.2 mm particle size filter after 30 minutes and deposited in a 60°C ultrasonic bath for 60 minutes. Analysis using HPLC–DAD (Agilent 1260) device. The Agilent C18 column (4.6 mm x 250 mm i.d., 5 m) was used to separate capsaicinoids. The mobile phase consisted of (A) 1% acetic acid and (B) 50% acetonitrile (V/V) at a flow rate of 1.5 mL/min. For each of the sample solutions, the injection volume was 20 l. At 280 nm, the MWD was modified. Using standard compound, the column temperature was maintained at 40 °C.

RESULTS AND DISCUSSION

Cultural, Morphological and Biochemical Characteristics and VITEK2 of Test Organisms:

Bacteria in general have the genetic potential to acquire and disseminate therapeutic drug resistance. Bacteria have developed many resistance mechanisms, including antibiotic efflux, as a result of the widespread use of antibiotics in human medicine (Hoffman, 2001).

Antibiotic-resistant microorganisms have reached an alarming level throughout the world, particularly in developing nations (Amann *et al.,* 2019; Omulo *et al.,* 2015; Williams *et al.,* 2018). Egypt is one of the countries that have less severe restrictions on antibiotic prescription (Khalil, 2012; Sabry *et al.,* 2014), the emergence of multidrug-resistant bacteria is unceasing very quickly. A major reason is that antibiotics can be bought directly from drug retailers and pharmacies without a prescription (Kariuki, 2010; Llor & Cots, 2009).

Ten bacterial isolates (S1-S10) were collected from Benha University Hospital's Microbiology and Immunology Department. These isolates were subjected to cultural characteristics shown in Table (1), that isolated (S1-S4) were gram-negative, non-motile, rod shape and Large, greyish, highly mucoid and lactose fermenting colonies. Isolates (S5-S6) were gram-negative, non-motile, Coccobacillus shape and non-pigmented, mucoid with smooth to the pitted surface. Therefore, isolates (S7-S8) were gram-negative, motile, rod shape and fishy odour, non-lactose fermenting colonies. Then, isolates (S9-S10) were gram-negative, motile, rod shape and bluish green pigment and non-lactose fermenting colonies.

Therefore, the physiological and biochemical characteristics of the bacterial isolates are shown in Table (2). isolated (S1-S4) were catalase-positive, citrate positive, coagulase not detected, Indol negative, MR (Methylred) positive, VP (Voges proskauer) positive, oxidase negative, Urease positive, TSI (triple sugar iron agar) positive, Negative for H2S, positive for nitrate reduction, positive for glucose, positive for lactose, and positive for sucrose, mannose positive, mannite positive. Isolate (S5-S6) was catalase-positive, citrate positive, coagulase-negative, Indol negative, MR negative, VP negative, oxidase negative, Urease negative, TSI negative, Negative for H2S, negative for nitrate reduction, positive for glucose, lactose, and sucrose, mannose positive and mannite positive. Isolate (S7-S8) was catalase-positive, citrate positive, coagulase-negative, Indol negative, MR positive, VP negative, oxidase negative, Urease positive, TSI positive, H_2S positive, Nitrate reduction positive, glucose positive, lactose negative, sucrose positive, mannose negative, mannite negative. Isolate (S9-S10) was catalase-positive, citrate positive, coagulase-negative, Indol negative, MR negative, VP negative, oxidase-positive, Urease negative, TSI negative, H2S negative, Nitrate reduction positive, glucose positive, lactose negative, sucrose negative, mannose negative and mannite negative.

While VITEK2 system version: 07.01 apparatus was used to confirm conventional

biochemical identification of the ten bacterial isolates as exhibited in tables (3-6). So, by morphological, cultural, biochemical characteristics and VITEK2 system isolates (S1-S4) were identified as *Klebsiella pneumonia,* isolates (S5-S6) were identified as *Acinetobacter baumannii*. Isolates (S7-S8) were identified as *Proteus mirabilis* and isolates (S9-S10) were identified as *pseudomonas aeruginosa*.

Table 1. Cultural and morphological characteristics of bacterial isolates.

