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Phytochemical Analysis, Antibacterial and Anticancer Activities of the *Physalis Peruviana* Calyces Growing in Egypt

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Abstract

Chemical composition, antimicrobial and anticancer activities of the agriculture wastes of *Physalis peruviana* calyces growing in Egypt was investigated. Phytochemical analysis of the calyces was determined by quantifying protein, ash, fats, fibres, moisture, crude protein, carbohydrates, minerals and amino acids. The methanolic extract of the *Physalis peruviana* calyces was analysed by GC-MS. The antimicrobial activity against Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella sp. and yeast were investigated. The cytotoxicity of the methanolic extract of calyces against human hepatoma (Hep-3B), human gastric (AGS) and human breast (MDA-MB-231) cancer cell lines were evaluated using fluorometric micro culture cytotoxicity assay. Calyces of *Physalis peruviana* was found to contain 9.7% ash, 2.1 % fats, 42.0% fibres, 7.2% moisture, 6.1% protein and 32% carbohydrates. The most abundant minerals detected in calyces were calcium (7.50 mg) and iron (1.38 mg). Essential and non-essential amino acids were also existed. The total of phenols, flavonoids, saponins and antioxidant capacity were evaluated as 525 mg Gallic acid/100 g, 61 mg quercetin /100g, 3.1% and 395 mg AAE/100 g, respectively. GC-MS analysis of the Calyces extract showed the presence of several metabolites. The methanolic extract showed moderate antimicrobial activity against Bacillus subtilis, Escherichia coli, Salmonella sp. and yeast. Additionally, the extract had a cytotoxic activity against human hepatoma (Hep-3B), human gastric (AGS) and human breast (MDA-MB-231) cancer cell lines.

Keyword: Antimicrobial Land Anticancer Activities; Calyces; Chemical Analysis; *Physalis Peruviana*

Introduction

Natural products from fruits source have promising biological activities as anti-ageing, anti-cancer, anti-inflammatory and against several infectious diseases [1,2]. The agriculture wastes of these fruits are produced in large quantities in most local and international markets and may cause several environmental problems [2,3]. Discarding of these wastes may occur by burning or unplanned landfilling. Therefore, researchers are looking for different ways to take the advantages of these agro-industrial wastes. As per literature, skins of different fruits contain several types of bioactive natural products which possibly have a high value of therapeutic importance [4]. Moreover, several types of outer peels as in kiwifruit peels, orange peels, lemon peels and pomegranate peels are used to produce vitamins, antioxidants antimicrobials and anticancer agents [5-7].

Physalis peruviana is one of the valued crops in Egypt [8]. The plant and its fruit are commonly called harankash, cape gooseberry and golden berry. It belongs to the *Solanacea* family and is closely related to the tomatoes. It is used in the traditional medicine as antimalarial, antispasmodic, a diuretic, anti-rheumatic and anti-hepatic therapeutic agent [9,10]. The fruit is rich with phenolic compounds, vitamins and minerals [11]. Several withanolides derivatives has been isolated from the fruit [12,13]. These compounds had several biological activities such as antitumor, antimicrobial, hepatoprotective and anti-inflammatory [14,15]. Additionally, the juice of harankash has dietary and antioxidant properties [16]. Peel and seeds of the fruit are also a rich source of oils, fibres and proteins [17,18]. The fruit of *Physalis peruviana* is surrounded by a papery calyx which protects the fruit against insects, pathogens, and adversative weather conditions. After eating harankash, the straw-colored calyces are thrown in the trash and become useless. As per literature, the calyx can be considered as agro-industrial wastes produced in fruit manufacture as well

as an unexplored source of bioactive compounds. This study was designed to assess the chemical composition, antimicrobial and anticancer activities of the waste calyces of *Physalis peruviana* fruit growing in Egypt.

Materials and Methods

Sample Collection

Physalis was purchased from the different Egyptian local markets during January 2017. Calyces of *Physalis peruviana* were collected, air dried and stored in plastic bags.

Preparation of the Methanolic Extract

Dried calyces of *Physalis peruviana* was grounded to a finely coarse powder. Five grams of the powder was mixed with 20 ml of 80% methanol (v/v) using a Turrax mixer set at 11,000 rpm for 20 seconds [19]. The extract was then centrifuged at 3000 rpm for 30 minutes to remove the residues. The solvent was evaporated under vacuum.

Chemical Analysis

Ash, fats, fibres, moisture and crude protein were determined according to the AOAC methods (1995) [20]. Carbohydrate content was calculated by difference [21].

