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Phytochemical Screening and GCMS Analysis of Bioactive Compounds in Ethanolic Leaf Extracts of *Physalis minima* (PMELE)

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ABSTRACT

Plants have been exploited for the benefit of humankind to cure diseases/ ailments since prehistoric times. Plant medicinal importance is due to secondary metabolites endowed with specific biochemical/ metabolic/ physiological action on the human/ animal system. A wide variety of functional activities has been attributed to the secondary metabolites or plant-based natural products (PBNPs) that cure common illnesses to cure chronic disorders and their associated long-term complications. PBNPs are bioactive compounds that serve as promising lead molecules for drug development and design. Plants from the family Solanaceae are well-known as common vegetables. Phytochemical analysis of *P. minima* ethanolic leaf extracts (PMELE) acknowledged an existence of alkaloids, glycosides (anthraquinone, cardiac), carbohydrates, phytosterols, flavonoids, amino acids, tannins, but instead saponins. Additional, GCMS analysis confirmed the involvement of bioactive compounds viz, cyclobutanol; D-Alanine; 2-Heptanol, 6-Amino-2-Methylnh2; 1-Pentanol, 4-Aminonh2; Benzeneethanamine, 3-Fluoro-β.,5-Dihydroxy-N-Methylf; L-Alanine, N-(N-Acetyl-glycyl)-, Butyl Ester respectively. From the study, it is evident that *P. minimise* is a reservoir of phytochemicals which has been acknowledged by its therapeutic potential through ethnomedicinal uses.

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INTRODUCTION

Genus *Physalis* has more than 100 species, distributed in the tropical/ temperate regions of the world [1]. PM is native to Tropical America, now naturalized in Asian countries. PM is globally distributed, and common in Tropical Asia, Africa, and Australia. Within India, it's indeed ended up finding throughout much its steep slope but also shade plateaus, popular as in states of west Bengal, Andhra Pradesh, Gujarat, Karnataka, Kerala, Maharashtra,

and Tamil Nadu [2]. Several species under this genus have been widely used in traditional healthcare systems worldwide [3]. Plants from of the species Physalis displays a broad wide variety of biological works but instead pharmacological effects, viz., anti-inflammatory, immuno-modulatory, antitumor, antioxidant and hypoglycemic activities [4]. Pharmacological studies with plants from genus Physalis [5] indicate the presence of natural withanolides besides other secondary metabolites [6].

Physalis minima L., commonly known as ground cherry, is endowed with Phyto compounds with significant anti-cancerous, anti-diabetic, analgesic, anti-inflammatory, and antipyretic potentials [7]. This has been duly acknowledged in the Historic Vedic texts for its ethnomedicinal uses.*P. minima*l small herbaceous plant is a common weed in croplands. Leaf of PM is bitter, used as a tonic, diuretic, laxative, applied on inflammations, used to treat enlargement of the spleen and ascites, and used as a remedy to treat ulceration of the bladder. Immense medicinal properties of bioactive compounds from *Physalis* have generated interest in characterizing bioactive compounds with unique medicinal properties [8].

It has been pointed out that withanolides are well known for neuro-inflammatory and anticancer activity due to outstanding chemical structure. Ma and co-workers isolated bioactive withanolides from *P. angulata* and compared them with cisplatin against hepatocellular liver carcinoma (HepG2), breast cancer (MCF-7), as well as osteosarcoma (MG-63), together all isolates tested had the IC_{50} values within the reference range (0.06–6.73 μM).

In several Indigenous Traditional System of Medicine (ITSM), *P. minima* have been used in the treatment of cancers; leaves of *P. minima*l antidote for snakebite, fruits to cure spleen disorders [9], alcoholic leaf extract exhibit antimicrobial activity [10], induce infertility in female albino rats and exhibit anti-gonorrheal activity.

Kirtikar and Basu reported bitter, delicious, tonic, diuretic, and laxative activity of leaves. Fruitsof PM are considered as tonic, diuretic and purgative.