Isolates	Characteristics			
	Culture Characteristics	Shape	Gram stain	Motility
S1	Large, greyish, highly mucoid and lactose fermenting colonies	Rods	(-)	Non-Motile
S2	Large, greyish, highly mucoid and lactose fermenting colonies	Rods	(-)	Non-Motile
S3	Large, greyish, highly mucoid and lactose fermenting colonies	Rods	(-)	Non-Motile
S4	Large, greyish, highly mucoid and lactose fermenting colonies	Rods	(-)	Non-Motile
S5	Non Pigmented, mucoid with smooth to the pitted surface	Coccobacillus	(-)	Non-Motile
S6	Non Pigmented, mucoid with smooth to the pitted surface	Coccobacillus	(-)	Non-Motile
S7	Fishy odour, non-lactose fermenting colonies	Rods	(-)	Motile
S8	Fishy odour, non-lactose fermenting colonies	Rods	(-)	Motile
S9	Bluish-green pigment and non-lactose fermenting colonies	Rods	(-)	Motile
S10	Bluish-green pigment and non-lactose fermenting colonies	Rods	(-)	Motile

Table 2. Biochemical characteristics of Bacterial Isolates

Characteristics	Bacterial isolates									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Catalase	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Citrate	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Coagulase	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Indol	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
MR (Methylred)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)
VP(Voges proskauer)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Oxidase	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)
Urease	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)
TSI (triple sugar iron agar)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)
H_2S production	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)
Nitrate reduction	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(+)	(+)
Glucose	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Lactose	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
Sucrose	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)
Mannose	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)

(+) Positive result – (-) Negative result

Table 3. Biochemical characteristics of samples (S1-S4) by VITEK2 system**.**

Biochemical Details																
2	APPA	-	3	ADO	+	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAIap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATK	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Table 4. Biochemical characteristics of samples (S5-S6) by VITEK2 system

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	Dmne	+	21	BXYL	-	22	BAIap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATK	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	+			

Table 5. Biochemical characteristics of samples (S7-S8) by VITEK2 system**.**

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	Dglu	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAIap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATK	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Table 6. Biochemical characteristics of samples (S9-S10) by VITEK2 system.

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAIap	+
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATK	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	+			

Antibacterial activity of extracts, MIC and MBC of the potential one

The fear of multidrug-resistant strains continuing to emerge prompted the researchers to come up with new therapeutic choices. Medicinal herbs have been employed in the suppression and treatment of pathogenic microorganisms for as long as anybody can remember (Bhuchar *et al.*, 2012), and likely, they are safe, cheaper and have low side effects (Singh *et al.*, 2013).

A total of 3 different plant materials (hot green Pepper, potato peel and ammi visnaga) were included in this study collected from a local market. The crude extracts of these plant materials which extracted with different

solvents were tested against altogether ten Gram-negative bacteria (Table 7-8). Generally, due to their extra protective outer membrane and other particularities, Gram-negative bacteria are usually considerably more resistant to antibacterial agents than gram-positive bacteria (Bamoniri *et al.*, 2010). So, that all crude extracts of ammi visnaga were ineffective against all Gram-negative bacterial isolates. While, the antimicrobial potentiality of obtained extracts of Potato Peel, revealed that only methanolic extract has significant activity against all test organisms. Peels of vegetables are the main byproduct of plant processing factories which have important organic compounds. Potato waste contains valuable chemical components like phenols which are suitable to apply in food preservation and pharmaceutical industries (Grunert, 2005). As well as, all the crude extracts of hot green pepper have strong antibacterial activity against all test organisms especially ethanolic extract which was the most active one gave a large inhibition zone of 13.8±0.1mm against Klebsiella pneumonia (S3). Previous studies showed that hot green Pepper extract is frequently used as a medicinal plant due to its antimicrobial activity especially against Klebsiella pneumonia due to its phenolic compounds (De Souza Nascimento *et al.*, 2013; Maharjan *et al.*, 2019).

By determination of the minimal inhibitory concentration for the ethanolic extract of hot green pepper which was the active plant material against examined *Klebsiella pneumonia* (S3) was 12 mg/ml. while the minimum bactericidal concentration was 18 mg/ml. Therefore, the antimicrobial activity of hot green pepper described MICs of 10 mg/l (De Souza Nascimento *et al.*, 2013) to 250 mg/ml. while, MBC was 500 mg/ml (Maharjan *et al.*, 2019).

Table 7. Antimicrobial activity of Potato Peel (clear zone in mm).

