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ORIGINAL ARTICLE

Physalis peruviana Juice and Seeds Methanolic Extracts; Gas Chromatography Mass Spectrometry; Antioxidant and Anticancer against Human A549, HepG2

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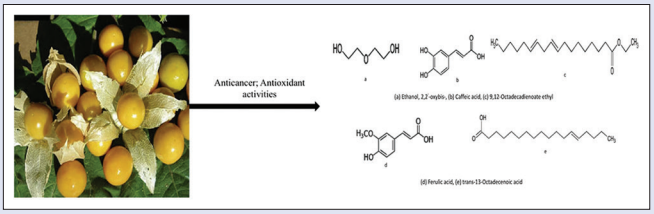
ABSTRACT

Objectives: *Physalis peruviana* L. is a medicinal herb and its consumption increases annually in The Middle East, also the scientific research on it increases due to its valuable nutrient. **Materials and Methods:** Methanolic extracts of *P. peruviana* L. seeds and juice were screened for their anticancer and antioxidant. Gas chromatography-mass spectroscopy profiling was performed for all extracts. . **Results:** The identification of seeds and juice methanolic extract showed the main sex compounds; ethanol, 2,2'-oxybis-, caffeic acid in both of the extracts. Octadecadienoate ethyl and octadecenoic acid have been found in seed extract, and octadecadienoic acid and ferulic acid were in juice extract. Seeds extract has phenolic and flavonoid content as 53.58 and 45.56, respectively, comparing to juice extract (26.58 and 7.30, respectively). The antioxidant activities of seeds extract using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP) (28.73 at 50 µg/ml and 1164.10, respectively) comparing to juice extract values (4.06 at µg/ml and 848.43, respectively). **Conclusion:** The conspicuous optimistic result is that seeds extract showed cancer inhibition against human HepG2 and A549 (81.45 and 85.34, respectively) comparing to juice extract (44.06 and 32.06, respectively). Therefore, the demand to increase the usage of *Physalis* or golden berry in people's diet is a demand to face the environmental oxidative stress.

Key words: 2,2-diphenyl-1-picrylhydrazyl, A549 cell, anticancer, ferric reducing antioxidant potential, gas chromatography mass spectrometry, golden berry, HepG2, phenolic compounds, *Physalis peruviana*

SUMMARY

- *Physalis peruviana* seeds extract could be a good source of anticancer phytochemicals that make the daily usage of golden berry in diet highly recommended to protect the human body from carcinogenic agents.



Abbreviations used: GC-MS: Gas chromatography mass spectrometry; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant potential; HepG2: Liver hepatocellular carcinoma; A549: Human lung carcinoma; TPC: Total phenolic content; TFC: Total flavonoid content; TPTZ: 2,4,6-Tripyridyl-S-triazine; DMSO: Dimethyl sulfoxide.

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INTRODUCTION

The medicinal herb, *Physalis peruviana* (*Solanaceae* family) has been commonly known in India and The Middle East and it was known as golden berry. *Physalis* extracts are mentioned to contain chemopreventive and chemotherapeutic compounds,[1] and it was evaluated as antihepatotoxic for the acute toxicity.[2] Extract administration proved as well to increase hepatic glutathione and reduce malondialdehyde. They disclosed this activity for the diverse constituents in the berries aqueous extract.[3] They showed the importance of *Physalis* ethanolic extract in treating cancer and hepatitis for high antioxidative activity.

The potent compounds in *Physalis* are not fully recognized, but they proved to catch or scavenge reactive oxygen species and their biological oxidative substances.[4,5] Since seeds represent approximately 27% of fruit weight,[6] our research is a trial to study the waste generated importance during juice processing. The biological activity of *P. peruviana* juice and seeds extract has been studied for their potent compounds, phenolic and flavonoid contents which are found to have reducing power, scavenging free radicals, and anticancer agents against human lung and hepatocellular carcinoma cell lines.

MATERIALS AND METHODS

Plant material

Ripen *P. peruviana* fruits were collected from public markets around Minya Governorate, Egypt, in March 2019. The plant was identified by Dr. A. Galal of the Botany Department, Minia University, Egypt. 61519.

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Preparation of *Physalis peruviana* fruit juice and seeds

Fresh fruits of *P. peruviana* were washed and blended in a speed blender (Moulinex Ovatio 3, France) for 15 min, to eliminate the seeds and skin remains (fruit pomace). The fruit juice was obtained by filtration through cheesecloth. Fruit seeds were separated from the skin and then freeze-dried reduce the moisture level to 15%. The dried seeds were ground and kept at 4°C until further process.