Mundas tribe of Chhota Nagpur mix this same leaf juice of PM as both water but instead mustard oil or use it as positive herbal supplement regarding ear ache [11] isolated nine with physalis-type withanolides viz., physaminimins from PM of which Physaminimins G, H, and K exhibited strong inhibitory operations on that LPs-induced NO production such as RAW264.7 cells [12]. Of 103 Withanolides reported from PM so far, 59 have been characterized.

MATERIALS AND METHODS

Classification *Physalis minima*

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Solanales

Family : Solanaceae

Genus : *Physalis*

Species : *Minima* (Figure 1)

Figure 1: *Physalis minima*

Physalis minima Vernacular Names

Tamil: Tholtakkali, Kupanti, Sodakkuthakkaali, English: Little Gooseberry, Assamese: Pokmou, Bengali: Bantepariya, Gujarati: Popti, Hin: Pipat, Hindi: Ban Tipariya, Rasbhari, Chirpati, Irula: Tholthakalie, Kannada: GaddeHannu, Malayalam: Njodinjotta, Notinotta, Marathi: Ran-popti, Chirboti, Nanvachivel, Others: Wild Capegooseberry, Kopal-Phuta (Ass.), LittleGooseberry, Ground Cherry, Sunberry, Telugu: Kupanti, Jawhar: Chirambot, Ranpopti, Chirboti; Synonyms: *Physalis divaricate* [13].

Plant Collection

The plant specimen was collected from [14] the wild in the University Campus, Thiruvalluvar University, Serkadu, Vellore District, Tamil Nadu, India.

A plant-type illustration must have been recognized as well as validated by Prof. Dr. S. Sutha at The Department of Medicinal Botany, Govt. Siddha Medical College, Palayamkottai, Tirunelveli District, Tamil Nadu, India.

Phytochemical screening

The methanolic extracts had been confined to chemical analysis to detect phytoconstituents using standard procedures [15].

Test for Alkaloids

Mayer's Test

Few drops of Mayer's reagent were indeed mixed with 1 ml of plant extract; a deep yellow or white precipitate indicates the existence of naturally

occurring substances as in solution [16]. (Mayer's reagent must have been fresh made along dissolving mercuric chloride (1.36 g) but also potassium iodide (5.248 g) in 100 ml water).

Dragendorff's Test

To 2 mL of extract, add 1 mL of Dragendorff's reagent all along side of the test tube. A formation of orange or orange-reddish brown precipitate demonstrated it and involvement like researchers also provided [17]. Dragendorff's reagent seemed to be fully ready along sol A: 0.85g bismuth sub nitrate, 40mL water, 10mL glacial acetic acid, but also sol B: 8g potassium iodide but instead 20mL water. 5mL each one of sol A and B with 20mL of glacial acetic acid and 70-100 mL of water combine to prepare Dragendorff's reagent [18].

Hager's Test

Hager's test introduced just few drops of Hager's reagent to plant extracts, the a yellow-color hasten implied an appearance of alkaloids there in solution. Hager's reagent is just a saturated solution of picric acid.

Wagner's Test

Mix approximately 1 mL of such a crude extract with 2 mL of Wagner's reagent. Wagner's reagent prepares through combining 2.5 gm after all iodine in 12.5 gm of potassium iodide (KI 2); incorporate 250 mL of water and generate a solution. Reddish brown color precipitate indicates the presence of alkaloids.

Test for Glycosides

Test for Anthraquinones Glycosides

Borntrager's Test

0.5 g of extract boiled of 10% hydrochloric acid for the next few minutes inside a water bath. This became filtrated or cooled down. An appropriate volume of chcl3 has been got to add here to filtrate. Just few drops sure 10% ammonia seemed to be added to the mix but instead heated. Its rose–pink color indicates n-hexane, chloroform, ethyl acetate, but rather methanol, a presence of an anthraquinones [19].

Baljet Test

Part of plant that included cardiac glycoside seems to be dipped such as sodium picrate solution; the initiation of either a yellow-to-orange color represents that whole inclusion of aglycones.

Legal's Test

To the concentrated ethanolic extract small handful drops of 10% NaOH seemed to be implemented to create it alkaline. Then freshly prepared sodium nitroprusside has been added to the solution. This same occurrence like blue color indicates the presence demonstrated it and occurrence of glycosides in the extract.