	H2O	Methanol	Ethanol	Acetone	Ethyl Acetate	Di-ethyl Ether
S_1	0.0	6.4±0.5	0.0	0.0	0.0	0.0
S_2	0.0	7.2±0.1	0.0	0.0	0.0	0.0
S_3	0.0	7.3±0.1	0.0	0.0	0.0	0.0
S_4	0.0	7.5±0.4	0.0	0.0	0.0	0.0
S_5	0.0	3.5±0.6	0.0	0.0	0.0	0.0
S_6	0.0	4.3±0.5	0.0	0.0	0.0	0.0
S_7	0.0	3.5±0.5	0.0	0.0	0.0	0.0
S_8	0.0	3.8±0.2	0.0	0.0	0.0	0.0
S_9	0.0	4.3±0.5	0.0	0.0	0.0	0.0
S_{10}	0.0	3.7±0.5	0.0	0.0	0.0	0.0

Table 8. Antimicrobial activity of hot green pepper (clear zone in mm).

	H2O	Methanol	Ethanol	Acetone	Ethyl Acetate	Di-Ethyl Ether
S_1	0.0	10.3±0.1	13.1±0.1	10.0±0.1	9.6±0.7	7.5±0.4
S_2	0.0	10.2±0.1	13.5±0.1	10.3±0.1	9.5±0.8	7.5±0.8
S3	0.0	9.0±0.5	13.8±0.1	10.8±0.7	8.7±0.5	8.1±0.1
S4	0.0	11.4±0.5	12.5±0.5	9.5±0.5	10.5±0.2	8.2±0.9
S5	0.0	8.7±0.5	9.5±0.5	7.5±0.8	6.4±0.2	6.5±0.4
S6	0.0	7.8±0.1	10.1±0.5	7.6±0.5	6.5±0.5	6.0±0.4
S7	0.0	6.5±0.5	10.2±0.1	8.4±0.5	8.0±0.1	6.0±0.1
S8	0.0	5.8±0.6	9.5±0.5	8.5±0.7	7.5±0.4	5.8±0.9
S9	0.0	3.7±0.1	6.7±0.5	7.1±0.5	6.5±0.7	3.3±0.5
S_{10}	0.0	2.6±0.5	5.8±0.4	6.4±0.5	7.0±0.6	3.0±0.6

Total Phenolic Content of Hot Green Pepper Crude Extract:

The Folin-Ciocalteu technique is a simple, rapid, and practical method for determining the total phenolic content of diverse materials (Singleton *et al.*, 1999). Several authors have evaluated the phenolic contents of different pepper species of the genus *Capsicum* using various techniques and solvents for the extraction of the chemical constituents of peppers. The total phenolic content of ethanolic extract of hot green pepper was 8.4±0.9 mg /g.

For centuries, capsaicin was used unknowingly in foods in order to enhance their taste, aroma, color and hotness (Goodwin & Hertwig, 2003). Besides it being used in the food industry, capsaicin has found its application in the pharmaceutical industry as well providing many health benefits and treatment strategies for medical conditions (Szolcsányi, 2004). Many analytical methods, such as HPLC, HPTLC, RP-HPLC, are suggested by many scientists for identification and quantification of Capsaicin (Cheema & Pant, 2011; Zahra *et al.*, 2016). In this study, we have proposed HPLC method for the determination of capsaicin and its derivatives obtained from ethanolic extracts of hot green pepper, it was characterized for the capsaicin content by HPLC (Table 9 & Fig. 1).

