



## Exploring quality control standards and potential antibacterial property of different extracts of the root of *Physalis minima* L.

Dibyendu Shil<sup>\*1</sup>, Tanmay Sarma<sup>2</sup>, Saumendu Deb Roy<sup>1</sup>, Jashabir Chakraborty<sup>1</sup>,  
Smriti Rekha Chanda Das<sup>2</sup>, Trishna Das<sup>2</sup>, Taslima Akhter Rohman<sup>2</sup>

<sup>1</sup>Department of Pharmacognosy, Mata Gujri College of Pharmacy, Mata Gujri University, Purabpali Road, Kishanganj, Bihar - 855107, India

<sup>2</sup>Department of Pharmacognosy, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati, Assam - 781017, India

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### ABSTRACT

*Physalis minima* (Fam. Solanaceae) is well known (local name: Pokmou) ethno-medicinal plant in north-east part of India. Traditional knowledge claimed that aerial parts of this plant has medicinal values like antiulcer, anti-inflammatory, antimalarial, antidiabetic, antimicrobial etc but there are lack of scientific evidence to support these properties. The aim of study is to established standard monograph of the root of *Physalis minima* and also evaluate antibacterial property from root extracts against selected Gram (+ve) (*S. aureus*) and Gram (-ve) (*E. coli*) bacteria strains by agar disc diffusion method. Morphological study revealed that root is tap root, greyish white in colour, slightly sweet taste. Microscopic evaluation showed the presence of phelloderm, cambium, medullary rays across the xylem and phloem, starch grains. Physicochemical parameters were found to be within limit. Different extracts have shown the existence of metabolites viz. Alkaloids, steroids, tannin, flavonoids. Phytoconstituents like flavonoid, phenolic and tannin were quantified. Pet ether and ethanol extracts at 5mg/ml concentration showed remarkable zone of inhibition against Gram (+ve) and Gram (-ve) microorganism. Recent study suggests standardized *Physalis minima* root contains bioactive compounds mainly flavonoids, tannins possess significant antibacterial potential against both Gram (+ve) and Gram (-ve) microorganism.



### \*Corresponding Author

Name: Dibyendu Shil  
Phone: 9435856199  
Email: drdibyendushil@gmail.com

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### INTRODUCTION

Bacterial infections have large effect on human health. Antibiotics are becoming less effective day by day may be because of drug resistant bacteria (Farnsworth, 1993). Traditionally claimed natural products also have significant role against bacterial infections (Houghton, 1995). Present study was focused on exploring natural antibacterial agent from root of medicinal plant *Physalis minima* (wild cape-gooseberry in English) (Figure 1 and Figure 2) belonging to Solanaceae family has been selected (Karpagasundari and Kulothungan, 2014). The plant is found in the wastelands and roadsides in India, Bangladesh, Afghanistan, Baluchistan, tropical Africa and Australia (Kallianpur et al.,

2016). Ethnomedicinally, plant is used as diuretics, purgative, cardioprotective, in carache, malaria, diabetes etc. (Fasihuddin and Ghazally, 2003). To establish the ethnotherapeutic property scientifically plants need to be standardized first (Shahidi, 2004). Standardization includes authentication and identification of the plant parts macroscopically and microscopically, categorize the bioactive constituents present in the plant responsible for pharmacological property (Prasad et al., 2012). Leaf of *Physalis minima* has reported to have alkaloids, glycosides, flavonoids, tannin, steroids as phytoconstituents (Shil et al., 2019) and literature said that the plants rich in such phytoconstituents have been found to have antibacterial properties (Cowan, 1999). But there was no scientific evidence describing the quality control standard and antibacterial property of root of *Physalis minima*. Therefore, the study was carried out to establish the standard monograph of root and to evaluate *in-vitro* antibacterial activity of different extracts of root of *Physalis minima*.



Figure 1: *Physalis minima* plant



Figure 2: *Physalis minima* root

## MATERIALS AND METHODS

### Chemicals

Mueller-Hinton agar media (Himedia, India), Ethanol (Merck India), petroleum ether (Merck India), chloroform (Merck India), Nutrient broth media (Himedia, India), Distilled Water.

### Instrument

Autoclave [(MSW-101 (FAD), Macro Scientific, Delhi], pH meter (FiveEasy Plus FEP20, Mettler Toledo), Bunsen burner, Laminar air flow, Thermometer, Micro pipette, BOD incubator (BTI-06,

Bio-Technics, India), Semi-micro Balance (CPA225D, Sartorius, Germany). Whatmann No.1 filter paper and aluminium foils are required during experiment.