Gas chromatography-mass spectrometry analysis

The most appearance valuable compounds in juice and seeds extract were identified operating Trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness). The column oven temperature was initially held at 50°C–200°C by 5°C/min and increased to 300°C by 25°C/min. EI mass spectra data were obtained at 70 eV ionization voltages above the range of *m/z* 50–650 in full scan mode. The chemical constituents were identified by similarity of their retention times and mass spectra data with WILEY 09 and NIST 14 mass spectral database.

Cell culture and reagents

A549 (human lung cancer cell) and HepG2 (human hepatocellular carcinoma) cell lines were obtained from ATCC (Manassas, VA, USA) for this study. The A549 and HepG2 cells were cultivated in RPMI 1640 medium improved with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. The two types of cancer cells were grown at 37°C in a humidified 5% CO_2 incubator. A Bio-Rad TC-20 automated cell counter (Hercules, CA, USA) was used to determine cell growing and viability.

Estimation of total phenolic content

Following the Folin-Ciocalteu colorimetric method, total phenolic content (TPC) of the samples were measured[7] with minor modification for 96-well microplates. Briefly, 15 µl of diluted samples was placed into wells of 96-well microplates (GS, USA). Consequently, 240 µl of Folin was added and left for half an hour in darkness at ambient temperature. Then, 15 µl of Na_2CO_3 (20%) was added to each well, adjust the micro-plate reader at shaken mode before start reading the TPC concentrations. The absorbance was measured at λ = 755 nm with the microplate reader ACCURIS Smart Reader (Edison, NJ, USA). TPC was calculated using a standard curve set of serial dilutions of gallic acid (GAE). TPC values were performed in triplicate and expressed as (mg GAE/g[FM]).

Estimation of total flavonoid content

Following previously described method,[8] to determine the content of total flavonoid with minor modifications, 25 µl of the samples was added to 75 µl of MeOH 96% (v/v). Then, 5 µl of 10% aluminum chloride and 5 µl of potassium acetate, and 140 µl with distilled water added to the previous solutions. The mixture Kept for half an hour in darkness at 25°C, the readings were measured at λ = 415 nm. Kept for half an hour in darkness at 25°C, the readings were measured at λ = 415 nm. Total flavonoid content (TFC) content was calculated using a standard curve prepared using gradient dilutions of quercetin. The TFC was presented as mg QE/g (FM).

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay

The antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.[9] The stock solution was prepared using 10 mg/1 ml dimethyl sulfoxide (DMSO). Serial dilutions for each extract were prepared. Readings were measured using at λ = 515 nm. % DPPH inhibition = $(1-[A_{sample} - A_{background}]/[A_{DMSO} - A_{background}]) \times 100$. Calibration curve was obtained using the inhibition rate values of the standard Trolox solution.

Ferric reducing antioxidant potential

Ferric reducing antioxidant potential (FRAP) assay was performed for evaluating the total antioxidant activity. The assay is established on the reducing power of the antioxidant. A powerful antioxidant reduces the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/2,4,6-tripyridyl-*S*-triazine [TPTZ]), which increases the absorption at 593 nm. Briefly, 20 µl of sample solution was added to the 96-well microplate followed by 280 µl of working FRAP solution. The mixtures were shaken and incubated at 37°C for 30 min in darkness, and then, absorbance was measured using a 96-well microplate reader.[10-13] FRAP working solution was prepared daily and warmed at 37°C for 10 min before use by mixing acetate buffer (300 mM, pH 3.6), TPTZ (40 mM dissolved with 40 mM HCl), and ferric chloride (20 mM in water) (10/1/1 v/v). The FRAP working solution was prepared. The calibration curve was obtained using the inhibition rate values of Trolox.

Cytotoxicity assay

Following the method by Mosmann[14] with slight changes. Using 96-well plates, cells were seeded at 1×10^5 cells/mL/24 h. incubation/5%CO_2/37°C. Then treated with serum-free medium containing extracts at different concentrations in DMSO <2% v/v per well. DMSO group was used as control. Cell viability was determined using automated cell counter (Bio-Rad TC-20) at 24, 48, and 72 h. Results are presented as cell viability percentage as compared to control.

Statistical analysis

Data are presented as means ± standard deviation. Student's *t*-test was used for means comparison at 95% level of confidence; $P < 0.05$.

