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Antibacterial activities of *Physalis peruviana* against some food-borne pathogenic bacteria

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Abstract:
The study looked at the antibacterial activity of water, methanol, acetone, and ethyl acetate extracts from cape gooseberry *(Physalis peruviana*) against some food-borne pathogens. Plant extracts were diluted to different concentrations (50, 100, 150, and 200 mg/L) and tested against three types of bacteria namely *Salmonella enterica* and *Escherichia coli* as Gram negative bacteria and *Enterococcus* faecalis as a Gram positive bacteria. The obtained result observed that, all the cape gooseberry extracts under examination contained saponins, terpenes, and flavonoids, according to the chemical analysis. Additionally total flavonoids and total phenols were detected. Microbiological analysis revealed that ethyl acetate extract was more efficient than the other extracts at both 50 and 200 mg/L against the *Salmonella enterica* strain. These results are promising to be applied the cape gooseberry extracts as bioactive agents in the field of biotechnology.

INTRODUCTION

The cape gooseberry, also known as *Physalis peruviana* L., is a member of the family Solanaceae, characterized by its annual growth pattern with the potential to live as a short-lived perennial reaching heights of up to one meter **(Ramadan, 2011)**. It boasts a nutritional profile rich in vitamins A and C, various B-complex vitamins, and essential minerals like phosphorus, iron, potassium, and zinc **(Salazar *et al*., 2008)**. Research confirms that the nutritional composition and bioactive elements present in gooseberries significantly contributed to their health benefits **(Hassanien, 2001)**. Fruit juices derived from gooseberries are intricate mixtures of colloidal insoluble particles dispersed within a soluble compound-rich medium, encompassing sugars, organic acids, soluble pectin, phenolic compounds, and salts (**Eraso-Grisales *et al*., 2021)**.The best antibacterial performance was obtained against Escherichia coli using the methanolic extract for *physalis peruviana*. **Ramadan *et al.* (2015)**, who discovered that the extract of Cape gooseberry fruit had stronger antioxidant activity than the hexane extract and hence tested positive for anticancer activity. Physalis peruviana's antioxidant action is attributed to its high polyphenol content as well as its high levels of vitamins A and C. **Narváez-Cuenca *et al.* (2014)** discovered that P. peruviana L possesses excellent antioxidant activity.The fruits' exceptional antioxidant capacity is most likely owing to their high content of oxygenated monoterpenes. Not ably, gooseberries exhibit considerable functional properties due to their antioxidant content and high levels of vitamin E (**Ramadan and Moersel 2009**). Phenolic compounds found in gooseberries have been extensively studied for their health benefits, with their functionality influenced by factors such as content, intake, and bioavailability, which can be impacted by fruit processing techniques and

microstructure **(Balasundram *et al*., 2006)**. The calyx of the cape gooseberry, characterized by five hairy sepals and veins, reaches a length of approximately 4-5 cm after fertilization, enveloping the fruit during its development and growth stages **(Flórez *et al*., 2000)**. Initially green, it transitions to a yellow orange hue upon ripening, serving as protection against birds, insects, harsh weather conditions, and physical damage while providing essential carbohydrates during the fruit's initial growth period (**Fischer *et al*.,2000)**. The study looked at the composition, antimicrobial and antioxidant activities of water, methanol, acetone, and ethyl acetate extracts of cape gooseberry *(Physalis peruviana*).

Materials and Methods

Collection and preparation of plant samples

Freshly ripened cape gooseberry (golden berry) (*Physalis peruviana*) fruits were bought in March 2022 from Damietta, Egypt's local marketplaces. The studies were carried out at the Agric. Biotechnol. Dep. Faculty of Agriculture, Damietta University, Damietta, Egypt. After being cleaned and given a tap water wash, the fruits were dried in an oven at 40 to 50 degrees Celsius for three to four days. A laboratory blender was then used to grind the fruits into a powder.