### Plant Material

The fresh roots of *Physalis minima* was collected from the naturally growing plants in Lankeshwar area in Kamrup (metro) district, Assam, during the month of January to March. The authentication of the entire specimen was done by Prof. Dr. N. Devi, HOD, Department of Botany, Gauhati University, India. The voucher specimen of *Physalis minima* roots (no. GUBH18504) was kept at the departmental museum for future reference.

### Processing of the Collected Plant Materials

The collected plant materials (roots) were washed thoroughly with running water to remove the earthy matter or adherent impurity followed by shade drying. Plant material was then pulverized to make powder and stored in air tight container until further use (Khandelwal, 2020).

### Morphology and Microscopy Evaluation

Macroscopy and microscopic characters of root of *Physalis minima* were established and documented as per the method demonstrated by Khandelwal (Khandelwal, 2020). Powder study also performed as per standard methods described by Brain and Turner (Brain and Turner, 1975).

### Physicochemical Evaluation

The powdered root of *Physalis minima* was examined and reported for the quantification of physicochemical parameter like loss on drying, ash value, foaming index, swelling index, extractive value, foreign matter, heavy metal content as per the standard method described in the WHO guidelines (World Health Organization, 2002).

### Fluorescence Analysis

Powdered drug was reacted with different reagents like NaOH (Aq), NaOH (Alcohol), HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, picric acid, acetic acid, NH<sub>3</sub>, KOH and examined under UV and visible light. Various colour radiation emitted were observed under different wavelength of 254 nm and 365 nm and noted down (Ahmed and Hasan, 2015).

### Quantification of Crude Fibre

The crude powdered was boiled with Nitric acid (10%) followed by filtration and washed the residue properly with hot water and further treated with sodium hydroxide (2.5%), filtered and weighed the marc as crude fibres. The crude fibres content was calculated in term of percentage yield (w/w) with

reference to air dried plant material (Ajeesh et al., 2014).

### Preparation of Extract

Soxhlet extraction process was carried out to obtain extracts from coarsely powdered drug by using pet ether and ethanol as solvents. The ethanol and pet ether extracts were stored properly in desiccators for further study (Shil et al., 2018).

### Preliminary Phytochemical Screening

Both the extracts were subjected to preliminary phytochemical screening as per standard methods to identify the various bioactive constituents like alkaloids, flavonoids, glycosides, protein, steroids etc., present in the plant part (Das et al., 2019).

### Quantitative Estimation of Polyphenolic Compounds

Different phytoconstituents present in extracts were quantified for total flavonoid content (where rutin used as standard) (Kumaran and Karunakaran, 2007), total saponin content (where Diosgenin used as a standard) (Hiai et al., 1976), total phenolics and tannin content (whereas tannic acid was used as reference standards) (Makkar, 2000). Results were calculated and expressed as the mean  $\pm$  S.E.M using a statistical linear regression method.

### Bacterial Strains

The antibacterial potency of both ethanol extract and petroleum ether extract were selectively tested against two bacterial strains belonging to gram positive and gram negative species viz. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). Strains were obtained from Guwahati Biotech park, Guwahati. All types of cultures of bacteria was sub-cultured on Mueller-Hinton agar media and stored at 4°C until further study.

### Standard Drug

Ciprofloxacin is an antibacterial drug approved by the U.S. Food and Drug Administration (US-FDA) taken as standard for the study.

### Determination of Antibacterial Activity

Antibacterial study of ethanol as well as petroleum ether extracts of *Physalis minima* root was carried out by disc diffusion method (Balouiri et al., 2016; Ahmad et al., 1998). The inoculums with bacteria were homogeneously seeded onto the 90mm petri dishes containing 20 ml cooled molten MHA medium using sterile glass spreader in such a way as to ensure through coverage of the plates. This inoculated plates were left to dry at least 15 min. The extracts were dissolved in the distilled water to obtain the different concentration of 5mg/ml and

1 mg/ml. Ciprofloxacin at concentration 1mg/ml was used as positive control and was dissolved in distilled water. Sterile filter paper disc (Whatman No.1, diameter 5mm) were impregnated with 10  $\mu$ l of each different concentration of root extracts. The discs were allowed to dry and then placed on the agar surface of each petri dish, which were already inoculated with bacterial suspension. The petri dishes were then placed in an incubator for 24 h at 37°C and the zone of inhibition is measured. The complete antibacterial analysis was carried out under strict aseptic condition.

