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Microbialactivity, Phytochemical Constituents and Total Phenolics Content of Physalis Angulata

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KEYWORDS

Anti-microbial activity, Correlation study, *Physalis angulata*, Phytochemical evaluations, TLC, HPLC.

ABSTRACT:

Introduction: One of the primary reasons of this issue is the widespread occurrence of acquired bacterial resistance to antibiotics, which poses a major threat to public health worldwide today in the form of pandemics and epidemics of antibiotic resistance.

Objective: This current research project involves conducting phytochemical, pharmacognostical, and antimicrobial studies on *Physalis angulata*.

Methods: The plant material in powdered form was extracted using different solvents including ethanol, ethyl acetate, chloroform, and water. The solvent extracts were comprehensively characterized through a series of evaluation tests, including phytochemical evaluation through chemical tests, proximate analysis, for bioactive compounds present in the extract. Furthermore, the antimicrobial potential of the extracts was evaluated using disc diffusion method. The antimicrobial activity was further assessed by determining the minimum inhibitory concentration.

Results: Result revealed that presence of alkaloids, glycosides, flavonoids, terpenoids, and phytosterols in ethanol solvent and other solvents contain limited bioactive compounds. Proximate analysis data showed presence of moisture of 9.42 %, total ash content 8.47 %, ethanol soluble extractive value 7.84%, water soluble extractive value 4.56%. Further, total phenolic content was estimated and revealed presence of 256.23 mg/g plant extract (GAE). Finally, antimicrobial activity of the extract was performed against Gram positive (*S. aureus* and *B. subtilis*), Gram negative (*E. coli*, and *Pseudomonas aeruginosa*) bacteria and two fungal organisms *Aspergillus niger*, *Candida auris* after performed MIC and MBC tests.

Conclusion: Result revealed dose dependent antimicrobial activity and finally result correlated with the content of total phenolics which showed positive correlation.

1. Introduction

Recently, individuals are increasingly vulnerable to microbial infections, which are the most lethal for humans and can lead to fatalities. Bacteria and fungi are the victims to cause various microbial infections like urinary tract infections, topical/skin infection and respiratory tract infections. *Physalis angulata* (PA) is an herbaceous plant classified under the nightshade category of the Solanaceae family. Commonly the plant is known as wild gooseberry and its roots and leaves are most useful parts (Mahklouf, 2019). Traditionally, the plant is used in intestinal and digestive problems, sores, boils, cuts, etc. (Jiangjie et al., 2019). The leaves are used as antiallergic, antiasthmatic, antileishmanial, antmalarial, and immunomodulatory activity (Shangguo et al., 2020). It also possesses versatile pharmacological activities like Anti-microbial activity (Jayachithra et al.,

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2022), Anti-cancer (Yang et al., 2017), Anti-inflammatory (Ramakrishna Pillai et al., 2022), Anti-nociceptive (Bastos et al., 2006), Immunosuppressive, Antileishmanial (Elisalva et al., 2009), Anti-asthmatic (Rathore et al., 2011), Anti-parasitic, Anti-mycobacterial (Pietro et al., 2000), Anti-malarial and Diuretics activities (Ankrah et al., 2003). All the therapeutic activities are due to presence of various phytoconstituents especially, alkaloids, flavonoids, terpenoids, etc. Apart from that, some specific constituents like withanolide such as physalins A–I, physagulin A–G, withangulatin A are important (Qinghong et al., 2019). In the present study, phytochemical screening, followed by TLC and HPLC studies were carried out for identification, separation and quantification of phenolic compound which was not carried out previous. Thereafter, investigations on the antibacterial characteristics of PA and its constituent parts have been motivated by the need to find novel, safer, and more affordable therapies that can tackle the problem of antibiotic resistance. The effects of PA against different Gram-positive and Gram-negative bacteria are still being studied. Furthermore, correlation study was also carried out with the total phenolic present in the extract with the microbial study.

2. Materials and Methods:

Collection and processing of plant

Aerial parts of *Physalis angulata* was collected from lake shores of Guntur district, Andhra Pradesh, and was authenticated by authenticated by Dr. K. Madhava Chetty, Plant Taxonomist, SV University, Tirupati, Andhra Pradesh, India (Voucher number-0327). The plant was dried by shade drying, which is employed to preserve antioxidants, flavonoids, alkaloids, and other valuable compounds. Following the drying process, the plants were ground into a powder using a grinder or mixer and further extracted by soxhlet method (500 g of dried aerial parts) using chloroform, ethyl acetate, ethanol and water as solvents. The yield of the extracts was determined and finally, phytochemical analysis was carried out using various chemical tests.