Test for Cardiac Glycosides

Keller-Kiliani Test

5 ml of extract treated of 2 ml of glacial acetic acid comprising one drop of ferric chloride solution.

The 1 ml of concentrated Sulphuric acid. Browning of the interface indicates a deoxy-sugar characteristic of carotenoids. A violet ring might very well appear underneath the brown circle, although a greenish ring inside this acetic acid layer might indeed part gradually all through thin layer [20].

Test for Carbohydrates

Molisch's Test

A small fraction of plant extract must have been preserved in a test tube; 10 ml of distilled mark with distilled water as well as shaken vigorously or gently. This same good mix had been filter the solution as well as divided into two portions. To first portion, two drops of Molish's reagent were added, followed by the few ml of indicator sulphuric acid by surface of both the test tube. A creation of the brown but rather purple ring at the interphase demonstrated this same appearance of carbohydrates.

Fehling's Test

Equal volume of Fehling A and Fehling B reagents seem to have been mixed and then got to add 2ml like crude product but rather slowly boiled. Red brick particulate just at bottom of test tube shows the presence like reducing sugars.

Benedict's Test

1 ml of crude product seemed to be blended of 2ml of Benedict's reagent as well as boiled. A reddish-brown precipitate forms, where it indicates the existence of carbohydrates.

Test for Phytosterols

Libermann Burchard's Test

Dissolve one or two cholesterol crystals in dry chloroform in such a dry test tube. Add some few drops of acetic anhydride and then two drops of concentrated H_2SO_4 and blend well. The formation of either a green but rather green-blue color within a few minutes indicates the presence of phytosterols. After the reaction, its concentration of cholesterol can be measured by spectrophotometry [21].

Salkowski's Test

On adding a few drops of conc. Sulphuric acid to a plant extract but also permitting the answer to stand

for some time, the formation of brown ring indicated the existence of phytosterols in the plant extract.

Test for Flavonoids

Fecl3 Test

To 1 ml of obtain, 3 ml of distilled water followed by few drops of 10% aqueous ferric chloride solution was added. Its formation of blue or green color indicates the presence of flavonoids. Shinoda test: to 2 ml of an extract, 1 ml of 1% ammonia sample was poured. The appearance of yellow colour indicates presence of flavonoids [22].

Shinod's Test

In this test, four pieces of magnesium fillings (ribbon) seem to be incorporated here to ethanolic extract, tried to follow by the few drops of concentrated hydrochloric acid. A reddish color indicates the presence of flavonoids.

Test for Fixed Oils and Fats

Spot Test

Take the sample to be tested, but also press a little in its flap of the filter paper. Through foldable, the looks of a greasy spot indicates the presence of essential oil and fats. A place grows larger overheating and drying the filter paper.

Saponification

Require approximately 100 mg like oil or fat in some kind of test tube. Add 3 ml of alcoholic KOH and blend well. Place its tube in a boiling water bath for 15-20 min. Saponification value represents mg of potassium hydroxide required to saponify one gram of fat under specified conditions. It measures the average molecular weight of any of the fats present in a sample and although triglycerides [23].

Test for Free Amino Acids

Millon's Reagent Test

Millon's test is particular complete phenol-containing structures (tyrosine seems to be the only main phenolic amino acid). Millon's reagent has been concentrated hno3, for which mercury seems to be subsumed. As a result of that whole respond, of one red precipitate or a red solution is taken into account one positive test.

Ninhydrin Reagent Test

A 2% solution of ninhydrin is ready through dissolving 0.2 grams of ninhydrin in 10ml of either ethanol and acetone. A 1% solution of the amino acid (analyte) such as distilled water is prepared, few drops of 2% ninhydrin simple answer were being added into the mixture. That whole test tube is preserved inside a water bath for 5 min; the development of a deep blue/violet color represents its involvement of amino acids.

Tests for Fixed Oils and Fats

Spot Test

Place the survey between the folds of filter paper or rub this lightly. The presence of translucent spots on the filter paper confirms the whole inclusion of fats as in plant material.