### Determination of Minimum Inhibitory Concentration (MIC)

The MIC is the lowest concentration of antibacterial agents that inhibit the growth of microorganism as determined by turbidity in the test tube after 24 hrs of incubation.

MICs were determined for antibacterial most efficient extracts by agar or broth dilution methods (Ashraf et al., 2018).

A series of dilutions were prepared to contain the same volume of medium inoculated with the test organism (the inoculums may vary from  $10^3$  to  $10^6$  cells/ml) (Ramasamy et al., 2014).

In present study, both ethanol and pet ether extracts were dissolved in 10% DMSO and then serial two-fold dilutions were made to get six different concentrations of 1000, 500, 250, 125, 62.5 and 31.25  $\mu$ g/ml in sterile test tubes (Ramasamy et al., 2014).

Each culture tube contains culture medium (2ml), plant extracts (2ml) and inoculums (individual microorganism) (0.1ml) were incubated at 37°C for 24 hours. Test tube contains nutrient broth incubated with bacteria used as positive control and nutrient broth with plant extract used as negative control.

The growth of microorganism in test tubes was determined visually by turbidity. Test tube showed no antibacterial activity will be turbid due to presence of microorganism whereas no turbidity indicates the growth of microorganism has inhibited by the presence of antibacterial agent in concentration sufficient.

The growth of the organism for each dilution was observed and thus the MIC was recorded.

## RESULTS

### Morphological evaluation

The macroscopic evaluation *Physalis minima* root is described in Table 1.

**Table 1: Morphological parameters of *Physalis minima* root**

Sl. No.	Features	Description
1	Colour	Grayish white
2	Size	Mature roots are 7-15 cm in length and about 10 mm in diameter
3	Shape	Branched tap root
4	Odour	Characteristic and slightly disagreeable
5	Taste	Slightly sweet
6	Texture	Slightly rough

**Table 2: Physicochemical parameters of *Physalis minima* root**

Sl. No.	Physicochemical Parameters	Results
1	Ash value	
	Total ash	12.5 % w/w
	Acid insoluble ash	3 % w/w
	Water soluble ash	7 % w/w
2	Loss on drying	8 % w/w
3	Foreign matter	0.05 % w/w
4	Extractive value	
	Alcohol soluble extractive	8.7 % w/w
	Ether soluble extractive	3.65 % w/w
5	Swelling index	Not less than 5
6	Foaming index	Below 100
7	Heavy metals	
	Lead (Pb)	Not more than 1 ppm
	Cadmium (Cd)	Not more than 1 ppm
	Zinc (Zn)	Not more than 1 ppm
	Mercury (Hg)	Not more than 1 ppm

**Table 3: Fluorescence analysis of powder drug of *Physalis minima* root**

Sl. No.	Powder + Reagent	Fluorescence in day light	Fluorescence in (254 nm)	Fluorescence in (365 nm)
1	Powder	Light grayish	Bluish green	Bluish green
2	Powder + NaOH (aqueous)	Yellow	Green	Grayish blue
3	Powder + NaOH (alcohol)	Light yellow	Light green	Light green
4	Powder + HCl	Bluish green	Light yellow	Yellow
5	Powder + H <sub>2</sub> SO <sub>4</sub>	Light yellowish	Yellow	Blue
6	Powder + HNO <sub>3</sub>	Light greenish yellow	Grayish yellow	Blue
7	Powder + Picric acid	Gray	Greenish blue	Light gray
8	Powder + Acetic acid	Light brown	Brown	Reddish brown
9	Powder + NH <sub>3</sub>	Yellowish green	Strong bluish	Green
10	Powder + KOH (alcohol)	Brownish gray	Grayish yellow	Intense gray



**Table 4: Phytochemical screening of both the extracts of *Physalis minima* root**

Sl. No.	Bioactive constituent	Petroleum Ether extract	Ethanol extract
1	Alkaloids	+	+
2	Carbohydrates	-	+
3	Steroids	+	-
4	Flavanoids	+	+
5	Phenolic compounds	+	+
6	Saponins	-	+
7	Glycosides	-	-
8	Ascorbic acid	-	-
9	Proteins	-	-
10	Tannins	-	+

