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SCREENING OF GENETIC VARIANTS, PHYTOCHEMICAL ANALYSIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF *Physalis minima* FRUITS EXTRACT

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ABSTRACT

Medicinal herbs are playing a major role in the therapeutic field due to the combination of phytochemicals and are the alternatives to chemical drugs. *Physalis minima* fruit has also exhibited multiple biological activities due to its richness in vitamins, minerals, and antioxidant chemicals. This work investigated the phytochemical variations of the genetic variants collected from four districts around Salem, Namakkal, Erode, and Dharmapuri with the help of polymerase chain reaction and random amplified polymorphic DNA methods. Phytochemicals of the fruits were examined through thin layer chromatography using chloroform, diethyl ether, ethanol, ethyl acetate, and methanol extracts. The polar solvent ethanol crude extract was characterized by FT-IR and GC-MS analysis to identify the functional groups. The antimicrobial activity of ethanolic extracts of Physalis minima fruits was evaluated against *Streptococci acidominimus*, *Pseudomonas aeuriginosa, Penicillium expansum,* and *Aspergillus fumigatus* strains by the cup diffusion method using serially diluted ethanolic extracts of *Physalis minima* fruit. The sample's inhibition zones were compared with 25µl of the standards such as tetracyclin and fluconazole. The genetic variants were identified through the RAPD analysis and proved to have both phenotypic and genotypic characteristics in *Physalis minima* fruits. The thin-layer chromatography outcomes exposed the presence of phytochemicals in an evident manner, and the antimicrobial activity of the fruits exhibited good inhibition against the selected pathogens. The fruit extracts showed good inhibition between 1.5 mm and 5 mm against bacteria. Likewise, 0.5mm to 2.18mm was observed against fungus pathogens.

Keywords: Physalis minima, Genetic Variants, Phytochemicals, Antimicrobial Activity, RAPD, Characterization.
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INTRODUCTION

In recent days, research interest in the phyto compounds of medicinal plants is increasing due to the low cost and its availability in nature, especially during the COVID-19 period. Especially in developing countries, herbal medicines' requirements are increasing due to various novel viruses and bacteria's impact. India is also one of the countries in which different plant-based medicinal fields such as Siddha, Ayurveda, and Unani are used for treatments. Since ancient times, the history of using medicinal plants in India for the treatment of various diseases, including asthma, stomach ailments, skin diseases, respiratory, urinary complaints, liver diseases, and cardiovascular diseases. Plant-based compounds with diverse molecular structures are superior to synthetic chemical drugs.[1] So, the research field is trying to identify the new plant-based molecules with the structure for a novel drug design. Also, they are the building molecules for the present allopathic drugs. *Physalis minima* fruit (Fig.-1) has various common names like Sunberry, Gooseberry, Ground Cherry, and Wild Gooseberry, which is in the Solanaceae family.[2] Organic matter enriched soil is a suitable habitat for *Physalis minima* and was spread in the non-agricultural

grasslands. They are pollinated through honeybees like insects. These fruits are enriched with vitamins, minerals, carbohydrates, and phytosteroids.[3] There is a gradual rise in demand for this plant because of its antioxidant and antimicrobial activities. A *Physalis minima* has been used as an ingredient with tea to treat diabetes. The boiled water extracts of this plant's roots and leaves are used to control high blood pressure. In addition, this plant is enriched with A and C vitamins, which is a very good source of ascorbic acid.[4] It is a very good medicinal product provider in nature.[5] It exhibits several pharmacological actions, like anti-inflammatory, anti-pyretic, anti-leishmaniasis, anti-fertility, anti-diabetic, and hypoglycemic.[6]