Proximate analysis:

Moisture content, ash contents and extractive values were determined as per the method described earlier and results were tabulated in result section (Adamu et al., 2017). Further, TLC was performed using toluene, ethyl acetate and formic acid to identify various components present in the extract followed by HPLC analysis for separation and estimation of the component.

Mobile phase preparation

Mobile phase was prepared by mixing Methanol: Water (60:40). This solution was filtered using a 0.45 micron Millipore filter paper and was sonicated for 10mins. The total volume of the mobile phase prepared was 500ml.

Standard preparation 10 mg of Gallic acid was taken in 10ml volumetric flask and make up the volume to 10 ml with methanol (the concentration of this solution is 1000 µg/ml). This is a working solution of 10 µg/ml. It was sonicated for 8 mins then the solution was filtered using 0.45 micron Millipore filters.

Sample preparation To 1mg of the given sample 1ml of respective solvent was added. The solution was vortex for 5mins. The sample was filtered using 0.45 micron Millipore filters. 20µl of this sample was injected in the HPLC system.

Determination of total Phenolics:

The Folin-Ciocalteu spectrophotometric method was used for determination of total phenolics content. To a 2.5 mL solution of Folin-Ciocalteu's reagent (0.2 M), a 0.5 ml aliquot from each extract was added. After allowing the solution to settle for five minutes, two milliliters of 7.5% p/v sodium carbonate (Merk, Mumbai, India) were added. After a 15-minute reaction time at 40 degree C, the final color absorbance was measured at 760 nm in a UV-Vis spectrophotometer. Gallic acid was used in a calibration curve with varying concentrations (10 to 70 mg/l) to determine the concentration data of the phenolic compounds. The amount of total phenolic compounds in each liter of sample was quantified in milligrams of gallic acid equivalents (Kalpoutzakis et al., 2023).

Antimicrobial Assay:

Test organisms:Two Gram positive (*S. aureus* and *B. subtilis*), Gram negative (*E. coli*, and *Pseudomonas aeruginosa*) and two fungal organisms *Aspergillus niger*, *Candida auris* were selected. All organisms obtained and well identified onto Microbiology Department, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur.

Disk diffusion method:

Each bacterium was isolated into pure colonies and kept in sterile saline until the turbidity matched McFarland tube number 0.5 (1.5×10^8 CFU/ml). On Muller Hinton

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agar, a loopful of each adjusted organism was swabbed. Sterile paper discs of 6 mm in diameter were impregnated with 100 µl of diluted extracts and conc. prepared with the range of 20 to 100 mg/ml. The discs were then dried in a hot air oven at 100 °C for two hours, and the surface of the infected agar plate was covered with them. The plates were then incubated in accordance with each organism's requirements for growth. The antibacterial activity of each sample was assessed by measuring and recording the zones of inhibition in millimeters (mm), taking into account the 6 mm disk. The tests were conducted in triplicate. To compare the antimicrobial potency of commercial antimicrobial agents with that of plant extracts, a parallel analysis study was carried out using standard Gentamicin (10 µg). Nystatin (10 µg) was used as standard for determination of antifungal activity. Similar way, the zones of growth inhibition was measured after incubation of 48 to 96 hours for fungi at 28°C.

Determination of MIC:

Using the dilution procedure, extracts were diluted twice in serial dilution at concentrations ranging from 600 to 1000 µg/ml. One milliliter of each extract dilution was combined with nine milliliters of Muller Hinton Agar. Each standardized broth culture, containing 1.5×10^8 CFU/ml, was grown in ten microliters on plates with different extract concentrations. After that, the plates were incubated in accordance with each organism's requirements for growth, and any apparent bacterial growth was noted. The minimum extract concentration (MIC) required to produce no discernible growth on the agar's surface.

Determination of MBC:

For the purpose of determining the MBC, agar plates that did not develop following MIC tests were inoculated with new nutrient broth, which served as the recovery medium. The broths were incubated in accordance with each organism's requirements for growth. The recovery medium's lack of turbidity served as proof that the bacteria had been killed.