Indications: [+] denotes present; [-] denotes absent

**Table 5: Quantitative estimation of polyphenolic components of both the extracts of *Physalis minima* root**

Sl. No	Polyphenolic compounds	PEPM	EPM
1	Total flavanoid (mg/g RE)	18.7 ± 0.5	79.7 ± 3.4
2	Total saponin (mg/g DE)	Nil	9.2 ± 2.5
3	Total phenolics (mg/g TAE)	12.2 ± 4.2	58.1 ± 2.1
4	Total tannin	Nil	25.7 ± 2.5

Abbreviations: PEPM: Petroleum ether extract of *Physalis minima*; EPM: Ethanol extract of *Physalis minima*; RE: Rutin equivalent; DE: Diosgenin equivalent; TAE: Tannic acid equivalent

**Table 6: *In-vitro* antibacterial activity of extracts of *Physalis minima* root**

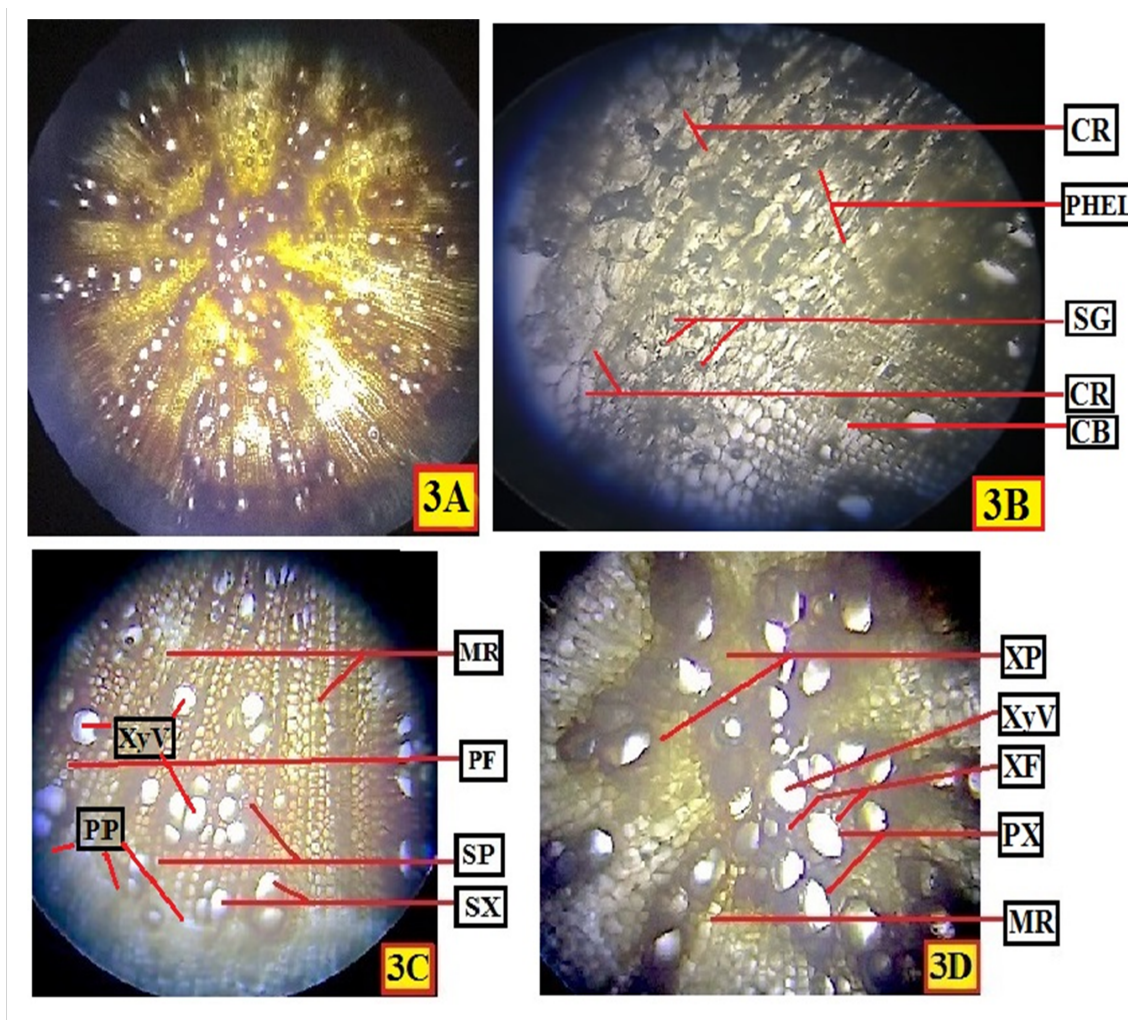
Sl. No.	Name of the compounds and concentration (100 µl)	<i>Staphylococcus aureus</i> (50 µl) (Zone of inhibition in mm)	<i>Escherichia coli</i> (50 µl) (Zone of inhibition in mm)
1	Standard Ciprofloxacin (1 mg/ml)	38.3 ± 3.7	39.6 ± 2.9
2	Petroleum ether extract (PE)(5 mg/ml)	12.6 ± 0.8	11.3 ± 0.3
3	Petroleum ether extract (PE)(1 mg/ml)	6.6 ± 0.3	7.0 ± 0.5
4	Ethanol extract (EE) (5 mg/ml)	14.3 ± 0.3	15.6 ± 0.3
5	Ethanol extract (EE) (1 mg/ml)	9.6 ± 0.3	7.6 ± 0.6