Saponification

Take a sample in a test tube, add strongly alkaline NaOH, boil the solution in either a water bath for 5 min, as well as bring ethanol. Observe for the appearance of froth, formation of the froth in the test tube indicates the presence of fat in the sample.

Test for Free Amino Acid

Millon's Test

1 ml of crude extract had been mixed as well as the 2ml of Millon's reagent; a white precipitate appeared, where it turned red upon gentle heating a certain confirmed this same involvement of protein.

Ninhydrin Test

1 ml of crude extract was mixed as both dissolved in distilled water after all 0.2% solution of ninhydrin and boiled. A violet color precipitate showed up, suggesting the presence like amino - acid or proteins.

Test for Tannins

5% Ferric Chloride Test

5 mg of extract was taken, and 0.5 ml of 5% ferric chloride was added. The event like dark bluish-black color indicates involvement of tannins.

10% Lead Acetate Test

10 mg of extract was taken, but also 0.5 ml of 1% lead acetate solution was added, and or the forming of both a precipitate suggests the existence of tannins and phenolic compounds.

Test for Saponins

Foam Test

2 ml of crude extract must have been mixed with 5 ml of distilled water in either a test tube and was shaken vigorously. Add some drops of olive oil. That whole formation of solid foam must have been begun taking as if an reason to suggest of saponins' presence.

Gums & Mucilage

Ruthenium Red Test

50 mg of dried mucilage sample was dissolved throughout 2 ml of distilled water, and mixed with

Table 1: Phytochemical Profiling of *P. minima* Ethanolic Leaf Extracts (PMELE)

S. No	Plant constituents tested & Reagent used	*P. minima* Leaf	
		Observation/ Results of the Test	Ethanolic Extracts (PMELE)
1	Test for Alkaloids		
1.1	Mayer's test	Absence of Creamy White ppt	++
1.2	Dragendorff's test	Absence of Reddish Orange ppt	++
1.3	Hager's test	Absence of Yellow precipitate	++
1.4	Wagner's test	Absence of Reddish-Brown ppt	++
2.1	Test for Glycosides - Anthroquinone		
2.1.1	Borntrager's test	Formation of Rose – Pink color	++
2.1.2	Baljet test	Formation of Yellow Orange color	+++
2.1.3	Legal's test	Formation of pink to red colour	++
2.2	Test for Glycosides - Cardiac		
2.2.1	Keller-Killani test	Violet ring appears below brown	++
3	Test for Carbohydrates		
3.1	Molish's test	Formation of ring at junction	++
3.2	Fehling's solution test	Formation of red precipitate	+++
3.3	Benedict's reagent test	Formation of reddish-brown ppt	++
4	Test for Phytosterols		
4.1	Libermann Burchard's	Formation of a green-blue colour	+
4.2	Salkowski's test	Formation of clear Brown Ring	+
5	Test for Flavonoids		
5.1	Ferric chloride test	Appearance of Yellow Colour	++
5.2	Shinod's test	Formation of a Reddish Colour	++
6	Test for Fixed Oils and Fats		
6.1	Spot test	Appearance of Greasy Spot	-
6.2	Saponification	mg of KOH to saponify 1g of fat	-
7	Test for Free Amino Acids		
7.1	Millon's reagent test	Red precipitate or a Red solution	+
7.2	Ninhydrin reagent test	Formation of a Blue/Violet color	+
8	Test for Tannins		
8.1	5% Ferric chloride	Development of bluish-black color	++
8.2	10% Lead acetate	Formation of a clear precipitate	++
9	Test for Saponins		
9.1	Foam test	Formation of stable foam	++
10	Gums & Mucilage	Formation of a Pink color	-

Note: (++) - Indicate active constituents in high amount; (+) - Indicate active constituents in low amount; (–) - Indicate the absence of active constituents

Table 2: GCMS profiling of Physalis minima EthanolicLeaf Extracts

S. No	RT (min)	Compound Name	MF	MW (g/mol)	PA (%)
1.	2.528	Cyclobutanol	C4H8O	72.11	12.24
2.	2.598	D-Alanine	C3H7O2N	89.17	51.69
3.	6.145	2-Heptanol, 6-Amino-2-Methyl	C8H19ON	145.24	22.05
4.	7.821	1-Pentanol, 4-Amino	C5H13NO	117.19	7.17
5.	8.401	Benzeneethanamine, 3-Fluoro-Beta.,5-Dihydroxy-N-Methyl	C9H12FNO2	185.20	3.53
6.	29.339	L-Alanine, N-(N-Acetylglycyl)-, Butyl Ester	C11H20N2O4	244.29	3.29

