

Study of phytochemical constituents and Antibacterial activity of Methanol Extract of *Physalis minima* Linn.

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Abstract: From prehistoric era, the plants are used as a healing for many alarming disease due to the presence of therapeutic value. Even in contemporary invention, many herbal plants are intent in research field to concise about the efficacy of plant in curing disease with fewer side effects in long term exposure. *Physalis minima* Linn is generally used in the indigenous system of medicine for various diseases like diuretic, fevers, etc. In the present study, an attempt was made to evaluate the phytochemical substances and bioactive compound of the aqueous methanol extract of unripe fruit of *Physalis minima* Linn. The crude extract was separated by soxhlet using methanol as solvent followed by the phytochemical screening to identify the presence of secondary metabolites. Volatile components present in the methanol extract were separated and identified using GC/MS. The Gas chromatography - mass spectrometry chromatogram result showed the presence of total of 18 bioactive compounds in crude extract of *physalis minima* Linn which are exhibiting different biological functions such as antioxidant, anti-inflammation, anticancer, antidiuretics etc. Antibacterial activity of *Physalis minima* Linn extracts were also studied using disc diffusion method. The activities of methanol extracts were tested and had showed good inhibition zone against *Staphylococcus aureus*.

Key words: *Physalis minima*, phytochemical constituents, Gas chromatography - mass spectrometry, antibacterial, *Staphylococcus aureus*.

1. INTRODUCTION:

Recently, traditional herbal medicines are progressively more used as remedies for devastating disease. (Chothani DL et al., 2012). The plant was used in habitual medicine as a remedial for nearly all the disorders before the advent of synthetically derived medicines. The plant has its uses in the field of old medicinal practices like Ayurveda (subhasri et al 2010) where the extracts of this plant in varied concentrations were used as analgesic and also as an anti-inflammatory agent. Herbal medicines are not only found to be non detrimental, but also believe to boost the immune response, improve unfavorable effects of conventional treatments, and possess distinct medicinal properties (Vardhana R 2008). Therefore, huge efforts in fighting against disease have focused on the identification of bioactive components for exploring the potential activity of plant extracts. According to World Health Organization medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicine which has bioactive compounds derived from medicinal plants hence such plants should be investigated for better understanding of their properties, safety, efficacy and efficiency.

Physalis minima Linn is a small herbaceous perennial annual plant belonging to Solanaceae family. The other common names are such as Native gooseberry, wild Cape gooseberry and pygmy ground cherry and in Tamil it is called Sodakkuthakkali. It grows mostly in the south Asian regions, it grows to about 20–50 cm and is formed like the tomato, making a variation that it is encapsulated inside a defensive membranous covering completely hence later part of the name refers to the native tomato. The fruit is a good source of vitamin C is considered to be a diuretic, purgative and is used to relieve pain (analgesic action) (Ramadan M.F., 2011). Due to bitter nature in taste it has action against inflammations, spleen disorder & in ulceration of the bladder. The therapeutic properties in plants are due to the hundreds of phytochemicals formed by them (Okwu DE et al 2006) as primary or secondary constituents. Secondary metabolites help the plant to survive in its atmosphere. In addition of therapeutic value of fruit of the plant, it can also be used in salads as flavoring agent in cooked dishes, desserts and jam. Presently, there are different products processed from the fruit of gooseberry, such as, jams and chocolate-covered candies. It can also be processed for juice & pomace (Ramadan and Moersel, 2007) and other products, sweetened with sugar as a snack. It used for treating diseases like cancer, leukemia, malaria, asthma, hepatitis, dermatitis and rheumatism. Research report were shown for *physalis minima* source for the presence of bioactive compound with the remarkable properties such as anti-pyretic, anti-inflammatory, anti-allergic, anti-ulcer, antimicrobial, anti-oxidant and hepatoprotective, to purify blood of kidneys, decrease albumin, clean the cataract, to calcify and control amoebiasis (Tammu and Ramana, 2015).