Mineral and amino acids profiles

Measurement of Fe, Zn and Cu was conducted according to the AOAC (2002) [22]. The amino acids were estimated by the AOAC protocol (2005) [23].

Gas chromatography analysis (GC-MS)

Analysis of the methanolic extract of the *Physalis peruviana* calyces was carried out using gas chromatography (Agilent Technologies 7890A) [24]. The components (Figure S1) were verified by matching their mass spectra and retention time with the database of National Institute of Standard and Technology (NIST) library.

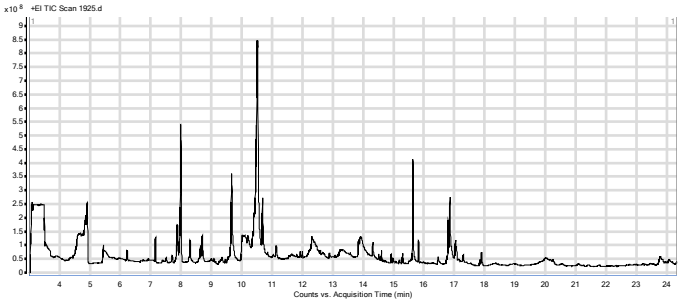

Figure S1: GC-MS chromatogram *Physalis peruviana* calyces crude extract.

Total phenolic, total flavonoids, saponins and antioxidant activity

The total phenolic, total flavonoids, saponins and antioxidant activity of the *Physalis peruviana* calyces were evaluated according to the reported methods in the literature [25-29].

Mycotoxin Determination

Total aflatoxin was performed according to AOAC (1998).

Antimicrobial Activity

Five microorganisms including two Gram-negative bacteria (*E. coli* and *Salmonella* sp.), two Gram-positive bacteria (*Bacillus cereus* and *Bacillus subtilis*) and one fungal strain (yeast) were used in our study. The strains were kindly supplied by Food Safety Laboratory, Regional Centre for Food and Feed, Agricultural Research Centre. The strains were maintained on slants of nutrient agar at 4 ˚C in our laboratory. The microorganisms were cultured in Brain Heart Infusion broth at 37 ˚C for 24 hours. The antibacterial activity of methanolic extract of *Physalis peruviana* calyces was evaluated by the disk diffusion method [30].

Cytotoxicity study

In this study, human hepatoma (Hep-3B), human gastric (AGS) and human breast (MDA-MB-231) cancer cell lines were supplied from the American Type Cell Culture Collection (ATCC, Manassas, VA, USA). The AGS and HeP-3B cells were cultured in Roswell Park Memorial Institute (RPMI) medium with 10% Fetal Bovine Serum (FBS). The MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4.5 g/L of glucose, 4 mmol/l of L-glutamine, and 10% heat-inactivated fatal calf serum [31]. Cancer cells (6000 cells/well) were seeded into a 96-well culture plate in 200 µl RPMI medium containing 10% FBS. Cells were cultured in a humidified atmosphere of 5% CO_2 at 37 ˚C. Extract concentrations ranged from 25-200 µg/ml were separately added to each well and monitored for their anticancer activity. After the 24-hourincubation period, cells were washed with of Phosphate-Buffered Saline (PBS). A 200 µl of PBS containing fluorescein diacetate (10 µg/ml) that was added to each well. The plates were incubated at 37 ˚C for one hour. Fluorescence was detected after one hour. Luteolin (17.5 µM) was used as a positive control [32].

Results and Discussion

Phytochemical Analysis

Phytochemical analysis of *Physalis peruviana* calyces that grows in Egypt is summarized in Tables 1-4. The percentages of ash, fat, fibre, moisture, protein, carbohydrates, minerals and amino acids are calculated based on dry weight of calyces. The results showed that calyces of *Physalis peruviana* are rich with carbohydrate

(42.0%). Carbohydrates are the most abundant nutrient in several fruit peels [33]. Additionally, calyces contain fibre (32.0%), fat (2.1%) and protein (6.1%). The protein content of the *Physalis peruviana* calyces was higher compared with the protein content (1.02%) found in physalis fruit juice. Moreover, the ash and moisture content of calyces is 9.7% and 7.2%, respectively. The percentage of ash content reflects that calyces are rich with some minerals. As shown in Table 2, the level of calcium (7.50 mg) and iron (1.38 mg) is the highest in the calyces of *Physalis peruviana*. Calcium and iron are the major minerals in calyces of *Physalis peruviana*. It was reported that raw physalis juice had high contents of potassium (1210 mg/100 g) and sodium (1210 mg/100 g) [34]. Calcium plays an important role in the interactions between cells walls and ensures the structure of cells by hardening them. It's also an important parameter in the synthesis of ethylene during maturing of the fruits [35]. Iron is vital for energy metabolism, temperature control and immune system [36].