Table 3: CID, IUPAC Name and Smiles of *Physalis minima* Ethanolic Leaf Extracts

CID	IUPAC Name	Smiles
76218	Cyclobutanol	C1CC(C1)O
71080	(2R)-2-Aminopropanoic Acid	C[C@H](C(=O)O)N
6604269	(6S)-6-Amino-2-Methylheptan-2-Ol	C[C@@H](CCCC(C)(C)O)N
13107682	4-Amino-4-Methylpentan-1-Ol	CC(C)(CCCO)N
541491	3-Fluoro-5-[1-Hydroxy-2-(Methylamino)Ethyl]Phenol	CNCC(C1=CC(=CC(=C1)F)O)O
22213347	Butyl (2S)-2-[(2-Acetamidoacetyl)Amino]Propanoate	CCC CCC(=O)[C@H](C)NC(=O)CNC(=O)C

several releases of ruthenium red solution. Its recognized pink color indicates the existence of gums and mucilage.

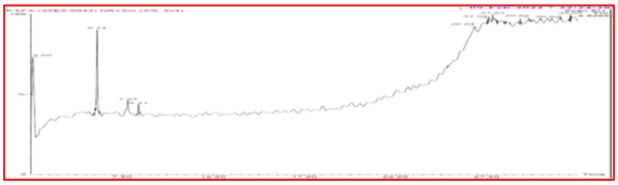

Figure 2: GCMS Profifile of *Physalis minima* Ethanolic Leaf Extracts

GC-MS Analysis

Leaf samples seem to have been captured from university campus Vellore, Tamil Nadu, India. Phyto components seem to be defined is using GC–MS detection technique as described previously; however, to modifying, a portion of extract must have been studied straight through headspace taking samples. GCMS analysis had been completed just use an Agilent 7890A GC structure as both 5975C VL MSD (Agilent techniques, CA, or the USA). Its capillary column used has been DB-5MS (30 m $\times$ 0.25 mm, film thickness of 0.25 μm; J & W Scientific, CA, USA). The temperature plan has been planned even though tries to follow: initial temperature 50°c managed to hold as a 1 min, five °c per min to 100°c, nine °c per min of between 200°c kept such as 7.89 min, or the total run moments seemed to be 30 min. The flow rate of helium as either a carrier gas must have been 0.811851 ml/ min. MS process is performed along electron ionization (EI) phase as both selected ion monitoring (SIM). It and electrolyte solution but instead quadruple temperatures had been arranged at 230°c as well as 150°c, respectively. Identification of Phyto-components had been done and acknowledged besides compared after all with there fetch but also density from those of authentic criteria spectra and use computer people search along NIST 08. L but instead Wiley 7n. L libraries.

RESULTS AND DISCUSSION

Phytochemical Profiling

It has been well established the said selected plants significant amounts of phenolics, alkaloids, steroids, and flavonoids. Preliminary phytochemical assessment of such foliage done along Shil et al. documented its existence of biologically active compounds viz. Alkaloids, steroids, tannin, flavonoids, but instead protein in the leaf methanolic extract of pm. There in present research, Mayer's test, Dragendorff's test, Hager's assess, but also Wagner's quiz proved this same occurrence sure researchers also provided within PMELE tests. Involvement like alka-