Table 9. Capsaicin and its derivatives content by HPLC

	Conc. mg/g
Capsaicin	0.66
DihydroCapsaicin	0.48
Nor DihydroCapsaicin	0.10

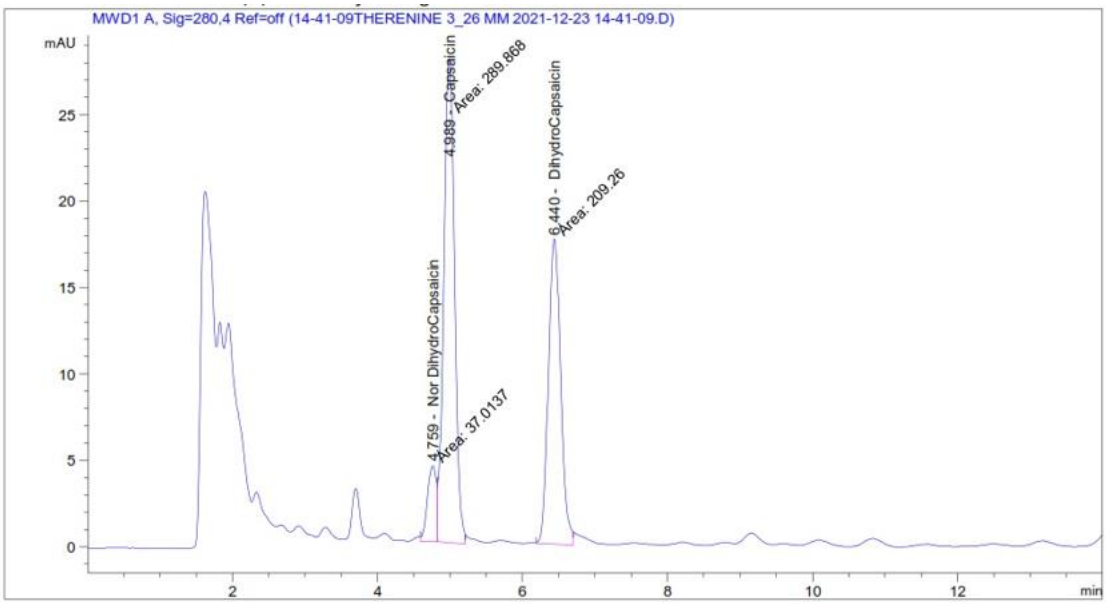

Fig. 1. Capsaicin and its derivatives content by HPLC

Conclusion

In conclusion, the hot green pepper flash could produce natural products with a strong antibacterial property against multidrug-resistant bacteria. Potato peel has limited antibacterial activity, and the Ammi Visnaga didn't show any antibacterial activity in our study. The crude extract was tested on ten different bacterial isolates. These isolates were identified morphologically, culturally, biochemically and by GNB cards and VITEK2 system. The Minimum inhibition concentration and minimum bactericidal inhibition were calculated. The total phenolic

content of the sample was estimated by The Folin-Ciocalteu method. The content of capsaicin and its derivatives was determined using high-performance liquid chromatography (HPLC).

REFERENCES

Amann, S., Neef, K., & Kohl, S. (2019). Antimicrobial resistance (AMR). *European Journal of Hospital Pharmacy* , *26*(3), 175–177. https://doi.org/10.1136/ejhpharm-2018-001820

Anago, E., Lagnika, L., Gbenou, J., Loko, F., Moudachirou, M., & Sanni, A. (2011). Antibacterial activity and phytochemical study of six medicinal plants used in Benin. In *Pakistan Journal of Biological Sciences*,Vol. 14(7), pp. 449–455). https://doi.org/ 10.3923/pjbs.2011.449.455

Anderson, D. J., Engemann, J. J., Harrell, L. J., Carmeli, Y., Reller, L. B., & Kaye, K. S. (2006). Predictors of mortality in patients with bloodstream infection due to ceftazidime-resistant Klebsiella pneumoniae. *Antimicrobial Agents and Chemotherapy*, 50(5), 1715–1720. https://doi.org/10.1128/ AAC. 50.5.1715-1720.2006

Bacon, K., Boyer, R., Denbow, C., O'Keefe, S., Neilson, A., & Williams, R. (2017). Evaluation of different solvents to extract antibacterial compounds from jalapeño peppers. *Food Science and Nutrition*, *5*(3), 497–503. https://doi.org/10.1002/ FSN3.423

Bamoniri, A., Ebrahimabadi, A. H., Mazoochi, A., Behpour, M., Kashi, F. J., & Batooli, H. (2010). Antioxidant and antimicrobial activity evaluation and essential oil analysis of Semenovia tragioides Boiss. from Iran. *Food Chemistry*, 122(3). https://doi.org/10.1016/j. foodchem.2010.03.009

Bhuchar, N., Sunasee, R., Ishihara, K., Thundat, T., & Narain, R. (2012). Degradable thermoresponsive nanogels for protein encapsulation and controlled release. *Bioconjugate Chemistry*, 23(1). https://doi.org/10. 1021/bc2003814

Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., Scheld, M., Spellberg, B., & Bartlett, J. (2009). Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. *Clinical Infectious Diseases*, 48(1), 1–12. https://doi. org/10.1086/595011

Calfee, D. P. (2017). Recent advances in the understanding and management of Klebsiella pneumoniae. *F1000 Research*, *6*. https://doi.org/ 10. 12688 /F1000RESEARCH. 11532.1

Cheema, S. K., & Pant, M. R. (2011). Estimation of capsaicin in seven cultivated varieties of Capsicum annuum L. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2(2).