Fig-1: *Physalis minima* Fruit

But, the plant phytochemical quantities differ based on the soil, water, and regions of the land with climate. This genetic variation in plants can be measured by polymerase chain reaction, which is a trusted methodology for DNA replication identification. RAPD (Random amplified polymorphic DNA) is also used to analyze the genetic variants like PCR (polymerase chain reaction) based method. In addition, the activity of the medicinal plants' metabolism is owing to the phytochemicals such as amino acids, proteins, common sugars, flavonoids, saponins, alkaloids, phenol compounds, and tannins.[7,8] So far, more than 200 phytochemicals have been isolated from the genus *Physalis*. These kinds of medicinally active phytochemicals are identified initially by crude biological activity and then isolated and identified by various techniques. Active chemicals are isolated through chromatographic separation methods such as thin-layer chromatography (TLC), and column chromatography and quantified by either gas chromatography or high-performance liquid chromatography.[9-11] From the reports and results, the present study investigated the genetic variants of *Physalis minima* fruits from various regions of Tamil Nadu by the RAPD method. This technique would be helpful to determine the taxonomic identity along with kinship relations to create specific probes among the samples gathered from *Physalis minima* fruits. The bioactive nutrients of *Physalis minima* fruits' were characterized using FT-IR and GC-MS techniques. From the phytochemical results, this work tested the *Physalis minima* fruit anti-microbial activity for the polar ethanolic extract where fewer reports were observed.[12,13]

EXPERIMENTAL

Chemicals, Collection of Samples, and Extraction

Important chemicals and solvents were purchased from Sigma-Aldrich, Biocorporals, India, and used as such for the extraction. *Physalis minima* fruits were collected from various districts such as Erode, Salem, Dharmapuri, and Kolli Hills of Namakkal District for genetic variation identification. They were washed with distilled water, and then sliced. The sliced fruits were dried for 12 days at 22˚C in the absence of sunlight and ground well up to the fine powdered form. 25 g of powdered fruits were treated with 1: 10 w/v of solvents such as water, chloroform, diethyl ether, ethanol, ethyl acetate, and methanol. Then, the extraction was conducted by the Soxhlet apparatus for 24 hrs and the extracted solvents were collected separately. The extract was concentrated under vacuum conditions and stored in the refrigerators.

Genetic Variants of *Physalis minima* Plants

The four district plants' genomic DNA was isolated with the help of the Macherey Nagel Nucleo spin plant II kit. In addition, Binding Buffer PC, Wash Buffer PCR, Elution Buffer PCR, RNAse A, filters, and columns were also utilized in the isolation process. The RAPD technique comprises preferential amplification of random sequences by PCR. One primer was used in screening to identify the specific marker through RAPD. RAPD band size was designated as amplified and was shared as dialectic

characters (present = 1, absent = 0). The numbers of polymorphic bands are calculated for each population.

Qualitative and Quantitative Phytochemical Analysis of *Physalis minima* Fruits

The concentrated extracts were dissolved in solvents like ethanol, methanol, chloroform, ethyl acetate, and diethyl ether. The phytochemical components of the extracts were identified using Mayer's and Wagner's tests (alkaloids), NaOH test or Ferric chloride test (flavonoids), Borntrager's test (glycosides), Ferric chloride test (phenols), NaOH test (quinines), foam test (saponins), Liebermann-Burchard test (steroids), Bromine water test, ferric chloride test (tannins), and Salkwoski's test (terpenoids). All the methodologies were executed as per the reported spot test methods.[14-16] The ethanolic extracts of *Physalis minima* fruits were estimated for the quantification of flavonoids, phenols, tannins, and steroids. Flavonoid content was determined by the slightly modified colorimetric method.[17] As per the reported methods, quantitative analysis was carried out on the extracts.[18-20]

Thin-layer Chromatography (TLC)

TLC is a rapid, accurate, and cheapest method to measure the chemical constituents in a crude mixture, as well as the purification of the substances. This method identifies the presence of phytochemical substances by staining with different types of spraying reagents.[21]

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis gives a clear output of the spectrum of various compounds and the components separated from the sample. The ethanolic extract of the *Physalis minima* fruit sample was injected into the injector port of the GC instrument. The sample was vaporized and separated into various components. The separated compounds gave spectral pea individually and have been recorded on a paper chart electronically. The retention time (RT), molecular weight (MW), molecular formula (MF), percentage of peak area, and peak height were measured using the Scion 436-GC Bruker model attached to a triple quadruple mass spectrophotometer.[22, 23]

FT-IR (Fourier Transform-Infrared Spectroscopy) Analysis

FT-IR analysis was performed using 2 mg of the ethanolic extract of *Physalis minima* was treated with 100 mg of potassium bromide and a salt disc of 3mm was prepared which was kept in the sample holder of FT-IR spectra. The spectrum was observed between 400 and 4000 cm^{-1} using Shimadzu-Affinity1, Japan.