Average values were calculated from triplicate measurements

**Table 7: Antibacterial activity (MIC, mg/ml) of different extracts of *Physalis minima* root**

Bacterial strains	Ethanol Extract (µg/ml)						Petroleum ether Extract (µg/ml)					
	1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25
<i>S. aureus</i>	-	-	-	+	+	+	-	-	+	+	+	+
<i>E. coli</i>	-	-	-	+	+	+	-	-	+	+	+	+

(+) = Growth (Turbidity); (-) = No growth (No turbidity)



[Abbreviations- CR: Cork (3B); PHEL: Phelloderm (3B); SG: Starch Grain (3B); CB: Cambium (3B); MR: Medullary rays (3C); PF: Phloem Fibre (3C); SP: Secondary Phloem (3E); SX: Secondary Xylem (3C); PP: Primary Phloem (3D); XyV: Xylem vessel (3D); PX: Primary Xylem (3D); MR: Medullary rays (3D)]

**Figure 3: Transverse section (40x and 10x) of the root of *Physalis minima* (3A)**

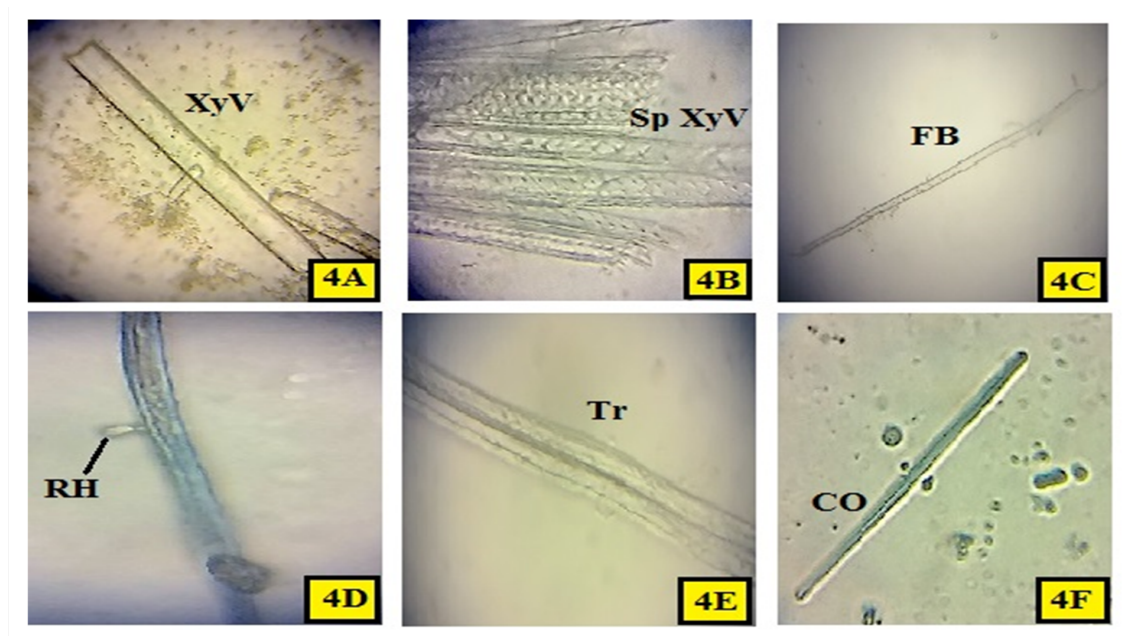
### Microscopical Evaluation

Transverse section (T.S) of the *Physalis minima* root in different magnification (40x and 10x) were established (Figure 3). T.S of whole root represented in Figure 3A. The outer most layer cork (CR) (Figure 3B) composed of more than ten layers of parenchymatous cells followed by three to five layers of radially arranged parenchymatous cell of phelloderm (PHEL) (Figure 3B) where few starch grains (SG) (Figure 3B) are observed. Secondary phloem (SP) (Figure 3C) consists of phloem fibres (PF) (Figure 3C), phloem parenchyma (PP) (Figure 3C) and cambium (CB) (Figure 3B). Multiseriate medullary rays (Fig 3C) were seen across the

secondary phloem (SP) (Figure 3C) and secondary xylem (SX) (Figure 3C) which is wider at phloem region and narrow in xylem region. Secondary xylem consists of xylem vessels (XyV) (Figure 3C), xylem parenchyma (XP) (Figure 3D). Central region consist of primary xylem (PX) (Figure 3D), xylem parenchyma (XP) (Figure 3D), xylem fibres (XF) (Figure 3D). Pith cells are absent. All the cells observed under compound microscope with different magnification (10x and 40x).