Epidemiological studies suggest that increased using up of fruits and vegetables are associated with lesser risk of chronic degenerative diseases (Reddy et al., 2010). Many medicinal properties are attributed to *physalis minima* plant such as antispasmodic, diuretic, antiseptic, sedative, analgesic, helping to fortify the optic nerve, throat trouble relief, elimination of intestinal parasites and amoeba. There are no studies that indicate possible adverse effects (Kirti Joshi, 2015). In spite of favorable abilities of the plant, the preliminary phytochemical screening, analysis of bioactivity compound and antimicrobial activity were examined for methanol extract of fruits of *physalis minima* Linn.

2. METHODOLOGY

Sample Collection & Processing:

The fruits of *Physalis minima* were procured from the street grown plants in Cuddalore district. The source was collected and washed, then packed in a plastic cover and used for extraction.

Extraction Procedure:

The fresh fruits (50g) were crushed using a motor and pestle, the extracted is soaked with 90% methanol make up to 150 ml and kept at room temperature (24–36°C) for 72 hrs. Solvents were filtered through a vacuum filter to get crude residues. The homogenate thus obtained was filtered & the extracts were evaporated at room temperature for 72 h to obtain pure crude residue. The crude extract was dissolved in dil.H₂O for further processes.

Preliminary Phytochemical Screening:

The preliminary screening was done by various tests for the qualitative analysis of identification of secondary metabolites (**Fatemeh Mirzaee et al 2019**).The methanol extract of fruit samples were used to find out the phytochemical constituents based on Harborne J.B 1973.

Antibacterial activity study:

The antibacterial activity of the fruit extract was analyzed by disc diffusion method. The target microorganism were cultured in Nutrient broth and incubated for 24 hrs. The petri dishes containing Mueller Hinton agar (MHA) medium were cultured with diluted bacterial strain (**Patel T et al 2011**).The prepared discs were placed on the culture medium. Test samples (250, 500, and 1000 µg) were injected to the sterile disc. Standard drug Streptomycin (20µg) was used as a positive reference standard to determine the sensitivity of microbial species tested. Then the inoculated plates were incubated at 37 °C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antibacterial activity.

GC-MS analysis:

The volatile compounds were analyzed by gas chromatograph mass spectrometer (GC/MS) by a Thermo Scientific trace GC Ultra Couple with single quadruple MS and a fused silica capillary column TG-5MS (30 m × 0.251 mm, 0.1 mm film thickness).The oven temperature was maintained initially at 40 °C for 3 minutes and then programmed from 40 to 280 °C with rate of 4 °C/min. Helium was used as carrier gas, at 1 ml/min flow rate. The determination of all the identified compounds was made using a percent relative peak area. A tentative identification of the components was made in function with the relative retention time and the mass spectra with those of The National Institute of Standard and Technology, NIST Willy library data of the GC/MS system (**Norhanizan Usaizan et al 2014**)

3. RESULTS & DISCUSSION:

Phytochemical screening:

The quantity of the fruit extract yield were shown in the table 1, the phytochemical constituents of methanol extract shows the presence of various phytochemicals such as flavonoids, phenols, Glycosides, steroids, terpenoids and protein (Figure 1). These secondary metabolites which are produced and used by the plants for safety and restore process within the natural environment. Flavonoids are recognized to possess powerful anticancer activity and antioxidants have ability to scavenge the free radicals which prevent oxidative cell damage (**Karpagasundari C et al 2014**). Phenol is a good antioxidant substance which has antidiarrhoeal activity and also prevents or control disorders related to oxidative stress (**Sumathy V et al 2011**).

Antibacterial Assay:

The antibacterial assay for methanol extracts of fruit were done against *staphylococcus aureus* using agar well diffusion method. The antibacterial results showed minimal inhibitory effect against the strains. The inhibition zone against *staphylococcus aureus* is: Streptomycin (positive control) – 20mm, Fruit methanol extract – 10mm. The diffusion method shows that there in an inhibition zone in *Staphylococcus aureus* when treated with *Physalis minima Linn* fruit extract. Various study report also gives strong support that the *Physalis minima Linn* plant extract has antibacterial activity done by disc diffusion method against both Gram positive and Gram negative bacteria (**Sukanya S L et al 2009**).

