

# Journal of Advanced Pharmacy Research



## Section A: Natural Products & Metabolomics

### Phytochemical Screening and Antimicrobial Activity of *Physalis peruviana* L. Calyces

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Submitted on: 03-11-2021; Revised on: 03-12-2021; Accepted on: 05-12-2021

To cite this article: Korany, E. M.; Soliman, H. S. M.; Abd El Gawwad, M. M.; El Naggar, N. M.; Ibrahim, H. A. Phytochemical Screening and Antimicrobial activity of *Physalis peruviana* L. Calyces. *J. Adv. Pharm. Res.* 2022, 6 (1), 1-5. DOI: [10.21608/aprh.2021.104236.1144](https://doi.org/10.21608/aprh.2021.104236.1144)

#### ABSTRACT

**Background:** The Goldenberry (*Physalis peruviana* L.) calyces are rich in phytochemical compounds which have promising biological activity. **Objective:** In the present study, a preliminary phytochemical screening was performed to explore the nature of calyces. The total methanolic extract and *n*-butanol fraction of *P. peruviana* calyces were investigated in terms of their antimicrobial activity. **Methods:** Methanol extract and *n*-butanol fraction were tested for their antimicrobial activity against Gram-positive, Gram-negative bacteria, and *Candida albicans* using the disc diffusion method. **Results:** Phytochemical screening showed that calyces are rich in saponins, flavonoids, Steroids and/or triterpenes, Carbohydrates and/or glycosides; while Alkaloids and/or nitrogenous base, tannins, Anthraquinones were absent. The *n*-butanol fraction showed inhibition zones ( $16 \pm 0.16$  mm) for *B. subtilis* and ( $10 \pm 0.17$  mm) for *S. aureus*, while the methanolic extract showed inhibition zones of ( $13 \pm 0.21$  mm) and ( $8 \pm 0.18$  mm) for *B. subtilis* and *S. aureus*, respectively. On the other hand, no inhibition zones were detected for Gram-negative bacteria or *C. albicans*. **Conclusion:** The total methanolic extract and *n*-butanol fraction displayed strong antibacterial activity against *B. subtilis* than *S. aureus*, while both of them didn't show activity against Gram-negative strains or *C. albicans*.

**Keywords:** *Physalis peruviana* L.; calyces; antimicrobial; inhibition zone.

#### INTRODUCTION

Family *Solanaceae* or nightshade is an important family of flowering plants that consists of nearly 100 genera and more than 3000 species. This family occurs in different regions of the world ranging from deserts to rainforests and contains different forms of annual herbs to rainforest trees. The family has large economic importance either in medicine as sources of

alkaloids (atropine, hyoscyne, etc.) or in foods (e.g., capsicum, tomato, potato, eggplants, etc.).<sup>1</sup>

*Physalis* is a genus of herbaceous plants belonging to the family *Solanaceae*. Such genus contains approximately 120 species, mainly distributed in tropical and temperate regions. Most species of *Physalis* have been used for a long time in folk medicine to treat different illnesses, such as dermatitis, hepatitis, liver disorders, malaria, asthma, *mycobacterium*, leukemia,

and as antipyretic, anticancer, and immunomodulatory agent.<sup>2</sup>

*Physalis peruviana* L. is an exotic fruit indigenous to South America in high altitude tropical Colombia, Ecuador, Chile and Peru where the fruit grows wild. It is cultivated in different climatic conditions of tropical, subtropical as well as temperate regions. The fruits are yellow in color and surrounded with papery calyx resembling tomato in appearance and flavor while the taste is sweet and sour<sup>3</sup>. The fruits are eaten raw or used in salads, cocktails, jams, jellies, ice cream, and in desserts.<sup>4</sup>

The fruits contain a lot of valuable constituents like minerals, carbohydrates, lipids, flavonoids<sup>5</sup>, polyphenols, vitamins, organic acids, withanolides<sup>6,7</sup>, carotenoids, flavors compounds, and phyosterols. The fruit has diverse activity as antioxidant<sup>8</sup>, anti-cancer<sup>9</sup>, anti-hepatotoxic<sup>10</sup>, anti-neurotoxic, renoprotective<sup>11</sup>, hypoglycemic, anti-hypertensive, anti-hypercholesterolemia<sup>12</sup>, anti-inflammatory<sup>13</sup>, and antimicrobial activities.<sup>14,15</sup>

Despite its health benefits, this fruit is still cultivated in limited areas of Egypt and no attention has been paid to utilize this fruit in food industries.<sup>16</sup>

The aim of this work is to evaluate the antimicrobial activity of *Physalis peruviana* L. calyces and to explore the nature of phytochemicals inside through phytochemical screening.