Bacterial strains

Three strains of bacteria namely *Salmonella enterica* ATCC 14028/NCTC 12023 sero types such as typhimurium and enteritidis , *Enterobacter faecalis* ATCC 29212/NCTC 12697, and *Escherichia coli* ATCC 8739. Prof. Husain El-Fadaly, Agriculture Biotech. Department, Faculty of Agriculture, Damietta University, Damietta, Egypt, kindly provided them. To examine the bacteria, they were inoculated onto nutritional broth and then incubated for 24 hours at 37°C. After that, the turbidity was calibrated to 0.5 optical density (OD) at 600 nm, according to **Doughari and Manzara (2008).**

Preparation of various extracts

With minor adjustments, the technique of **Jaca and Kambizi (2011)** was carefully followed for removing the husks from *Physalis peruviana* fruits by hand. After giving the fruits, a thorough wash with tap water and followed by distilled water, then the fruits were carefully dried to preserve their active ingredients and pulverized into a powder using an electronic blender in a laboratory. The methanolic extract of *P. peruviana* was made in a flask with 150 g of powdered golden berries and 450 mL of 70% methanol. Following a 48-hour incubation period under dark conditions at room temperature, this extract was filtered through Whatman no. 1 filter paper and concentrated at 40°C in an oven until all the solvent had been eliminated, leaving behind a solid residue that was kept in a freezer. After the filtrate dries, 56.41 g is weighed, then 169.23 mL of acetone is added, then filtration is done, then we take the filtrate and dry it at 40°C, then it is preserved (acetone extract). Then the precipitate is taken and put into the oven at 40°C, 600 mL of ethyl acetate is put on it, and the filter is taken (ethyl acetate extract). The water extract is made through 300 g of dry plant and distilled water at 60°C with Stellier and puts the holes in a distilled water for 5 hours with continuous stirring then filtered (water extract).

All analyses were conducted at the laboratories of Damietta University, Faculty of Agriculture. Damietta, Egypt, including phytochemical screening **(Sumathy and Sumathy 2011)** .

Phytochemical screening

detection of tannins

The **Gonzalez and Delgado (1962)** technique was used to identify tannins. A tiny amount of distilled water and crude methanolic extract and filtrate were combined. One milliliter of a 5% ferric chloride solution was added to this combination. The presence of tannins was suggested by the emergence of a yellowish-green tint. The creation of a blue, green-black, or grey product is the outcome of this reaction, which is dependent on the interaction between phenolics and iron salts.

Detection of flavonoids

The crude methanolic extracts were determined using the **Geissman and waiss (1962)** procedure. One milliliter of 90% methanol was used to dissolve about 100 mg of the extract, which was then put to filter paper, left to dry, and then sprayed with 1% aluminum chloride solution. Flavonoids were detected by the appearance of a yellow tint. Aluminum chloride reacts with flavones and flavanols by forming stable complexes with their C-4 keto group and either their C-3 or C-5 hydroxyl group.

Determination of total phenolic compounds

Folin-Ciocalteu reagent technique was used to determine the total poly phenolic content in the plant extracts according to **El-Zayat *et al* (2021)**. First, 10 uL of the extract sample and 250 uL of Folin-Ciocalteu's reagent were mixed together. Next, 3.5 mL of deionized water was added. After three-minute

incubation period, one milliliter of 20% sodium carbonate was added then, the mixture was vortexed and given a 40-minute incubation period. After cooling in a dark cabinet, a spectrophotometer was used to measure the reaction mixtures' absorbance at 685 nm. A standard curve for gallic acid was created, with values ranging from 0 to 300 µg/mL. The unit of measurement for total phenolic content (TPC) was gallic acid equivalents (ug/g of fresh weight).