Statistical analysis

Means ± SEm were used to calculate the inhibition zones. Using the Microsoft Excel program, an analysis of variance (ANOVA) was used to determine the significance. The data were found to differ significantly at the 5% level of significance.

Results and Discussion

Yield of the extract

Different solvents such as chloroform, ethyl acetate, ethanol and aqueous solvents were used for the extraction and the extract was higher in case of ethanol solvent (28.34 g of dried extract) followed by aqueous extract (18.34 g), ethyl acetate extract (15.42 g) and chloroform extract (9.23 g). The solvent ethanol is most suitable for extraction of plant constituents. Many literatures revealed the same and based on that the similar effect observed in the present investigation. This was may be due to solubility of the constituents in ethanol was more than other solvents (Sulaiman et al., 2015; Das et al., 2022). *Phytochemical analysis*

Various extracts were further screened for presence of group of bioactive compounds in the plants. It was observed that maximum groups of phytochemicals were present in ethanol extract (Table-1).

Table-1: Screening of Phytoconstituents by chemical tests

Tests performed for the presence of Phyto-constituents	**OBSERVATIONS**			
	Ethanolic extract	**Ethyl Acetate extract**	**Chloroform extract**	**Aqueous extract**
Alkaloids	+	-	-	+
Carbohydrates and Reducing sugars	-	-	-	+
Glycosides	+	-	-	-
Proteins and Amino acids	-	-	-	-
Steroids and Triterpenoids	+	+	-	-
Phenolic compounds	+	+	+	+
Flavonoids	+	+	-	+

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Fixed oils and Fats	-	-	-	-
Volatiles oils	+	+	+	+
Gums and mucilage	-	-	-	-
Tannins	-	-	-	-

Results

Proximate analysis

Crude powdered drug was estimated for moisture content, total ash content and extractive values and the result was tabulated in table-2.

Table-2: Proximate analysis of PA

Component	**% content**
Moisture content	9.42 ± 0.23
Total Ash content	8.47 ± 0.12
Water soluble ash	3.47 ± 0.41
Acid insoluble ash	2.19 ± 0.20
Ethanol soluble extractive	7.84 ± 0.62
Water soluble extractive	4.56 ± 0.15

• Values are mean ± SEm (n =3)

It was seen that aerial part of plants content moisture per cent higher of 9.42 where many earlier scientific literatures are also reported similar kind of report (Forkel et al., 2023). Water soluble ash and acid insoluble ash content were lower that indicated that the plant content lower impurities. Further, ethanol soluble extractive values was higher than of water soluble extractive values which indicated that ethanol is the suitable solvent for the extraction of the PA plant drug. The result of the present investigation was followed the similar trend with the previous literature reported (Onwuka, 2005; Iheanacho and Ubebani, 2009).

TLC separation and identification:

Toluene, ethyl acetate and formic acid (4.5: 4.5: 1) was applied as solvent system and TLC was carried out to identify various phenolics components present in the extract. Result revealed some five components were separated at various Rf values (Figure-1).

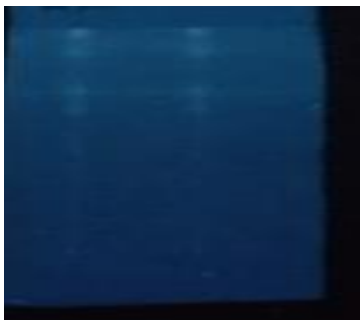

Figure-1: TLC of PA ethanol extract

HPLC determination:The condition for HPLC was as follows:

Detector: Shimadzu spd10A uv-vis, Japan

Pump: Shimadzu LC-10ATVP, Japan

Injection valve: 7725i Rheodyne 20µl, USA

Syringe: 50µl Hamilton, Switzerland,

Column: Phenomenex Gemini-NX-5 µm C18(2) 110 Å, LC Column 250 x 4.6 mm

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Dimensions: 250 x 4.6 mm ID

Elution Type: Isocratic
Elution A: Methanol
Elution B: Methanol:water(60:40)
Flow Rate: 1mL/min
Col. Temp: ambient
Detection: UV-Vis Abs.-Variable Wave. (UV) 203nm