### Microscopical Evaluation of Powder Drug

Fine powdered drug sample was reacted with phloroglucinol and HCl mixture and observed under compound microscope with 40x magnification (Fig-



[Abbreviations- XyV: Xylem Vessels (4A); Sp.XyV: Spiral shaped Xylem Vessels (4B); FB: Fibre (4C); RH: Root Hair (4D); Tr: Trachieds (4E); CO: Calcium Oxalate crystal (4F)]

**Figure 4: Powder study of the root of *Physalis minima* [40x magnification]**

Figure 4) showed the presence of lignified xylem vessel (XyV) (Figure 4A), spiral shaped xylem vessels (Sp XyV) (Figure 4B), fibre (FB) (Figure 4C), root hair (RH) (Figure 4D), trachieds (Tr) (Figure 4E), and calcium oxalate crystal (CO) (Figure 4F).

#### Physicochemical Characteristics

Results of the physicochemical parameters of the powdered root of the *Physalis minima* are shown in the Table 2.

#### Fluorescence Analysis of Powder Drug

Result of the fluorescence analysis of the powdered root of *Physalis minima* with different reagents are represented in Table 3.

#### Quantification of the Crude Fibre Content

Crude fibre content was found to be 32.0 % w/w per gram of the plant root.

#### Quantification of the Extractive Value of Different Extracts

The yield value of ethanolic extract and petroleum ether extract of root of *Physalis minima* were found to be 8.7% w/w and 3.65% w/w respectively.

#### Preliminary Phytochemical Screening

The results of the preliminary phytochemical screening of the both the extracts of *Physalis minima* root are represented in Table 4.

#### Quantitative Estimation of Polyphenolic Compounds

Quantitative estimation of various polyphenolic compounds of both the extracts are represented in Table 5.