Total alkaloid content

Identified alkaloids were done after **Harborne (1998)**. This required combining 2.0 mL of diluted hydrochloric acid (1.0%) with 1.0 mL of plant extract. Then, 1.0 mL of the resultant solution was gradually mixed with five drops of Wagner's reagent, stirring between drops. Alkaloids were present because a precipitate formed after 15 minutes of incubation. Wagner's reagent was made by slowly heating 50 mL of distilled water to dissolve one g. of iodine and 10 g of potassium iodide. Two mL of acetic acid were added to this solution, and the remaining 100 mL were made up with distilled water.

Total carotenoid content

The carotenoid levels was evaluated according to the protocols described by **Jeyanthi *et al*.,(2014)** . A spectrophotometer was used to measure the absorbance of carotenoids in the 450–480 nm region. Carotenoids were extracted from *Physalis peruviana* fruits using a 1:1 volume ratio of hexane to acetone.

Total anthocyanin content

The total anthocyanin content of the fruit was ascertained in accordance with **Mazumdar and Majumder (2003).** Using 10 mL of ethanolic-hydrochloric acid mixture made up to 15 parts 1.5 N hydrochloric acid and 85 parts 95% ethanol, a half gram of fresh fruit was first extracted.After three minutes of incubation at 40°C, the resultant solution was filtered through Whatman No. I filter paper. For two hours, the filtered section was kept in the dark. A spectrophotometer was then used at a wavelength of 535 nm.

The fruit skin's overall anthocyanin absorbance calculated using the formula:

Total absorbance (per 100 g) =(EXbXc)/(dXa).

(e×b×c)/ (d×a).

where,

a = weight of sample, b= volume made for color measurement, c =total volume, d = volume of aliquot taken for estimation and e = specific optical density (OD) value at wavelength 535 nm. 1 mg per mL of the solution is equivalent to the absorbance of 98.2.

Therefore, the amount of total anthocyanin present in the sample (mg/100g) = Total absorbance for the sample / 98.2.

It is based on that, the UV-visible spectrum absorbance of sample was measured to determine the presence of compounds . The absorption of the U.V or the visible light of these compounds is due to the electron excitation .

Assessment of antioxidant activity

The plant material was subjected to the DPPH assay to evaluate the antioxidant activity. Freshly made, a methanol solution containing 0.004%w/v of 2,2diphenyl-1picrylhydrazyl (DPPH) radical was kept at 10 °C in the dark. Methanol was used to dissolve the tested extracts. Subsequently, 40 µL of the methanolic solution were combined with 3 mL of the DPPH solution, and the spectrophotometer was used at -695 nm to promptly measure the absorbance **(Yen and Duh, 1994).** Furthermore, the absorbance measurements were obtained for the reference compound ascorbic acid and the DPPH radical in the absence of antioxidants as a control. Every determination was made three times. **Yen and Duh's (1994)** formula was used to calculate the percentage of inhibition (PI) of the DPPH radical.

PI=[(AC-AT)/AC,×100]

Where, AC= the absorbance of the control at : t=0 min

AT = the absorbance of the sample +

D.P.P.H at t=16 min

DPPH free radical scavenging activity (RSA)

The antioxidant activity of the fruit extract from *Physalis peruviana* was assessed by measuring its capacity to scavenge or donate hydrogen to radicals, utilizing the stable DPPH technique that was modified by **Park *et al*. (2006).** One milliliter of the extract at different concentrations (50, 100, 150, and 200 ug/ml) and one milliliter of DPPH made up the reaction mixture. It was thoroughly mixed and allowed to sit at room temperature for half an hour in the dark. At 517 nm, absorbance measurements were made with a UV-visible spectrophotometer. A percentage of inhibition was used to express the radical scavenging activity and calculated using the formula:

% DPPH = (Absorbance of control)

- Absorbance of sample/ Absorbance of control x 100

Antibacterial activities Growth conditions

P. peruviana extracts was used to determine antibacterial activity against the tested food -borne pathogenic bacteria namely *Escherichia coli*,

Salmonella enterica and *Enterococcus faecalis*. peptone and 3.0 g/L beef extract) and incubated at 37 °C for 18 h at 150 rpm using an incubator with orbital shaking LOM-80 (MRC Lab, London, UK).