## MATERIAL AND METHODS

### Plant material

*Physalis peruviana* L. calyces were collected from Nesmaya Farm, Shebin El-Qanater, Qalyubia, Egypt in April 2019. The plant was identified by Dr. Therese Labib, former plant taxonomy specialist at El Orman Botanical Garden, Giza, Egypt. A voucher specimen was kept in the herbarium of Pharmacognosy department in Helwan university with a serial number 27Ppe1 /2021.

### Preliminary phytochemical screening

Phytochemical screening was performed using standard procedures.<sup>17,18</sup>

Air dried powdered calyces of *P. peruviana* L. were screened for the following constituents: tannins, saponins, flavonoids, steroids and/or triterpenes, alkaloids and/or nitrogenous base, cardiac glycosides, Anthraquinones, combined Anthraquinones and carbohydrates and/or glycosides.

### Microbial Strain

The microorganisms used in this study were obtained from the American Type Culture Collection (ATCC) as well as the culture collection of the Microbiology Lab, Cairo University Research Park

(CURP), Faculty of Agriculture, Cairo University. In this sense, **Table 1.** illustrates the microorganisms and cultivation conditions used in this study.

**Table 1. Microbial strains and their cultivation conditions**

Microbial group	Indicator strain	Cultivation conditions
Gram-positive bacteria	<i>Staphylococcus aureus</i> (ATCC 25923)	Muller-Hinton agar, 30°C/24h
	<i>Bacillus subtilis</i> (ATCC 6051)	Muller-Hinton agar, 30°C/24h
Gram-negative bacteria	<i>Escherichia coli</i> (ATCC 8739)	Muller-Hinton agar, 30°C/24h
	<i>Pseudomonas aeruginosa</i> (ATCC 15692)	Muller-Hinton agar, 30°C/24h
Fungus	<i>Candida albicans</i> (ATCC 10231)	Malt agar, 25°C/3 days

### Extraction Method

100 g of the air-dried and coarsely grounded calyces were firstly defatted with *n*-hexane then extracted with 500 mL 80% aqueous methanol at room temperature. The methanol extract was dried under reduced pressure to obtain a crude residue (10 g). 5 g of the total methanolic extract was suspended in 100 mL distilled water and partitioned with (3 x 100 mL) *n*-butanol. The combined *n*-butanol extracts were dried under reduced pressure to obtain an *n*-butanol fraction (1.5 g).

### Antimicrobial activity

*Physalis peruviana* L. calyces total methanol extract and *n*-butanol fractions were tested for their antimicrobial activity using the disc diffusion method.<sup>19,20,21</sup> The inoculum suspension was made by inoculating Mueller-Hinton broth with colonies grown overnight on an agar plate (fungi using malt broth). A sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates (fungi using malt agar plates). The extracts were dissolved in dimethyl sulfoxide (DMSO) with a concentration (20 mg/ml). 5 mm diameter filter paper discs were sterilized in an autoclave and immersed in the solution of the prepared extract then applied to the surface of the agar plates. The plates were incubated at the optimum temperature for each indicator strain (**Table 1**). The inhibition zones were measured around each disc after 24, 48, and 72 h. Growth inhibition was scored positive in the presence of a clear detectable zone (ZI) around each disc and measured by a ruler in mm. Experiments were carried out

using DMSO as negative control and in triplicates where the inhibition zone was recorded as the average of the replicates  $\pm$  SD.

#### Determination of minimum inhibitory concentration (MIC)

MIC was determined by serial dilution in a microtiter plate (two-fold dilution).<sup>22</sup>

### RESULTS

#### Results of phytochemical screening

Results of phytochemical screening as compiled in **Table 2**. Phytochemical screening showed the presence of saponins, flavonoids, Steroids and/or triterpenes, Carbohydrates and/or glycosides. Alkaloids and/or nitrogenous base, tannins, anthraquinones and Combined anthraquinones are absent from the calyces.

**Table 2. Results of preliminary phytochemical screening of *P. peruviana* L. calyces**

Tested compounds	Results
Tannins	(-)
Saponins	(+)
Flavonoids	(+)
Steroids and/or triterpenes	(+)
Alkaloids and/or nitrogenous base	(-)
Cardiac glycosides	(-)
Anthraquinones	(-)
Combined Anthraquinones	(-)
Carbohydrates and/or glycosides	(+)

N.B. (+) = present, (-) = absent

#### Results of antimicrobial activity

Results of antimicrobial activity are compiled in table 3 and represented as the diameter of the inhibition zone (ZI) of the tested extracts.