Components	% (based on dry weight basis)
Ash	9.7
Fats	2.1
Fibers	42.0
Moisture	7.2
Protein	6.1
Carbohydrates	32.0

Table 1: Chemical analysis of the dried *Physalis peruviana* calyces.

Minerals	(mg/100g calyces)
Calcium	7.50
Iron	1.38
Copper	0.03
Zinc	0.02

Table 2: Minerals content of the dried *Physalis peruviana* calyces.

Amino acid	%
Aspartic	0.48
Glutamic	0.47
Therionine	0.22
Serine	0.23
Proline	0.25
Glycine	0.30
Alanine	0.32
Valine	0.29
Isoleucine	0.21
Leucine	0.35
Tyrosine	0.18
Phenylalanine	0.23
Hisitidine	0.11
Lysine	0.28
Arginine	0.27
Cysteine	0.16
Methionine	0.10

Table 3: Amino acid profile of the dried *Physalis peruviana* calyces.

Constituent	Results
Total phenolics content (mg gallic acid/100g)	525
Total flavonoids content (mg quercetin /100g)	61
Saponin (%)	3.1
Total antioxidant capacity (mg AAE/100g)	395

Table 4: Phytochemical analysis of the dried *Physalis peruviana* calyces.

To get more data about the phytochemical composition of *Physalis peruviana* calyces, the amino acids were determined and presented in Table 3. From the results, aspartic and glutamic acid were the major free amino acids in calyces with concentrations of 0.48% and 0.47%, respectively. Following in the order is leucine, alanine, glycine and the other amino acids. The amount of total phenolic, flavonoid, saponin and antioxidant capacity of the calyces from *Physalis peruviana* are presented in Table 4. The total phenolic contents were determined as mg gallic acid/100 g extract on comparison with a standard gallic acid curve. The extract showed a high total phenolic content (525 mg gallic acid/100 g). The total flavonoid content (61 mg quercetin /100 g) was determined as mg quercetin /100 g extract after comparison with the quercetin calibration curve. The extract also has 3.1% of crude saponin. As per literature, these compounds can be found not only in the eatable part of the fruits but also in the non-eatable portions and have different biological activities such as antioxidant, antihepatotoxic effects and anti-inflammatory activity [37-39]. The total antioxidant activity of the *Physalis peruviana* calyces was 395

mg Ascorbic Acid Equivalence (AAE)/100 g. The total antioxidant capacity may due to its flavonoids and phenol contents, compared to the fruit [40]. The lower value of the total antioxidant capacity of *Physalis peruviana* fruit was reported in the literature [41]. The dried calyces were free from aflatoxins.

The GC-MS analysis of the methanolic extract of *Physalis peruviana* calyces is shown in Table 5. Twenty-four compounds were identified, where the major predominant constituents were 2',5'-dimethoxyflavone (43.7%), 2',4'-dimethoxy-3-hydroxy-6-methylflavone (12.06%), 3-hydroxy-7,8,2'-trimethoxyflavone (6.39%) and xanthine (2.85%). Several polyphenols (phenolic and flavonoid) were identified in Physalis fruit by GC-MS [42]. According to the best of our knowledge, this research work is the first report about the phytochemical composition of calyces of *Physalis peruviana* cultivated in Egypt.

No.	Retention time	Compound Name	Area (%)
1	4.904	2',5'-dimethoxyflavone	43.7
2	5.804	4-Hydroxychalcone	1.84
3	5.559	7-Hydroxycoumarin-3-carboxylic acid	2.68
4	6.814	3,6,3',4'-Tetramethoxyflavone	1.15
5	7.70	3-(3,4-Dimethoxyphenyl)-7-hydroxy-4-methylcoumarin	1.87
6	7.735	Phytol	1.03
7	7.767	Isovitexin	0.6
8	8.505	Biotin	1.54
9	8.961	Gardenin	1.02
10	10.31	Spironolactone	0.65
11	10.76	3,6,2',3'Tetramethoxyflavone	0.8
12	10.87	Quercetin	0.85
13	11.23	3,2',4',5',6-Pentamethoxyflavone	0.81
14	12.68	3-Hydroxy-7,8,2'-trimethoxyflavone	6.39
15	12.96	Vitexin	1.94
16	14.71	2',4'-Dimethoxy-3-hydroxy-6-methylflavone	12.06
17	15.36	3,5,3',5'-Tetra-tert-butyldiphenoquinone	1.70
18	15.79	Methylprednisolone succinate	1.32
19	17.73	2-Hydroxy-2',4',6'-trimethoxychalcone	1.02
20	18.82	Isovitexin	0.9
21	19.22	Xanthine	2.85
22	19.89	3'-Benzyloxy-5,6,7,4'-tetramethoxyflavone	1.20
23	20.34	3-(3,4-Dimethoxyphenyl)-6-methyl-4-phenylcoumarin	0.65
24	21.31	3,5,3',5'-Tetra-tert-butyldiphenoquinone	2.26