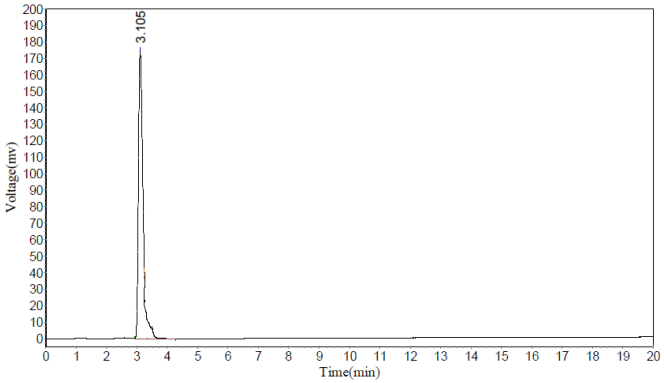

Figure-2: HPLC of standard Gallic acid

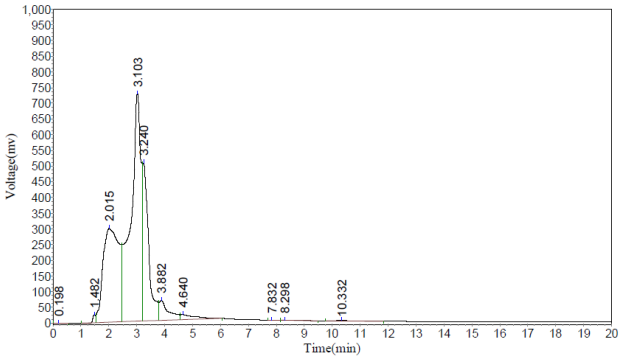

Figure-3: HPLC of presence of Gallic acid in PA extract

HPLC data showed the presence of gallic acid in the extract was 0.872 mg when compared with the standard value of gallic acid which was eluted at 3.103 and 3.105 min respectively.

Determination of total Phenolics:The total phenolics content (mg/g) of all the extracts was determined from a standard curve of Gallic acid (R^2 = 0.997) and expressed as gallic acid equivalents (GAE), and it varied from 38.48 to 256.23 mg GAE/g of the extract. It was observed that ethanol extract showed higher phenolic content (2 mg) followed by ethyl acetate (167.33 mg), aqueous (98.23 mg) and chloroform extract (38.48 mg).

Antimicrobial Activity:

Various concentrations of different PA extracts were performed for antimicrobial efficacy. The whole result was tabulated in table-3. It was observed that concentration dependent activity resulted by the extracts for all the organisms. Among the extracts, ethanol extract showed higher results for all the cases but showed higher inhibition for Gram negative organisms than Gram positive organisms. Thereafter, it was also resulted that all the extracts were also effective against fungal infection when tested against *Aspergillus niger*, and *Candida auris*. Interestingly, chloroform extract showed good result than aqueous extract. But similar trend followed for the fugal inhibition as that of antibacterial activity. All the results were compared with standard drugs i.e Gentamicin for bacterial inhibition whereas, Nystatin for fungal inhibition. Earlier many literatures resulted antibacterial activity against broad spectrum antibiotic such as Gentamicin (Parvez et al., 2019; Hemeg et al., 2000). Further, many literatures also used Nystatin as standard antifungal drug and revealed potent antifungal activity (Bhalodia and Shukla, 2011; Badea et al., 2015). In the present investigation, ethanol extract showed comparatively better activity against all

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the bacteria as well as fungal species. Earlier literature also reported that ethanol extract was the best solvent to perform potent antimicrobial activity (Borges et al., 2020; Das and Singirikonda, 2023) and the same trend followed in the present experiment. Further, *S. aureus* reveals MBC value of 1000, 978, 956 and 800 µg*l*mI for PA ethanol extract. In same manner, MIC value of the extracts for *S. aureus* was 978, 956, 847 and 600 µg*l*mI in the same order. The MIC value for *B. subtilis* at examination of PA extract was 995, 845, 734 and 600 µg/ml whereas MBC was 1243, 1000, 984 and 879 µg/ml. It was reported that MBC values were higher than that of MIC value (Kowalska-Krochmal and Dudek-Wicher, 2021) and the same trend followed in the present study and showed potent bacteriostatic activity.