The inhibition zone (ZI) diameter of the *n*-butanol fraction is (16  $\pm$  0.16 mm) for *B. subtilis* and (10  $\pm$  .17 mm) for *S. aureus* where the inhibition zone (ZI) diameter of the methanolic extract is (13  $\pm$  0.21 mm) for *B. subtilis* and (8  $\pm$  0.18 mm) for *S. aureus* on, the other hand no clear zones were detected for Gram-negative bacteria or *C. albicans*.

As for the minimum inhibitory concentration of the extracts they are compiled in **Table 4**. and represented as  $\mu$ g /mL.

The minimum inhibitory concentration of the *n*-butanol fraction against *B. subtilis* and *S. aureus* is 0.585 and 1.17  $\mu$ g/mL respectively also the minimum inhibitory concentration of methanol extract against *B. subtilis* and *S. aureus* is 1.17 and 2.342  $\mu$ g/mL respectively while the minimum inhibitory concentration of clindamycin reference against *B. subtilis* and *S. aureus* is 0.25 and 0.50 respectively.

### DISCUSSION

Due to the resistance of human pathogenic microorganisms to the major antibiotics it was crucial to search for new antibacterial agents from natural sources. Results of phytochemical screening showed that calyces of *P. peruviana* are rich in saponins, flavonoids, Steroids and/or triterpenes. Many research articles attributed the antimicrobial activity of many medicinal plants to its content of phytochemicals. Terpenoidal compounds are used in the treatment of bacterial infections due to their lipophilic properties which allow them to be easily interacting with the bacterial cell wall, interfering with the biosynthesis of its components as well as they can penetrate the bacterial cell and may also interfere with protein synthesis and DNA replication and repair mechanisms.<sup>23</sup>

Flavonoids are a large class of natural compounds, have been extensively studied for their antibacterial activity, and more than 150 articles have been published on this topic since 2005. Over the past decade, some promising results were obtained with the antibacterial activity of flavonoids through different mechanisms. In some cases, flavonoids showed up to sixfold stronger antibacterial activity than standard drugs in the market.<sup>24</sup> So it was motivating to investigate the antimicrobial activity of *P. peruviana* calyces seeking for new natural antimicrobial extracts.

The data obtained from this study showed that the *n*-butanol fraction has higher antibacterial activity against Gram-positive *Bacillus subtilis* (ZI= 16 mm) than *Staphylococcus aureus* (ZI= 10 mm) also methanol extract has higher antibacterial activity against Gram-positive *Bacillus subtilis* (ZI= 13 mm) than *Staphylococcus aureus* (ZI=8 mm). On the other hand, the methanol extract or *n*-butanol fraction has no antimicrobial activity against Gram-negative *E. coli* or *P. aeruginosa* and no antifungal activity against *C. albicans*.

Also, the data obtained from MIC showed that the *n*-butanol fraction has a lower concentration (0.585  $\mu$ g/mL) than the methanol extract (1.17  $\mu$ g/mL) to inhibit *B. subtilis* growth while in the case of *S. aureus* the MIC of the *n*-butanol fraction is (1.17  $\mu$ g/mL) and of the methanol extract is (2.342  $\mu$ g/mL) Therefore, the *n*-butanol fraction has the lowest concentration to inhibit microbial growth.

Table 3. Results of the antimicrobial activity of *Physalis peruviana* L. calyces extracts

Samples	(ZI) Inhibition zone (mm)				
	Gram-positive bacteria		Gram-negative bacteria		Fungus
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>p.aeruginosa</i>	<i>C. albicans</i>
Methanol extract	8 ± 0.18 mm	13 ± 0.21 mm	NA	NA	NA
<i>n</i> -Butanol fraction	10 ± .17 mm	16 ± 0.16 mm	AN	NA	NA
Positive control	Clindamycin		Gentamycin		Polymyxin
	20 ± 0.11 mm		22 ± 0.21 mm		14 ± 0.32 mm
Negative control	DMSO				

Table 4. Results of MIC for the active fractions

MIC (µg/mL)	<i>S. aureus</i>	<i>B. subtilis</i>
Methanol extract	2.342	1.17
<i>n</i> -butanol fraction	1.17	0.585
Clindamycin	0.50	0.25

## CONCLUSION

The total methanolic extract and *n*-butanol fraction of *Physalis peruviana* L. calyces displayed strong antibacterial activity against *B. subtilis* than *S. aureus*, while both of them didn't show activity against Gram-negative strains or *C. albicans*. The activity is expected to be contributed to the active constituents like flavonoids and triterpenoidal saponins whereas their concentration is higher in the *n*-butanol fraction than the methanolic extract, so the *n*-butanol fraction is more active than the methanolic extract.

## Recommendations

From the obtained results it is worthy to perform further investigations on *Physalis peruviana* L. calyces.

## Funding Acknowledgment

No external funding was received.

## Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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