Determination of antibacterial activities

The selected stock cultures (*Salmonella enterica* ATCC 14028 / NCTC 12023, *Escherichia coli* ATCC 8739 and *Enterococcus faecalis*_ATCC 29212 / NCTC 12697) were used for preparation of 0.5 MacFarland standard fresh bacterial cultures, which sub-cultured in Muller Hinton agar plates. By sterile forceps, extract disks were distributed on the surface of the plate, and positive control was vancomycin antibiotic discs (20 g), while negative control was DMSO, then incubated at 37℃. The clear zone obtained was measured by a clean ruler from the center of the extract disk to the edge of the area with zero growth (**Zaki *et al*., 2022)** .

Statistical analysis:

Bacteria were inoculated into nutrient broth (5.0 g/L Descriptive statistics, including the standard deviation and mean. The standard deviation was computed using the Statistical Package (SPSS) version 21. The findings were also organized into tables, and statistical significance was determined when $p < 0.05$ **(Jayawardana *et al*.,2015)**.

RESULTS AND DISCUSSION

Phytochemical screening of various plant extracts

Crude methanolic, water, acetone and ethyl acetate extracts of cape gooseberry were analyzed to phytochemical screening (**Table 1**). Results indicated that the methanol extract contained terpenes,tannins , flavonoids ,saponins and alkaloids . In contrast ,the water extract contained

the same components except for resins and alkaloids with all extracts being free off resins.

Table 1 . Preliminary qualitative screening of examined extracts of *Physalis peruviana*

Samples	Plant extracts			
Component	Methanol	Water	Acetone	Ethyl acetate
Terpenes	+	+	+	+
Tannins	+	+	–	–
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Resins	–	–	–	–
Alkaloids	+	–	+	+

Table 2 . Total polyphenols and flavonoid content in *Physalis peruviana*

Plant extract	Total polyphenols (mgGAE/g)	Total Flavonoids (mgQE/g)
P.peruviana	288.88	53.57

From Table 2 ; Physalis peruviana has a very high percentage of phenols , these phenols have the ability to kill or inactivate bacteria by effecting the bacterial cell cytoplasm or DNA **Javed *et al*.(2018).**

Table 3. Anthocyanin and carotenoids content of extracts of *Physalis peruviana* .

Examined extracts	Total anthocyanin µg/g	Total carotenoids µg/g
Methanolic extract	0.68	4.91
Water extract	0.52	1.28
Acetone extract	0.16	2.31
Ethyl acetate extract	0.12	2.12

From the results in Table 3, it can be showed a very high percentage of carotenoids in methanolic extract and a high percentage of anthocyanins. These results apply with those of **Gowri *et al* .(2010)** .

Table 4 .The inhibition percentage of the radical scavenger DPPH and IC_{50}values of extracts of *Physalis peruviana*

Examined extracts	Inhibition (%)	IC_{50} Value µg/ml
Methanolic extract	55.2	1.54
Water extract	21.4	3.2
Acetone extract	30.0	3.9
Ethylacetate extract	38.9	2.5
Vitamin C	45.7	1.89
Negative control	-	-

Results recorded DPPH had a maximum absorbance at 517 nm and an antioxidant concentration needed to reduce the initial DPPH concentration by 50% (IC_{50}). A lower IC_{50} value indicates a higher antioxidant power. The methanolic extract of *Physalis peruviana* against DPPH roots using 1.54 µg /mL while the acetone extract of *Physalis peruviana* gave the inhibition value of 3.9 mg/mL at 30% the same concentration.

Antibacterial activity of the extracts

The antibacterial activity of the obtained extracts of *Physalis peruviana* were assessed against three bacterial strains. The evaluation involved measuring the presence or absence of inhibition zones and recording the diameters where no growth was observed after incubation at 37°C for 24 and 48, hours. Results showing in **Table 5** the effect of extracts in the form of methanol, ethyl acetate acetone and aqueous extracts on the bacterial strains in the concentration of 50,100,150,200 ppm.