Antimicrobial Activity of Physalis Minima Fruits

Antibacterial activity of *Physalis minima* was evaluated using *Streptococci acidominimus* and *Pseudomonas aeuriginosa.* The bacterial strains were incubated along with serially diluted concentrations such as 25µl, 50 µl, and 100 µl at 37°C for 24 hours. The cup diffusion method and pour plate methods were adopted for the antibacterial activity. The cup diffusion and pour plate are carried out with the help of Muller Hinton agar along with the standard tetracycline. Similarly, *Penicillium expansum, Aspergillus fumigatus* are used to evaluate the fungal strains different concentrations of ethanolic extracts of plant fruits were used such as 25µl, 50 µl, and 100 µl with fluconazole as a standard antifungal agent. In vitro antifungal activity has been identified by testing the medicinal plants which were traditionally utilized in treating the diseases. Antifungal agents can be assumed as drugs that are helpful in eradicating the fungal organisms with a very low risk of toxicity to the host.

RESULTS AND DISCUSSION

This work observed that environmental factors like climatic and geographical distributions have shown an impact on genetic diversity in *Physalis minima*. The genetic bottleneck effect and weed management were understood with the information gathered from this investigation.[24] The role of genetic variants creates a significant change in the ecosystem, which is associated with species diversity. The efficiency of this variation can be described by the benefits like ecological value, economic importance, and medicinal value of the genus. The result exposed the low variation among the selected region's fruits. The RAPD analysis is associated with the geographical location of *physalis minima* in various soils of the Dharmapuri, Namakkal, Sathyamangalam, and Salem zones, which are presented in Fig.-2(a) and Table-

1. Phytochemicals such as alkaloids, flavonoids, terpenoids, glycosides, tannins, and steroids present in the crude extracts of the plant *Physalis minima* show antiviral, anti-cancerous, anti-diarrhoeal activity, antiseptic properties give defence mechanism to the plant. Similarly, the results of the phytochemical analysis of various extracts such as chloroform, diethyl ether, ethanol, ethyl acetate, and methanol are presented in Table-2. The ethanolic extracts of *Physalis minima* fruits showed clear spots for the respective secondary metabolites such as flavonoids, phenols, and tannins, which are shown in Fig.-2(b). The TLC analysis of ethanolic fruit extract of *Physalis minima* shows the presence of flavonoids, phenols, and tannins as qualitative analysis.

Table 1: Band Formation in RAPD Analysis

Location	Polymorphic bands	Monomorphic bands
Dharmapuri	6	5
Kolli hills (Namakkal)	5	5
Sathyamangalam (Erode)	7	5
Salem	6	5

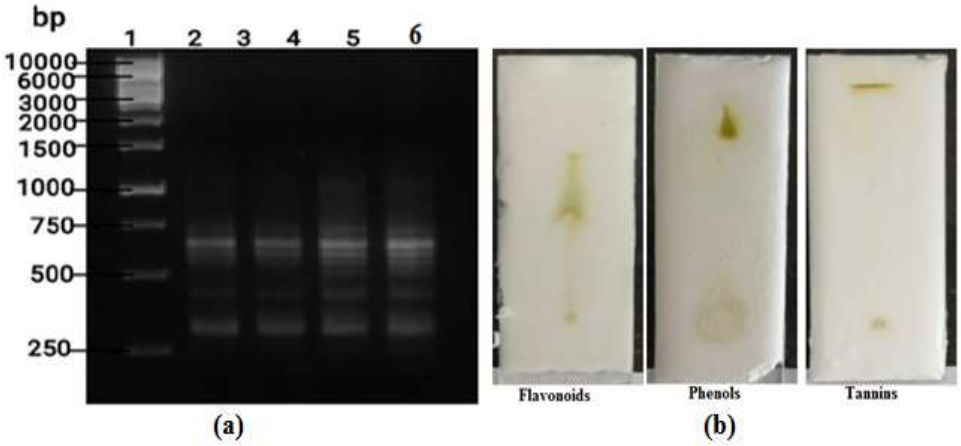


Fig.-2: RAPD Profile Analysis (a)1kb DNA Ladder (1), *Physalis minima* Dharmapuri (2), *Physalis minima* Kolli Hills (3), *Physalis minima* Sathyamangalam (4), *Physalis minima* Salem (5), OPI07 – CAGCGACAAG (6), (b) TLC Analysis of The Ethanolic Extract of *Physalis Minima* Fruits