Table-3: Antimicrobial activity of various PA extracts

Organism	CONC. (mg/ml)	Zone of inhibition of PA ethanol extract	Zone of inhibition of PA ethyl acetate extract	Zone of inhibition of PA chloroform extract	Zone of inhibition of PA aqueous extract
B. subtilis	20	17.7±1.32	15.1±0.63	11.1±0.50	12.9±1.87
	40	18.1±1.33	16.6±0.37	11.4±0.33	13.3±0.24
	60	18.3±0.14	17.8±0.38	12.9±1.11	13.6±0.42
	80	18.8±0.23	18.1±0.19	13.4±2.01	14.8±0.55
	100	19.9±0.13	19.5±0.17	14.3±0.33	15.2±0.51
S. aureus	20	19.1±0.15	17.6±0.13	11.1±0.41	12.9±0.23
	40	19.4±0.45	18.2±0.33	11.4±0.21	14.3±0.28
	60	20.1±0.56	18.8±2.31	12.9±0.11	14.8±0.84
	80	20.5±0.72	19.4±1.22	13.4±0.32	15.3±1.82
	100	21.6±0.03	20.7±0.04	14.3±0.14	16.1±1.00
E. coli	20	18.2±0.06	18.4±0.45	16.1±0.23	18.1±0.22
	40	18.8±0.13	18.7±0.23	16.5±1.22	18.3±1.31
	60	19.1±0.12	19.3±0.11	17.2±0.19	18.6±1.30
	80	19.5±0.32	19.6±0.46	17.7±0.81	19.1±2.11
	100	20.1±0.22	19.8±1.92	17.9±0.10	19.4±0.17
Pseudomonas	20	18.6±0.33	18.1±2.10	14.5±0.30	16.9±1.10
	40	18.9±0.14	18.5±1.40	14.8±0.33	17.3±0.13
	60	19.2±0.32	18.8±2.02	15.2±0.67	17.7±2.10
	80	19.8±0.11	19.2±1.13	15.5±0.77	18.1±2.15
	100	20.1±0.05	19.6±0.14	15.8±0.23	18.4±1.12
Aspergillus niger (Fungi)	20	18.2±0.03	18.1±0.17	18.4±0.01	17.9±1.33
	40	18.6±0.12	18.4±1.02	18.7±0.12	18.1±0.40
	60	18.8±0.21	18.5±1.34	19.1±0.15	18.4±0.15
	80	19.1±0.13	19.0±1.42	19.3±1.10	18.7±0.13
	100	19.8±0.44	19.3±1.20	19.6±2.03	19.2±0.11
Candida (Fungi)	20	18.8±0.56	18.5±0.22	18.6±0.11	18.1±0.60
	40	19.1±0.72	18.8±0.44	18.9±0.45	18.3±0.73
	60	19.4±0.10	19.2±0.16	19.2±0.35	18.5±0.12

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	80	19.7±0.40	19.7±0.11	19.5±0.43	18.9±0.32
	100	20.2±0.52	19.9±0.33	19.8±0.22	19.4±0.20
Gentamicin	(10 µg)	24.5±0.10 (*B.subtilis*)	23.3±0.21 (*S. aureus*)	22.7 ±0.43 (*E coli*)	22.4 ±1.23 (*Pseudomonas*)
Nystatin	(10 µg)	22.4 ±0.22 (*A. niger*)	22.8 ±0.13 (*Candida*)		

• Values are mean ± SEm (n =3)

It was reported that phenolic content of plants having direct correlation with the antimicrobial activity (Li et al., 2014; Tako et al., 2020). In food science, natural phenolic compounds have drawn interest as potential growth inhibitors of foodborne pathogenic and spoilage microorganisms. Numerous phenolic-enriched plant extracts and individual phenolics can inhibit the growth of biofilms and the manufacture of toxins by food-related diseases, and they also show promising anti-quorum sensing capability. Therefore, further correlation study was performed among the total phenolics with the higher potent antimicrobial activity and revealed positive correlation with significant values (Figure-4 and 5).