RESULTS

Identification of compounds from *Physalis peruviana* L. juice and seeds methanol extract using gas chromatography-mass spectrometry

Identification of the potent compounds in juice and seeds extracts has been done using gas chromatography mass spectrometry (GC-MS) [Figure 1a and b]. Integration identification was applied for the highest four compounds in each extract [Figure 1 and Table 1]. Such as structural elucidation of expected bioactive phytochemicals based on accurate mass data, ion source fragmentation, generated molecular formula, and mass database with a bibliographic search.

Diethylene glycol (a) was the highest concentration in both seeds and juice methanolic extract. MS fragments (*m/z*) were 75 (-$HOCH_2$) and 45 represented CH_2CH_2OH fragment. Compounds such as octadecadienoate ethyl, octadecenoic acid, and octadecadienoic acid are found in GC-MS analysis for *Melastomastrum* leaf methanol extract.[15] Octadecadienoate ethyl (c) appeared in seeds extract chromatogram has MS fragments 234, 220, 208, and 129. Fragment *m/z* represents 234 (-CH_3CH_2OCO), 220 represents (-$CH_3CH_2OCOCH_2$), 208 represents (-$CH_3CH_2OCOCH_2CH_2$), and 129 represents $CHCHCH_2CHCH$.

Octadecenoic acid (e) which appeared in seeds extract at 38.61 min showed 111, 97, 83, and 69 *m/z* fragments. First fragment 111 represents $CH_3(CH_2)_3CHCHCH_2CH_2$, 97 represents $CH_3(CH_2)_3CHCHCH_2$, 83 represents $CH_3(CH_2)_3CHCH$, and 69 fragment represents $CH_3(CH_2)_3C$.

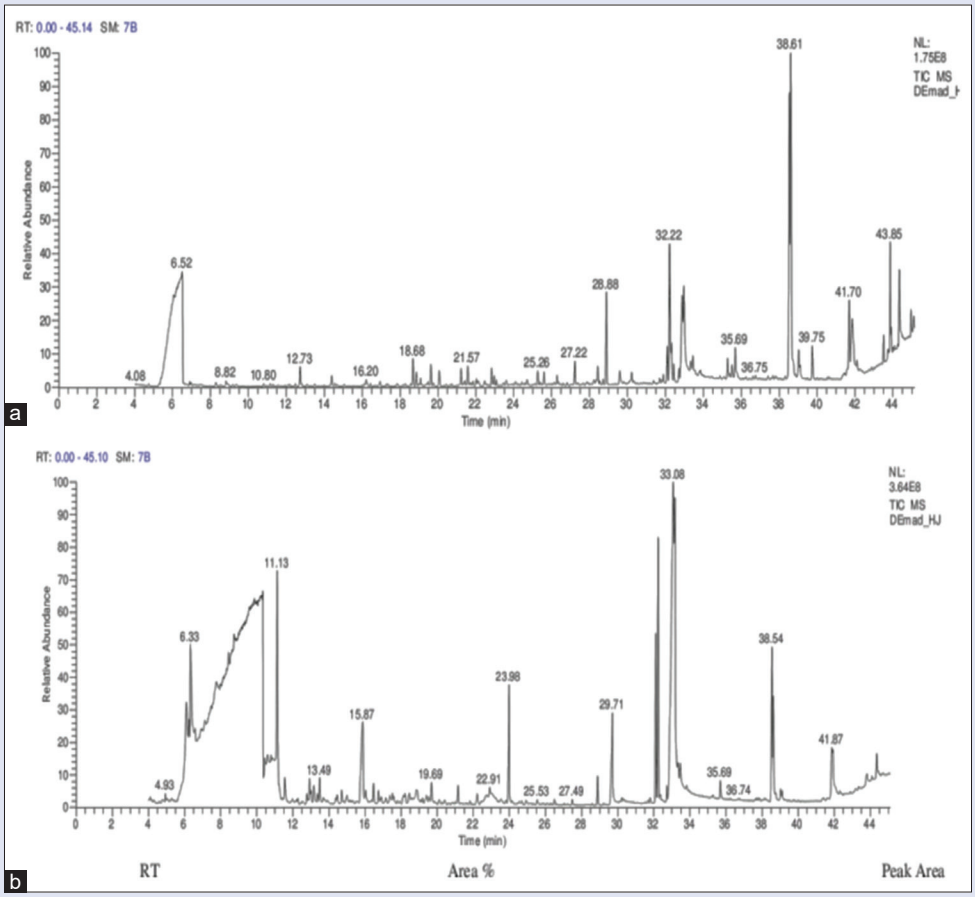

Figure 1: Gas chromatogram of *Physalis* (a) seeds and (b) juice extracts

Table 1: Main compounds in golden berry seeds and juice extracts retention time, peak area percentage, molecular weight, molecular formula, and references