The analysis reveals the presence of the above-mentioned phytochemicals in the fruits of *Physalis minima.* The analysis results revealed the content of phenols and quinones present in all extracts except in the ethanol extract. Comparative studies between the quantitative estimation of crude may be used to explain the preparation of copper, zinc, silver, and magnesium nanoparticles with pharmacological effects.[21] Table-3 describes the quantitative analysis of phytochemicals in *Physalis minima* fruit extract. The results show that the quantity of phenol and steroid concentration is high compared to flavonoids and tannins. High phenolic and steroid content in the plant sample indicated the plant source would act as better antioxidants, and signal compounds, essential for growth, and reproduction, and protect the plant from pathogens and UV radiation. FT-IR analysis of the ethanolic extract of *Physalis minima* fruits shows the presence of respective phytochemical functional groups. FT-IR analysis of the ethanolic extract of *Physalis minima* fruits is presented in Fig.-3 (a). The vibrational frequencies of the extract such as 3405.42 (-OH), 2922.77 (-alkane), 2852.39 (aldehyde), 1632.02 (amines and amides), 1514.45 (-NO_2), 1155.20 (-NH), 1024.99 (S=O), 864.68 (-CH aromatics) and 711.95 (-Cl) confirm the presence of phytochemicals functional groups.

Following functional group confirmation, the extract was subjected to GC-MS analysis, which revealed the presence of a variety of pharmacologically valuable phytoconstituents. The GCMS result is presented in Fig.-3(b). The GCMS library was compared and the chemicals are shown in Table-4. The phytoconstituents present in *Physalis minima* fruits confirmed their retention time, molecular formula, structure, and peak area. Also, the ethanolic extract of *Physalis minima* fruits contains various biological active chemicals such as antimicrobial, anticancer, anti-inflammatory, anti-cancer, immunomodulatory, and antiproliferative activities, etc.

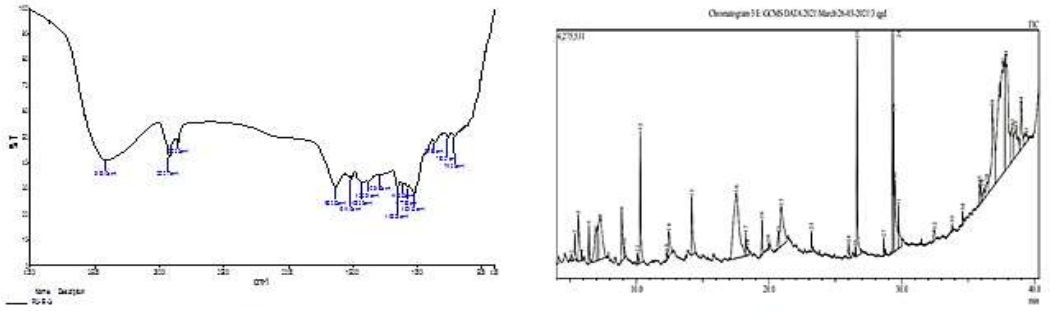
Table-2: Qualitative Phytochemical Analysis of *P.minima* Fruit Extracts

Organic Solvents	Alkaloids	Glycosides	Flavonoids	Phenols	Quinones	Saponins	Steroids	Tannin	Terpenoids
Chloroform	++	++	+	+	+	+	+	++	++
Diethyl ether	+	++	+	+	+	+	+	+	++
Ethanol	++	++	+	+	-	+	+	++	+
Ethyl acetate	++	+	+	+	++	+	+	+	+
Methanol	+	++	++	+	+	+	+	++	++

+ indicates the presence, - indicates absence, and ++ indicates high presence.

Table-3: Quantitative results of *P.minima* Fruit Extracts

S. No.	Phytochemicals	Concentration(mg/dl)
1	Flavonoids	15.583 ± 2.919
2	Phenols	49.523 ± 0.825
3	Steroids	60.833 ± 1.443
4	Tannins	8.426 ± 2.192



(a) **(b)**

Fig.-3: FT-IR (a) and GC-MS (b) Analysis of The Ethanolic Extract of *Physalis minima* Fruits