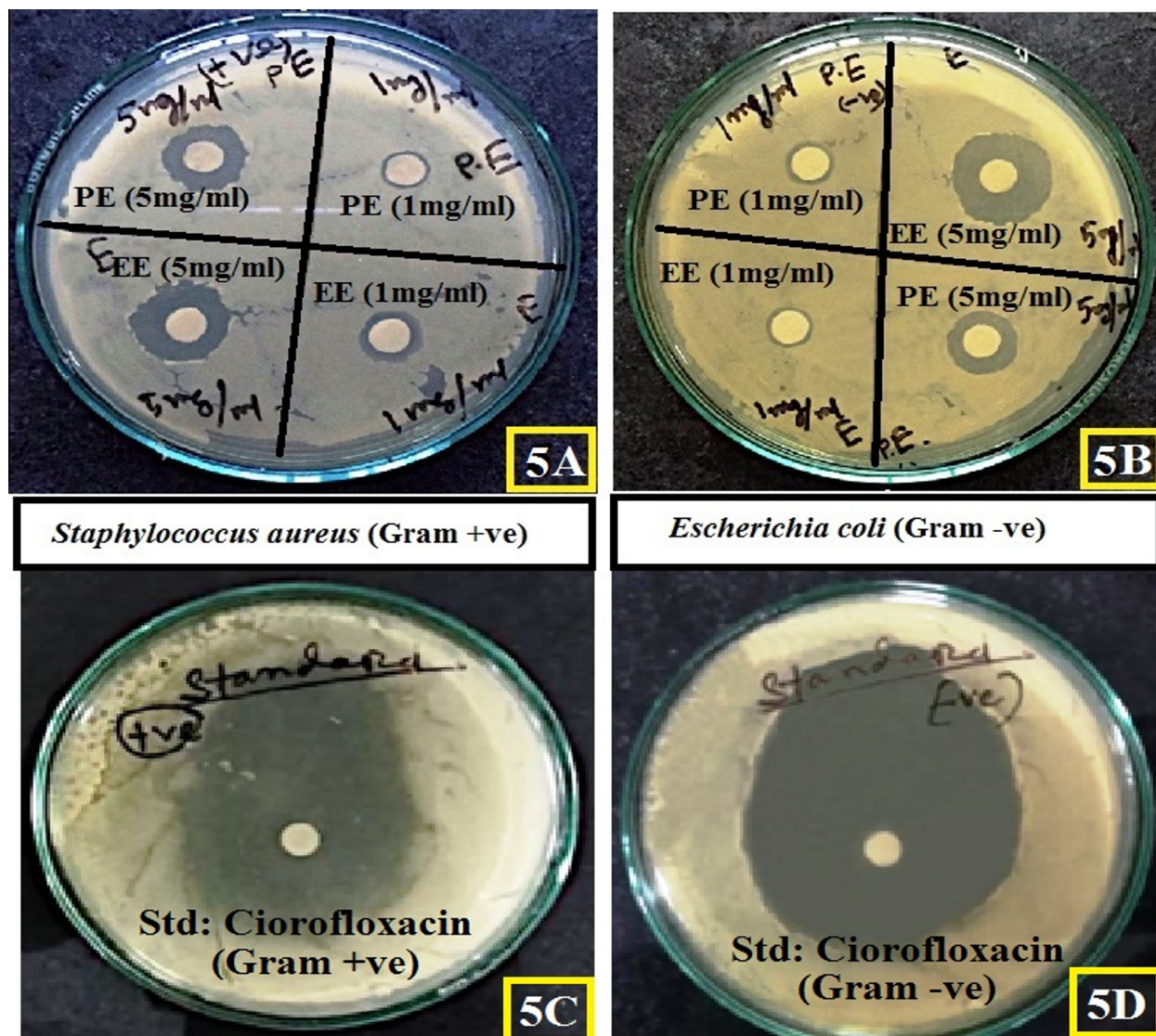
#### Antibacterial Activity

The *in vitro* antibacterial activity of root of *Physalis minima* showed (Figure 5) a remarkable zone of inhibition in both petroleum ether extract and ethanol extracts at higher concentration of 5mg/ml contrast to lower concentration of 1mg/ml against both gram +ve (*S. aureus*) and gram -ve (*E.coli*) microorganisms. Maximum zone of inhibitions were observed in ethanol extract (5mg/ml) of the plant i.e.  $14.3 \pm 0.3$  mm against *S. aureus* and  $15.6 \pm 0.3$  mm against *E. coli* where as petroleum ether extract with same concentration also showed potent zone of inhibition i.e.  $12.6 \pm 0.8$  mm and  $11.3 \pm 0.3$  mm against gram +ve and gram -ve microorganism respectively. Experimental data established the antibacterial property of root of *Physalis minima* but three fold less than that of standard ciprofloxacin (Table 6).

#### Minimum Inhibitory Concentration (MIC) for Antibacterial Activity

It shows that the tubes containing ethanol extract of 31.25, 62.5 and 125  $\mu$ g/ml and petroleum ether





[Indications: P.E.: Petroleum Ether extract; EE: Ethanol Extract]

**Figure 5: Antibacterial activity (5A and 5B) of Ethanol and Pet ether extract of root of *Physalis minima* on *S. aureus* (Gram +ve) and *E. coli* (Gram -ve). Antibacterial activity (5C and 5D) of ciprofloxacin as a standard drug on *S. aureus* (Gram +ve) and *E. coli* (Gram -ve)**

extract of 31.25, 62.5, 125 and 250  $\mu\text{g/ml}$  exhibited turbidity for both *S. aureus* and *E. coli* microorganism. Hence, 250  $\mu\text{g/ml}$  and 500  $\mu\text{g/ml}$  were found to be the minimum inhibitory concentration (MIC) for ethanol extract and petroleum ether extract respectively (Table 7).