Table 5: Chemical constitutions of the methanolic extract of *Physalis peruviana* calyces by GC-MS.

Antimicrobial and Cytotoxic Activities

The antibacterial activity of the methanolic extract of *Physalis peruviana* calyces (Table 6 and Figure 1) indicated that the extract had effect against Bacillus subtilis, Salmonella sp. and E. coli with inhibition zones of 27 mm, 24 mm and 24 mm, respectively. The extract showed no activity against Bacillus cereus but exhibited effect against yeast with inhibition zone diameter of 23 mm. The biological activities of calyces could be attributed to the presence of phenols, flavonoids, xanthine and saponins. Flavonoids had been demonstrated to possess antimicrobial, antifungal and antitumor activities [43].

Test organism	Zone of inhibition (in mm)
Bacillus subtilis	27
Bacillus cereus	NZ
Escherichia coli	17
Salmonella sp.	24
Yeast	23
NZ = no zone of inhibition	

Table 6: Antimicrobial activity of *Physalis peruviana* calyces methanolic extract.

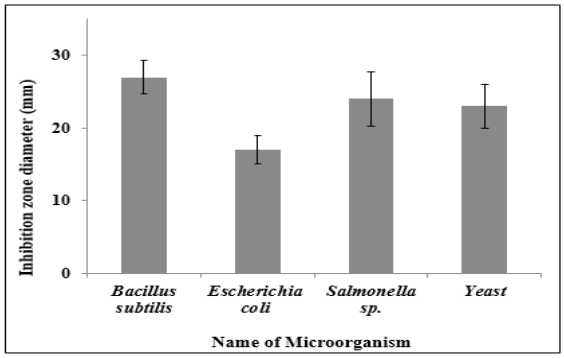

Figure 1: Antimicrobial activity of the methanolic extract of *Physalis peruviana* calyces.

The crude extract of *Physalis peruviana* calyces (Figure 2) were evaluated for their antitumor activities against a human hepatoma (Hep-3B), human gastric (AGS) and human breast (MDA-MB-231) cancer cell lines. Four different concentrations (25, 50, 100 and 200 µg/ml) were used in our study. Interestingly, the antitumor activity of the extract against a human gastric cancer cell line (AGS) is superior compared to the other cell lines. The anticancer activity of plants may be due to minerals, phenols and flavonoids [44]. As per literature, saponins had antibacterial, antioxidant and ant carcinogenic properties [45].

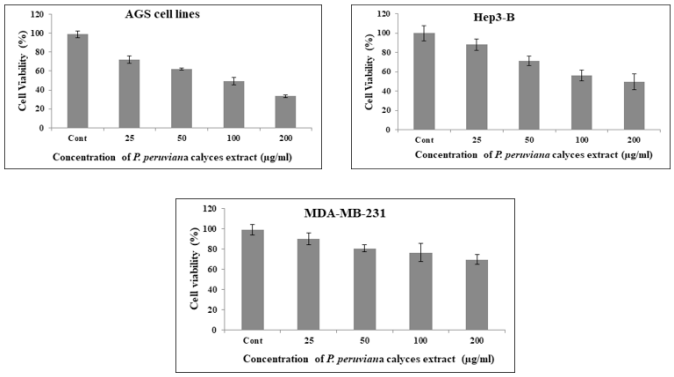

Figure 2: Cytotoxic activity of the crude extract of *Physalis peruviana* calyces against AGS, Hep3-B and MDA-MB-231cell lines.

Conclusion

The agriculture waste of *Physalis peruviana* calyces cultivated in Egypt may be recommended as a good source of antimicrobial, antioxidant and anticancer agents. Toxicological and *in-vivo* studies should be carried out to evaluate the potential effectiveness and safety usage of the extract.

Acknowledgments

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Conflict of Interest

There is no conflict of interest.

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