Rt	Percentage peak area	MW	MF	Reference
Seeds				
6.52 (a)	16.14	106	$C_4H_{10}O_3$	Wiley
32.22 (b)	5.70	180	$C_9H_8O_4$	Ballesteros-Vivas *et al.* (2019)
38.54 (c)	10.15	308	$C_{20}H_{36}O_2$	Wiley
38.61 (e)	11.87	282	$C_{18}H_{34}O_2$	Mainlib
Juice				
10.34 (a)	13.31	106	$C_4H_{10}O_3$	Wiley
32.23 (b)	6.99	180	$C_9H_8O_4$	Ballesteros-Vivas *et al.* (2019)
33.09	12.73	280	$C_{18}H_{32}O_2$	Replib
33.17 (d)	6.89	194	$C_{10}H_{10}O_4$	Ballesteros-Vivas *et al.* (2019)

Rts values remarked by identified compounds (a, b, c, d, and e). Rt: Retention time; MW: Molecular weight; MF: Molecular formula

However, 9,12-octadecadienoic acid (z, z) at 33.09 retention time for juice extract chromatogram showed 123, 109, 95, 81, and 67 *m/z* fragments. These fragments were represented by 123 as $CH_3(CH_2)_4CHCHCH_2C$, 109 as $CH_3(CH_2)_4CHCHC$, 95 as $CH_3(CH_2)_4CC$, 81 as $CH_3(CH_2)_3CC$, and 67 as $CH_3(CH_2)_2CC$.

Phenolic acids (caffeic and ferulic acids) were confirmed comparing retention time and MS data with the agreement with the reported data.[16] It was noticed that the percentage peak area of phenolic compounds was between 5.70% and 6.99%. Mass product ions (m/z) fragments for caffeic acid (b) were identified as 151, 135, 122, and 107.[17] Fragments of caffeic acid are represented as 151 (-OOH), 135 (-COOH), 122 (-CH COOH), and 107 (refer to dihydroxy benzene ring fragment). On the other side, mass product ions (*m/z*) fragments for ferulic acid (d) were identified as 178, 134, and 106.[18] Fragments of ferulic acid are represented as 178 (-OH,-2H+), 134 (-CH COOH,-H+), and 106 (-CH CH COOH, -hydroxyl group attached to benzene ring fragment).

Total phenolic acid and flavonoid contents

The methanol extract of *P. peruviana* L. seeds showed high TPC and flavonoids contents (53.58 ± 2.81 and 45.56 ± 7.15, respectively) compared to the methanol extract of juice [Table 2].

The imbalance of the reactive oxygen species and the antioxidant defense system could lead to the need for natural sources of phenolic and flavonoids. Daily intake of valuable foods or health drinks with high phenolic and flavonoids is required [19]

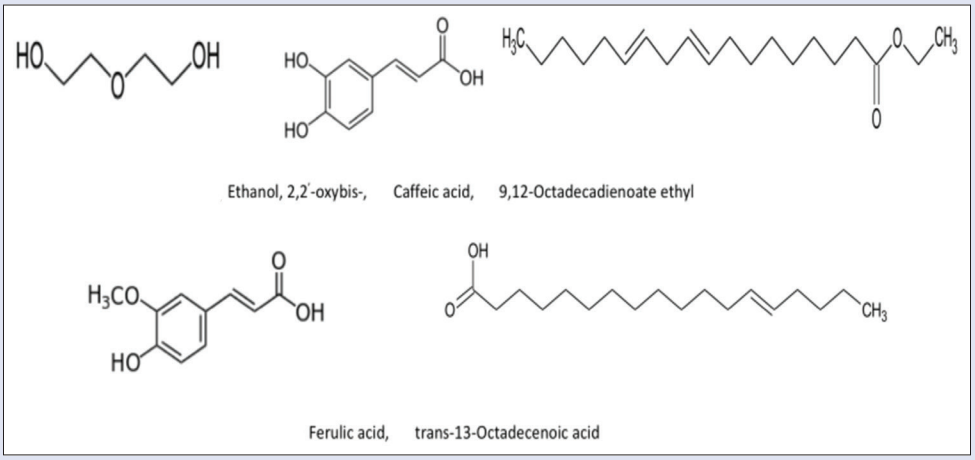

Figure 2: Main expected compounds chemical structure identified using gas chromatography mass spectrometry

Total antioxidant power of extracts from *Physalis peruviana* L. fruit juice and seeds by the ferric reducing antioxidant potential assay and radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl)

Table 3 shows that the methanol extract of the fruit seeds inhibits the potent antioxidant activity at extract concentration 50 µg/extract with percentage value (28.73% ± 0.32%) compared to the standard Trolox (95.5% ± 0.70%). FRAP assay presented the highest antioxidant power in fruit seed extract with value (1164.10 ± 14.73 µM) compared to the standard Trolox (3707.77 ± 4.50 µM).