Data from **Table 5** showing that, methanolic extract at a concentration of 150 mg/L had the highest effect on *Enterococcus faecalis*, Gram positive (G+) which inhibited by zone diameter equal to 31.0mm. On the other hand, it was found that the acetone and ethyl acetate extracts did not affect this bacteria .

For Gram negative (G-) bacterial strains , the results indicates that *Salmonella enterica* was less resistant than *E. coli* to all the tested extracts .On the other hand, It was found that *Salmonella enterica* was more susceptible to treatment with various plant extracts,

especially acetone extract at concentrations of 150 and 200 ppm.

Table 5 . Evaluation of antibacterial activities of *Physalis peruviana* extracts

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Microorganism	**Extract**	**Diameter of clear zone (mm) of antibacterial after 24h incubation period**			
		50 mg/L	100 mg/L	150 mg/L	200 mg/L
Salmonella	Acetone	28.6	28.6	32.3	32.3
	Water	29	29	31.6	31.6
	Methanol	13.3	17.6	32.6	28
	Ethyl acetate	30	31.3	35	32.3
Enterococcus faecalis	Acetone	0	0	0	0
	Water	11	14	27	24
	Methanol	19	29	31	22
	Ethyl acetate	0	0	0	0
Escherichia coli	Acetone	24.6	26.3	24	28.6
	Water	19.3	16.6	19	20.6
	Methanol	16.3	20	21.3	17.3
	Ethylacetate	22.3	24.6	22.3	22.6

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This research did not receive any funding.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

AUTHORS CONTRIBUTION

Sahar Hamed; Husain EL-Fadaly ; Sherif EL-kadi; and Nada shabara. Developed the concept of the manuscript .All authors checked and confirmed the final revised manuscript.

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الملخص العربي

النشاط الضد بكتيري لنبات الحرنكش ضد بعض البكتيريا الممرضة الموجودة في الغذاء

سحر حامد و حسين الفضالي وشريف القاضي وندي شبارة

قسم البيوتكنولوجيا الزراعية ،كلية الزراعة ،جامعة دمياط ،مصر

اهتم البحث بدراسة النشاط المضاد للبكتريا الممرضة لمستخلصات المياه والميثانول والأسيتون وأسيتات الإيثيل لنبات الحرنكش (*Physalis peruviana*). حيث تم تخفيف المستخلصات النباتية إلى تركيزات مختلفة (٥٠ و ١٠٠ و ١٥٠ و ٢٠٠ ملجم/لتر) واختبارها ضد ثلاثة أنواع مختلفة من البكتيريا وهي السلمونيلا المعوية والايشيريشيا كولاى (سالبة لجرام) والمكورات المعوية البرازية، وهي بكتيريا إيجابية لجرام. وبتحليل تلك المستخلصات وجد أنها تحتوي على سابونين، تربين، وفلافونويد، وفقا للتحليل الكيميائي. بالإضافة إلى ذلك تم اكتشاف مجموع الفلافونويد ومجموع الفينول كما أنه وجد لمستخلص تلك النباتات نشاط مضاد للأكسدة وخاصة المستخلص الميثانولى. وكشف التحليل الميكروبيولوجي أن مستخلص أسيتات الإيثيل كان أكثر كفاءة من المستخلصات الأخرى عند كل من ٥٠ و ٢٠٠ PPM ضد سلالة السالمونيلا المعوية. وتبشر النتائج التي تم الحصول عليها بإمكانية تطبيقها في مجال البيوتكنولوجي.

الكلمات المفتاحية : سابونين ، تربينات فلافونيدات ، الفينولات الكلية، النشاط المضاد للاكسدة، النشاط المضاد للبكتريا، البكتريا الممرضة المحمولة بالغذاء.