Gas chromatography-Mass spectrometry analysis:

The volatile compounds of fruit extract were analyzed by gas chromatograph mass spectrometer (GC/MS). An identification of the different components of the methanol extract was made in function with the relative retention time and the mass spectra (Figure 2). The data obtained from GC/MS analysis revealed that the major components found in the methanol extract of *Physalis minima Linn* were identified as alpha-D-glucopyranoside, beta-D-fructofuranosyl (29.42%), hexadecenoic acid methyl ester (13.05%) and 9-octadecanoic acid methyl ester (11.63%). The dominant compounds found in *Physalis minima Linn* methanol extract were enlisted in table 2. Hexadecenoic acid methyl ester is considered an inhibitor to 5-Alpha reductase, an enzyme whose inhibitors can be used in benign prostatic hyperplasia and prostate cancer (**Akpuaka et al., 2013**). One of the similar research were also provides support for our work, based on the GC-MS analyses, the ethanol extractions of leaves, roots and fruits of *Physalis minima* contained numerous numbers of phytochemical compounds. There are 9 major compounds that had been identified from the ethanol extract of leaves, whereas in root extraction it shows about 6 peaks as major compound and in fruit extract it's about 10 different peak of bioactive compounds were observed from chromatogram. The phytochemical compounds separated from the leaves, roots and fruits of *Physalis minima Linn* indicated the presence of various antimicrobial and antioxidant compounds that are very important for health.

4. Conclusion:

Medicinal plants were of great importance to the health of individuals and communities. Our study concludes that the phytochemical analysis of the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Additional understanding about the plant will be useful in the finding of various drugs in treating many dreadful diseases. Hence, the bioactive compound has to be isolated for further studies to explore the potential activity of *physalis minima* fruit.

Table 1: Extract yield of *Physalis minima* Linn.