These results correlated to that of Wu *et al.*,[3] who found that 10–100 µg/ml had potent inhibition rate on $FeCl_2$–ascorbic acid–induced lipid peroxidation in rat liver homogenate. It showed stronger antioxidative activity than α-tocopherol. The radical scavenging activity as mentioned was increased with higher concentrations of *Physalis* seeds extract. That was in a complete agreement with our results which showed that seed methanolic extracts showed dramatic increase for capturing DPPH radicals with increasing the concentration from 12.5 up to 50 µg/ml. The water-soluble Trolox, the analog of Vitamin E is used in biological or biochemical applications to reduce oxidative stress or damage.

Cytotoxic activity of *Physalis peruviana* L. fruit juice and seeds extracts using two different cancer cell lines HepG2 and A549

In the HepG2 and A549 cancer cells, the fruit seed extract was found to be more effective than the juice extract with values (81.45 ± 0.32 and 85.34 ± 1.23, respectively) as shown in Table 4. Furthermore, juice and doxorubicin were more efficient toward human hepatocarcinoma, but for seed extract, the opposite happened in the potential effect.

Comparing our extracts with the standard anticancer drug Doxorubicin, seed extract showed a promising anticancer activity against human lung and hepatic carcinoma cells.

The most essential four compounds appearing in seeds and juice methanolic extracts contain hydroxyl groups and double bonds in identified compounds [Table 1 and Figure 2], with the potent phenolic and methoxy groups found in phenolic acids (caffeic and ferulic acids) explaining the potential efficiency as reducing, free radical scavenging, and inhibition carcinogenic effects.

Table 2: Total phenolic and flavonoid contents of the methanolic extracts from *Physalis peruviana* L. fruit juice and seeds

Extracts/fractions	Total phenolic content	Total flavonoid content
Juice	26.58±0.63	7.30±0.06
Seed	53.58±2.81	45.56±7.15

TPC expressed in mg gallic acid equivalents/100 g dry weight of PP; TFC expressed in mg quercetin equivalents/100 g dry weight of PP; Each value is the mean±SD of triplicate measurements. The data are presented as the mean±SD of technical replicates (*n*=9). SD: Standard deviation; TFC: Total flavonoid content; TPC: Total phenolic content

Table 3: Percentage of DPPH inhibition and FRAP values for *Physalis peruviana L.* fruit juice and seeds extracts

Extracts/ Fractions	DPPH inhibition (%)			FRAP (µM Trolox)
	50 µg/ml	25 µg/ml	12.5 µg/ml	
Juice	4.06±0.59	2.44±1.63	1.84±0.02	848.43±35.56
Seed	28.73±0.32	12.55±1.62	4.17±1.01	1164.10±14.73
Trolox (µM)	95.5±0.70	57.1±1.44	29.8±0.80	3707.77±4.50

The data are presented as the mean ± SD of technical replicates (*n*=9). FRAP expressed in µM Trolox/100 g dry weight

Table 4: Cytotoxic activity (%) of *Physalis peruviana* L. fruit juice and seeds extracts

Extracts/fractions	100 µg/ml	
	HepG2	A549
Juice	44.06±0.59	32.06±2.12
Seed	81.45±0.32	85.34±1.23
Doxorubicin (µM)	98.33±0.70	96.10±1.42

The inhibition expressed as the mean±SD of 3 technical and 3 biological replicates (*n*=9). SD: Standard deviation

The inhibition activity of seeds extract was more than that for juice extract against human lung and hepatocellular carcinoma. This result was much clearer in the scavenging activity against DPPH radicals and the reducing power against ferric ions. These data are due to the valuable structures for having the methoxy and phenolic groups. The widespread properties of *Physalis* extracts encourage scientists to progress in studying the molecular mechanism of the compounds of this promising herb as a modern phytomedicine against many ailments that need more studies.

CONCLUSION

Our results showed that the medicinal plants like *Physalis peruviana* L. are a good source of antioxidant and anticancer phytochemicals. Future studies will be conducted to study the different compounds responsible for the antioxidant and anticancer activities.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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