Cichewicz, R. H., & Thorpe, P. A. (1996). The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. *Journal of Ethnopharmacology*, 52(2), 61–70. https://doi.org/10. 1016/0378-8741(96)01384-0

Committee, E., Testing, S., Microbiology, C., & Escmid, I. D. (2003). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clinical Microbiology and Infection*, 9(8), ix–xv. https://doi.org /10.1046/j.1469-0691.2003.00790.x

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564–582. https://doi.org/10.1128/cmr. 12.4.564

De Souza Nascimento, S., Desantana, J. M., Nampo, F. K., Ribeiro, Ê. A. N., Da Silva, D. L., Araújo-Júnior, J. X., Da Silva Almeida, J. R. G., Bonjardim, L. R., De Souza Araújo, A. A., &

Quintans-Júnior, L. J. (2013). Efficacy and safety of medicinal plants or related natural products for fibromyalgia: A systematic review. In *Evidence-based Complementary and Alternative Medicine*, Published online 2013 Jun 4. doi: 10.1155/ 2013/149468

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88(2), 308–316. https://doi.org/10.1046/J.1365-2672 .2000.00969.X

Dutta, T. C., & Dutta, A. C. (Atul C. (2001). *Botany for degree students*. 708. https://global.oup.com/academic/pro duct/botany-for-degree-students 9780195637489?lang=en&cc=gb

Funke, G., Monnet, D., DeBernardis, C., Von Graevenitz, A., & Freney, J. (1998). Evaluation of the VITEK 2 System for Rapid Identification of Medically Relevant Gram-Negative Rods. *Journal of Clinical Microbiology*, 36(7), 1948. https://doi.org/ 10.1128 /jcm.36.7.1948-1952.1998

Goodwin, D. C., & Hertwig, K. M. (2003). Peroxidase-catalyzed oxidation of capsaicinoids: Steady-state and transient-state kinetic studies. *Archives of Biochemistry and Biophysics*, 417(1). https://doi.org/ 10.1016/S0003-9861(03)00321-7

Grunert, K. G. (2005). Food quality and safety: Consumer perception and demand. *European Review of Agricultural Economics*, 32(3). https://doi.org/10.1093/eurrag/jbi01 1

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. In *Journal of Applied Microbiology*, 86(6):985-90.

Hoffman, S. B. (2001). Mechanisms of Antibiotic Resistance. *Compendium on Continuing Education for the Practicing Veterinarian*, 23(5). https://doi.org/10.1128/ microbiolspec.vmbf-0016-2015

Ibrahim, E. H., Sherman, G., Ward, S., Fraser, V. J., & Kollef, M. H. (2000). The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. *Chest*, 118(1), 146–155. https://doi.org/10. 1378/CHEST.118.1.146

Kariuki, S. (2010). Antimicrobial resistance in enteric pathogens in developing countries. In *Antimicrobial Resistance in Developing Countries* (Vol. 9780387893709). https://doi. org/10.1007/978-0-387-89370-9_11

Khalil, R. B. (2012). PRM58 Turning the Implausible to the Plausible: Towards a Better Control of Over the Counter Dispensing of Antibiotics in Egypt. *Value in Health*, 15(4). https://doi.org/10.1016/j.jval.2012.0 3.913

Kianbakht, S., & Jahaniani, F. (2003). Evaluation of Antibacterial Activity of Tribulus terrestris L. Growing in Iran. *Iranian Journal Of Pharmacology & Therapeutics*, *2*(1), 22–24. http://ijpt.iums.ac.ir

Llor, C., & Cots, J. M. (2009). The sale of antibiotics without prescription in pharmacies in catalonia, Spain. *Clinical Infectious Diseases*, 48(10). https://doi.org/10.1086/598183

Maharjan, R., Thapa, S., & Acharya, A. (2019). Evaluation of Antimicrobial Activity and Synergistic Effect of Spices against Few Selected Pathogens. *Tribhuvan University Journal of Microbiology*, *6*. https:// doi.org/10.3126/tujm.v6i0.26573

McGowan, J. E. (2006). Resistance in Nonfermenting Gram-Negative Bacteria: Multidrug Resistance to the Maximum. *The American Journal of Medicine*, 119(6), S29–S36. https://doi.org/10.1016/J. AMJMED.2006.03.014

Mojarad, T. B., Roghani, M., & Zare, N. (2005). Effect of Subchronic

Administration of Aqueous Artemisia annua Extract on-Adrenoceptor Agonist-Induced Contraction of Isolated Aorta in Rat. *Iranian Biomedical Journal*, 9(2), 57–62.