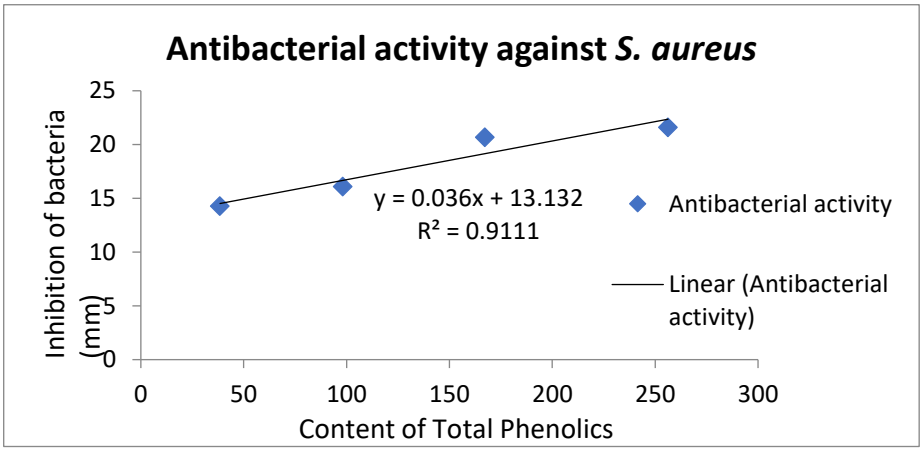

Figure-4: Positive correlation among total phenolics with *S. aureus* (100 µg/ml)

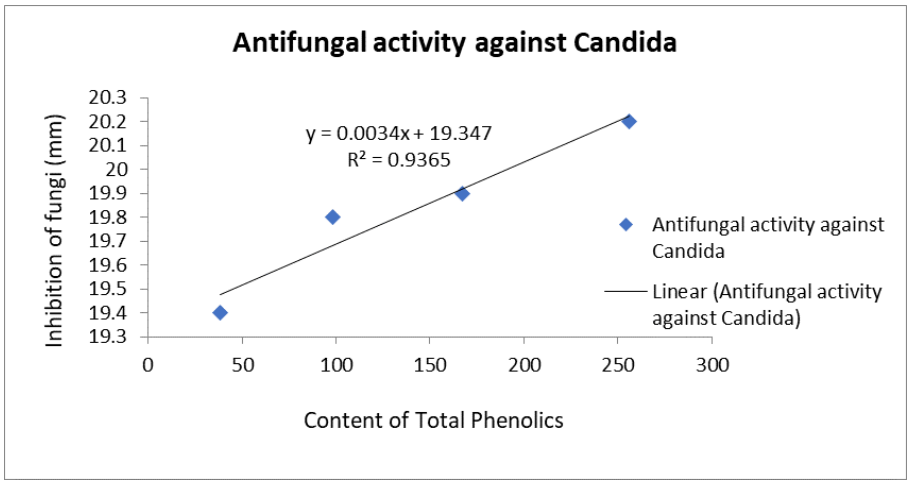

Figure-5: Positive correlation among total phenolics with *Candida species* (100 µg/ml)

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Finally, overall correlation study was performed and showed significant positive correlation among the activity and phenolics content (Table-4). This study indicated that plant phenolics have significant role in combating microbial contamination which was similar as per earlier reports.

Table-4: Correlation study among the activity with total phenolic content

	Total Phenolics	*B.subtilis*	*S. aureus*	*E. coli*	*Pseudomonus*	*A. niger*	*Candida*
Total Phenolics	1						
B.subtilis	0.931	1					
S.aureus	0.955	0.995**	1				
E. coli	0.892	0.848	0.891	1			
Pseudomonus	0.916	0.895	0.930	0.995**	1		
A.niger	0.988*	0.970*	0.979*	0.855	0.893	1	
Candida	0.968	0.863	0.906	0.961	0.963	0.925	1

- S

ignificant at *p<0.05; **p<0.01

Conclusion

In conclusion, our study highlights the significant antimicrobial potential of *Physalis angulata*, a weed plant abundant in various regions. Through comprehensive investigations, we have identified a rich array of chemical constituents within *Physalis angulata*, including carbohydrates, alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, and volatile oils. These compounds collectively contribute to its antimicrobial activity. Our findings demonstrate that *Physalis angulata* exhibits dose dependent inhibitory effects against a range of microbial species, including both bacteria and fungi which were significantly dependent on phenolic content. This suggests the therapeutic potential of *Physalis angulata* in addressing microbial infections and underscores its relevance in pharmaceutical and medicinal applications.

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