**DISCUSSION**

Standardization of crude drugs is one of the practices to establish the standard monograph in terms of identity, quality and purity of medicinal plants which help to diagnosis the adulterated one. It has been seen from past few years that there is increas-

ing interest of world population on herbal medicine for its significant therapeutic value against various diseases (Muniappan and Savarimuthu, 2011). Therefore it is important to obtained correct quality control profile to establish therapeutic activity for various medicinal plants used traditionally. Hence in this study the root part of selected plant *Physalis minima* was standardized as per WHO guidelines and explored the potential antibacterial property of the same which was not reported with supportive documentation in the past.

Pharmacognostical evaluations in terms of morphological and microscopical study were preliminary

steps for identification of plants materials (Kala et al., 2016). Morphologically root of *Physalis minima* showed branched in taproot system, with rough in texture and grayish white in colour. Taste is slightly sweet with characteristic odour. Microscopic study revealed the important diagnostic characters of Solanaceae family (Metcalf and Chalk, 1957) which is characterised by the presence of cork cell, phelloderm, multiseriate medullary rays, needle shape calcium oxalate crystals, lignified and large xylem vessels etc. and absence of pith cells in the root.

The physicochemical parameters were performed to ensure the quality and purity of drug. Ash value was determined to measure the percentage of inorganic substance namely salt of carbonate, phosphate and silicates of sodium, calcium and magnesium, silica present in the drug sample (Shil et al., 2017). In this study, inorganic substances were found within limits which confirm the quality of drug. Extractive values were performed to quantify as well as identify the character of metabolites present in crude drug (Sonia and Jessykutty, 2016). Result showed that various metabolites present in both the extracts. Result satisfies with low limit of moisture content in crude drug. Higher level of moisture may leads to the microbial growth as well as deteriorates the nature of metabolites present in plant (Khare et al., 2017). Swelling factor indicates the mucilage, pectin, gum content in drug (Poojari et al., 2014). In this study swelling index (Kala et al., 2016) was found to be not less than 5ml revealed that the above mentioned parameters were present in root part. Foaming index result confirmed the low quantity of saponin content in root part. Heavy metal existence interfere the quality and purity of crude drugs. So it is important to estimate the quantity of heavy metal presence in crude drug with atomic absorption spectroscopy method (Shil et al., 2017) and experimental report confirmed that heavy metals in *Physalis minima* root found to be within the limit as prescribed by WHO. Fluorescence analysis of powdered drugs is an important parameter to recognize the nature of metabolites exhibit fluorescence under day light or UV rays (Poojari et al., 2014). Crude fibres include indigestible cellulose, lignins, pentosans etc signify the nutritional value of medicinal plant (Shil et al., 2017). Root of *Physalis minima* contain high amount of crude fibres. Phytochemical screening of both the extracts of root of *Physalis minima* confirmed the presence of phytoconstituents like flavonoids, alkaloids, steroids, phenolic compounds, carbohydrates, tannin.

Antibacterial activity of roots of *Physalis minima* plant has been evaluated. The study was made

against both the Gram (+ve) and Gram (-ve) bacteria strains, which were selected for screening antibacterial property of the extracts (Sen and Batra, 2012). Results of this study showed that both ethanolic extract and petroleum extract of root of *Physalis minima* at 5mg/ml concentration was highly successful in producing a desired effect against both the Gram (+ve) and Gram (-ve) bacteria in agar disc diffusion method. Literature said that plant rich in bioactive constituents like flavonoids, tannins, alkaloids were used to achieve defense mechanism against different types of microorganisms (Sahraei et al., 2014; Elisha et al., 2017). Phytochemical study of ethanol and pet ether extract of root of *Physalis minima* confirmed the presence of such metabolites which is the strong evidence to support antibacterial activity of said plant.

## CONCLUSION

The present investigation is concluded that *Physalis minima* root contains potent antibacterial agents like flavonoids, tannins which possess significant inhibitory effect against tested Gram +ve and Gram -ve microorganisms. Therefore, further studies are necessitated for the isolation of the active principles responsible for this property. The results of the study sustain the ethnomedicinal claim along with the advancement of new antibacterial drugs from the root part of plant.

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## Conflict of Interest

The authors declare that there is no conflict of interest.

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