Table 4: GC-MS Analysis of The Ethanolic Extract of *Physalis minima* Fruits

RT	Compound Name	Mol. Formula	Mol. Wt.	Peak Area %
18.258	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220	0.48
19.472	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.89
20.907	1,3,4,5-Tetrahydroxy-cyclohexane carboxylic acid	$C_7H_{12}O_6$	192	3.8
23.202	Tetra decanoic acid	$C_{14}H_{28}O_2$	228	0.42
25.993	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.38
29.4	Oleic Acid	$C_{18}H_{34}O_2$	282	2.46
29.755	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.55
33.794	Carbamic acid, 2-(diethylamino) ethyl ester	$C_5H_{12}N_2O_2$	132	0.11
34.59	Hexadecenoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	0.31
35.945	dl-alpha-Tocopherol acetate	$C_{31}H_{52}O_3$	472	0.5
36.828	9,12-Octadecadienoic acid (Z, Z)-, 2,3-Dihydroxy propyl ester	$C_{36}H_{68}O_2$	532	6.3
37.637	Nonacosan-10-one	$C_{29}H_{58}O$	422	19.08
37.834	Octadecanoic acid, 9,10-dihydroxy-, methyl ester	$C_{19}H_{38}O_4$	330	10.05
38.611	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	2.67
39.001	Stigmasta-5,22-dien-3-ol, (3.beta.,22E)	$C_{29}H_{48}O$	412	2.22
39.39	4H-1-Benzopyran-4-one, 2-(3,4-Di methoxyphenyl) -3,5-Dihydroxy-7-methoxy	$C_{18}H_{16}O_7$	344	0.73

The reported antibacterial activity of *Physalis minima* fruit methanolic extract showed antibacterial activity against gram (+ve) and gram (-ve) bacteria such as *Staphylococcus aureus* and *E. coli* by the disc diffusion method.[25,26] Hence, this work also investigated antibacterial activities against *pseudomonas*

aeruginosa (gram-negative), and *streptococci acidominimus* (gram-positive), which have been examined through the pour plate method and cup diffusion method using ethanol extracts of the *Physalis minima* fruit.[27, 28] Table-5 explains both the pour plate and cup plate methods' results. The pour plate method showed the good inhibition (Fig.-4a, b, c) tendency of the *Physalis minima* fruit extract. Similarly, the cup plate method exposed a good zone of inhibition which is high at 50 µl and 100 µl. Antibacterial activity of *Physalis minima* ethanolic extract by the cup diffusion method shows a higher zone of inhibition at 50 µl and 100 µl plates with tetracycline as an antibiotic (Fig.-4d, e). [29]

Table-5: Antibacterial Activity of *Physalis minima* Ethanolic Extract

Microorganism	Pour plate method.		Diameter of zone of inhibition(mm) cup diffusion method			
	Control	Extract	Std (25µl)	25µl	50µl	100µl
Streptococci acidominimus	+	-	5 ± 1.0	1.5 ± 0.0	3.8 ± 0.3	4.7 ± 1.25
Pseudomonas aeruginosa	+	-	5 ± 1.2	2.5 ± 0.5	4 ± 0.25	5 ± 1.1

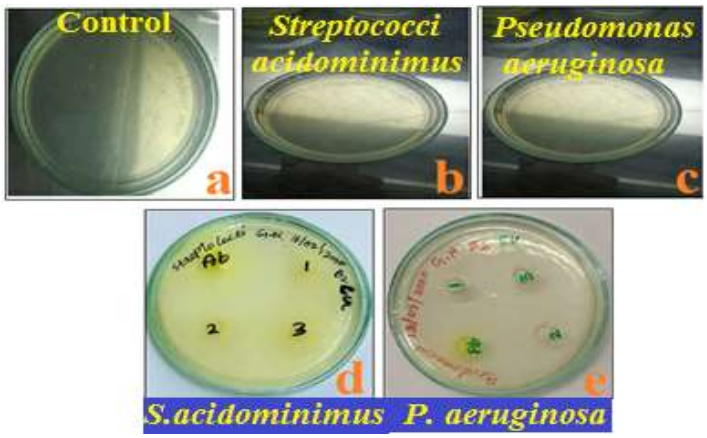


Fig.-4: Antibacterial Activity of Ethanolic Extracts of *Physalis minima* Fruits for control (a), *Streptococci acidominimus* (b&d). *Pseudomonas aeruginosa* (c&e)

Table-6 shows nil fungal growth against *Aspergillus fumigatus* and *Penicillium expansum* in the ethanol extract of *Physalis minima,* which has been done through the pour plate method (Fig.-5a, b, c). Similarly, the zone of inhibition was examined through the cup diffusion method (Fig.-5d, e) against the *A. fumigatus* and *P. expansum,* which are presented in Table-6.