Solvent system	Fruit quantity(g)	Extract weight (g)
Methanol	50	2.817

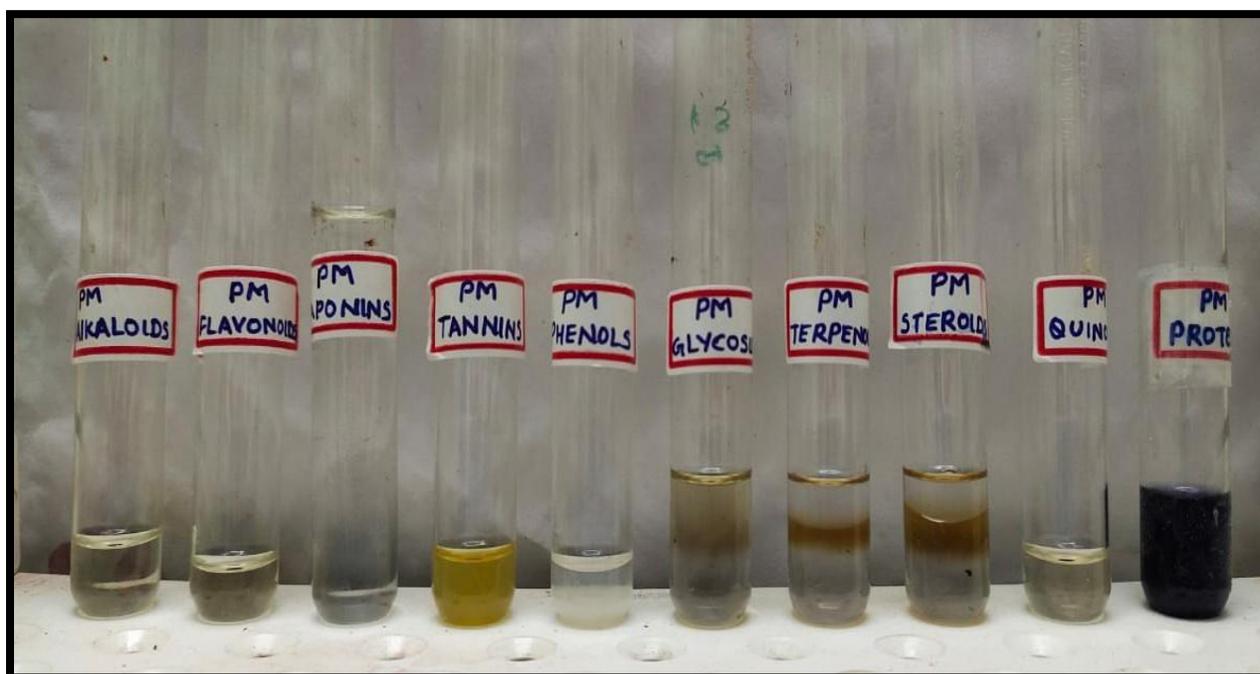


Figure 1: Phytochemical analysis of methanol extract of *Physalis minima* Linn

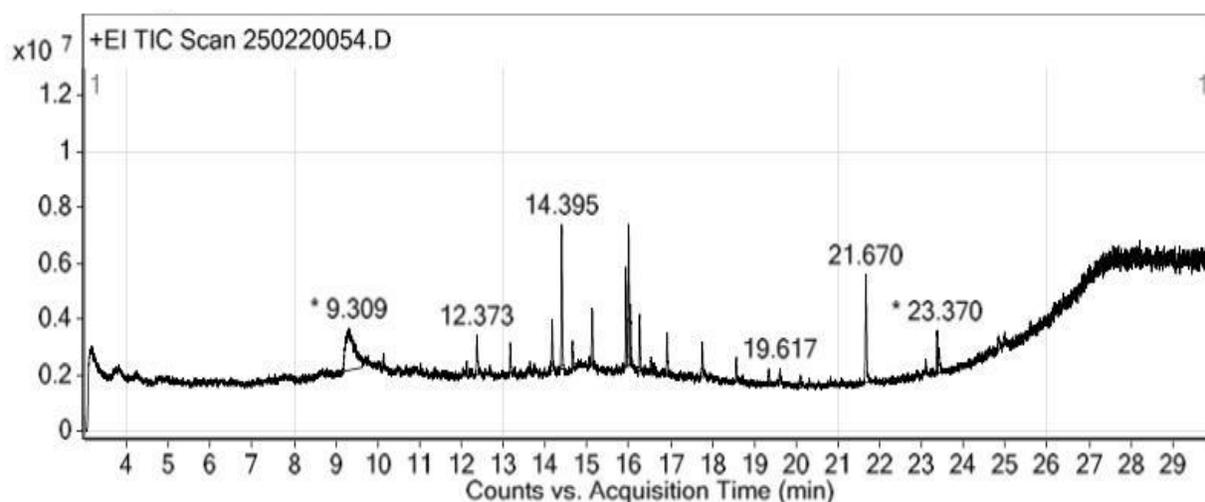


Figure 2: Chromatogram for methanol extracts of *Physalis minima* Linn.

RT	Name	Area	Formula	Mass	Area %
9.31	.alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	21639464	C18H32O16	504.2	29.42
10.15	10-Hydroxydecanoic acid, methyl ester	862540	C11H22O3	202.2	1.17
12.37	Methyl tetradecanoate	2945852	C15H30O2	242.2	4.00
14.17	tert-Hexadecanethiol	3259424	C16H34S	258.2	4.43
14.40	Hexadecanoic acid, methyl ester	9598781	C17H34O2	270.3	13.05
14.66	Phthalic acid, butyl oct-3-yl ester	1746098	C20H30O4	334.2	2.37
15.93	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	5921785	C19H34O2	294.3	8.05
16.00	9-Octadecenoic acid, methyl ester, (E)-	8555901	C19H36O2	296.3	11.63
16.04	Geranyl isovalerate	4083967	C15H26O2	238.2	5.55
16.27	Methyl stearate	3422770	C19H38O2	298.3	4.65
16.54	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	826412	C14H24O4	256.2	1.12
18.57	7-Methyl-Z-tetradecen-1-ol acetate	1397650	C17H32O2	268.2	1.90
19.62	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	921989	C9H9F3O2	206.1	1.25
20.11	1-Heptatriacotanol	952126	C11H14N2OS	536.6	1.29
23.10	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl-	783774	C30H50	222.1	1.07
23.37	Urs-12-ene	3288489	C28H48	410.4	4.47
23.42	17.alpha.,21.beta.-28,30-Bisnorhopane	2231269	C14H24O3Si2	384.4	3.03
24.84	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether	1121055	530683	296.1	1.52

Table 2: List of Bioactive compound of methanol extracts of *Physalis minima* Linn.

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