N'guessan, J. D., Dinzedi, M. R., Guessennd, N., Coulibaly, A., Dosso, M., Djaman, A. J., & Guede-Guina, F. (2007). Antibacterial activity of the aqueous extract of <I>Thonningia sanguinea</i> against Extended-Spectrum-b-Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains. *Tropical Journal of Pharmaceutical Research*, 6(3), 779–783. https://doi.org/10.4314/tjpr.v6i3.14659

Okigbo, R. N., & Ogbogu. (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. *Biotechnology and Molecular Biology Reviews*, 3(6), 127–134. http://www.academicjournals.org/BMBR

Omulo, S., Thumbi, S. M., Njenga, M. K., & Call, D. R. (2015). A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: What can be done better? *Antimicrobial Resistance and Infection Control*, 4(1). https://doi.org/10.1186/s13756-014-0041-4

Onu, I., & Aliyu, M. (1995). Evaluation of powdered fruits of four peppers (Capsicum spp.) for the control of Callosobruchus maculatus (F) on stored cowpea seed. *International Journal of Pest Management*, 41(3), 143–145. https://doi.org/10.1080/09670879509371939

Otunola, G. A., Oloyede, O. B., Oladiji, A. T., & Afolayan, A. J. (2010). Comparative analysis of the chemical composition of three spices - Allium sativum L. Zingiber officinale Rosc. and Capsicum frutescens L. commonly consumed in Nigeria. *African Journal of Biotechnology*, 9(41), 6927–6931. https://doi.org/10.5897/ajb10.183

Pitout, J. D., & Laupland, K. B. (2008). Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *The Lancet Infectious Diseases*, 8(3), 159–166. https://doi.org/10.1016/S1473-3099(08)70041-0

Rice, L. B. (2010). Progress and Challenges in Implementing the Research on ESKAPE Pathogens. *Infection Control & Hospital Epidemiology*, 31(S1), S7–S10. https://doi.org/10.1086/655995

Sabry, N. A., Farid, S. F., & Dawoud, D. M. (2014). Antibiotic dispensing in Egyptian community pharmacies: An observational study. *Research in Social and Administrative Pharmacy*, 10(1). https://doi.org/10.1016/j.sapharm.2013.03.004

Singh, R., Kaur, N., Kishore, L., & Kumar Gupta, G. (2013). Management of diabetic complications: A chemical constituents based approach. In *Journal of Ethnopharmacology*,Vol. 150 (1). https://doi.org/10.1016/j.jep.2013.08.051

Slinkard, K., & Singleton, V. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28(1).

Szolcsányi, J. (2004). Forty years in capsaicin research for sensory pharmacology and physiology. In *Neuropeptides*, Vol.38(6). https://doi.org/10.1016/j.npep. 2004.07.005

Taylor, R. S., Manandhar, N. P., & Towers, G. H. N. (1995). Screening of selected medicinal plants of Nepal for antimicrobial activities. *Journal of Ethnopharmacology*, 46(3), 153–159. https://doi.org/10.1016/0378-8741(95)01242-6

Williams, P. C. M., Isaacs, D., & Berkley, J. A. (2018). Antimicrobial resistance among children in sub-Saharan Africa. In *The Lancet Infectious Diseases*,Vol.18(2). https://doi.org/

10.1016/S1473-3099(17)30467-X

Zahra, N., Kalim, I., Hina, S., Javed, A., Manzar Inam, S., Muhammad Malik, S., & Arshad, F. (2016). Estimation of capsaicin in different chilli varieties using different Extraction Techniques and HPLC method: A Review. *Pakistan Journal of Food Sciences*, 26(1).

Zhuang, Y., Chen, L., Sun, L., & Cao, J. (2012). Bioactive characteristics and antioxidant activities of nine peppers. *Journal of Functional Foods*, *4*(1). https://doi.org/10.1016/j.jff.2012.01.001