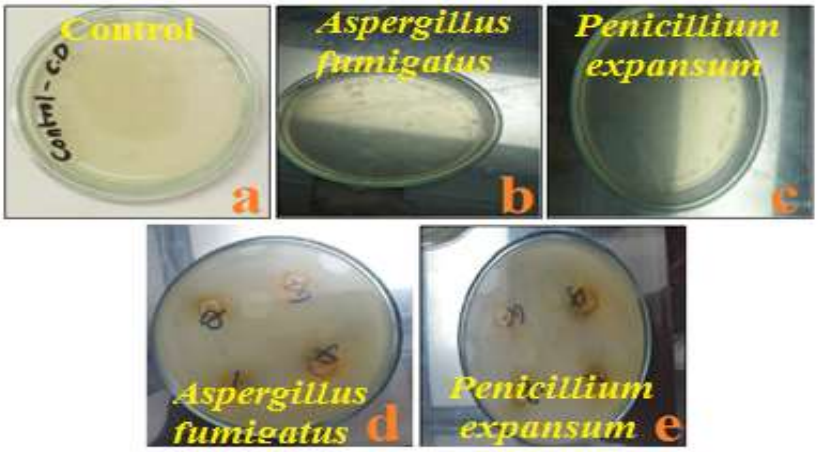


Fig.-5: Antifungal Activity of Ethanolic Extracts of *Physalis minima* Fruits for control (a), *Aspergillus fumigatus* (b and d). *Penicillium expansum* (c and e)

Table-6: Antifungal Activity of *Physalis minima* Ethanolic Extract

Microorganism	Pour plate method.		Diameter of zone of inhibition(mm) cup diffusion method			
	Control	Extract	Std (25µl)	25µl	50µl	100µl
Aspergillus fumigatus	+	-	1.5 ± 0.0	0.55 ± 0.0	0.97 ± 0.2	1.25 ± 0.11
Penicillium expansum	+	-	2.5 ± 0.2	0.74 ± 0.13	1.45 ± 0.15	2.18 ± 0.2

CONCLUSION

This investigation was successfully completed as reported works on *Manihotesculanta* and *Vetiveria zizanioides* plant extracts. The genetic variants that have been identified through the RAPD analysis prove the presence of variation in the phenotypic and genotypic characteristics in the fruit samples of *Physalis minima* that were collected from the districts of Salem, Erode, Dharmapuri, and Namakkal. These variations were influenced by geographical and climatic distribution. The qualitative and quantitative phytochemical analysis, including TLC analysis, reveals the presence and quantification of phytochemical compounds like alkaloids, flavonoids, cardiac glycosides, phenols, saponins, steroids, tannins, and terpenoids that are present in the organic solvents that include ethanol, methanol, chloroform, ethyl acetate, and diethyl ether. The current study of FTIR and GC-MS analysis shows the presence of a variety of functional groups and constituents such as aldehydes, amines, hydroxyl, nitro, chloride, and sulfoxide groups.[30] The growth of bacteria in the pour plate method is nil and hence it could be concluded that there is an effective antibacterial activity present against *Pseudomonas aeruginosa* (gram-negative), *Streptococci acidominimus* (gram-positive). Similarly, the zone of inhibition is high in the antimicrobial screening of ethanolic extract of *Physalis minima* fruit extracts, which is evident through the cup diffusion method. The antifungal activity against *A. fumigatus* and *P. expansum* also seems to be effective, as a nil fungal growth has been reported in the pour plate method and the zone of inhibition is high in the antimicrobial screening of ethanolic extracts of *Physalis minima* fruit extracts. The genetic variants are found to be evident through the analysis and the fruit extract has produced positive results in the presence of phytochemicals and antimicrobial activities in the *Physalis minima*. Further research and studies would support the medicinal importance of the plant, which would help in treating more diseases caused by microbial organisms. Extension studies could also be carried out in relevant fields like pharmacology and phytochemistry. The fruit extract showed good inhibition against *Streptococci acidominimus* (4.7 ± 1.25mm)*, and Pseudomonas aeruginosa* (5 ± 1.1mm). Similarly, the extract showed good inhibition against *Aspergillus fumigatus* (1.25 ± 0.1mm)*,* and *Penicillium expansum* (2.18 ± 0.2mm) respectively. These results proved the efficiency of the extracts and their ability to be extended for various pharmacological activities.

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