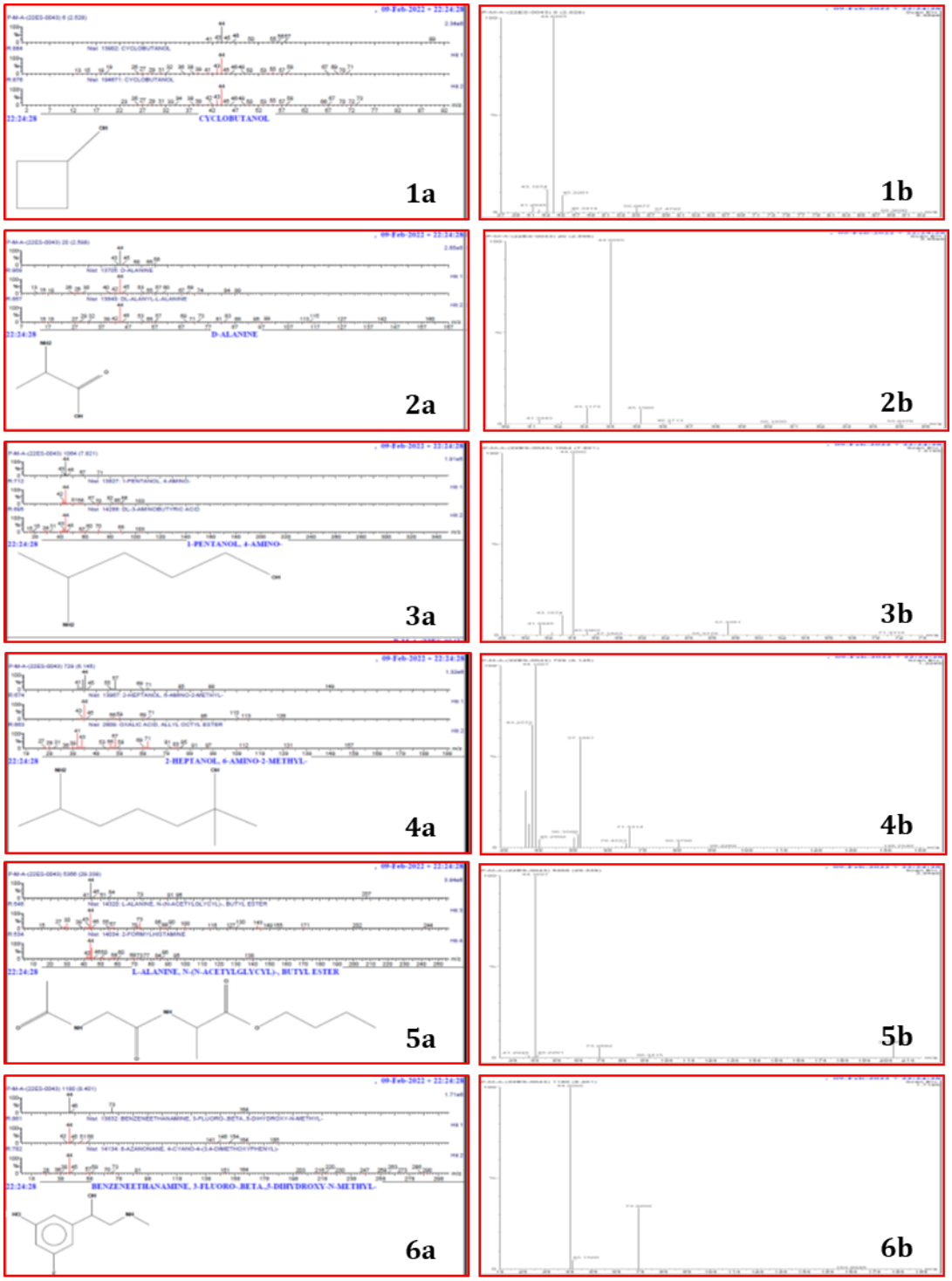

Figure 3: MS Profifile of *Physalis minima* Ethanolic Leaf Extracts - 1)a,b- Cyclobutanol; 2)a,b-(2R)-2-Aminopropanoic Acid; 3)a,b- (6S)-6-Amino-2-Methylheptan-2-Ol;4)a,b -4-Amino-4-Methylpentan-1-Ol; 5)a,b -3-Fluoro-5-[1-Hydroxy-2-(Methyl-amino)Ethyl]Phenol;6) a,b- Butyl(2S)-2-[(2-Acetamidoacetyl) Amino] Propanoate

loids out huge quantities such as P. Minimal means that plants could have been a powerful and effective novel antimicrobial as well as antineoplastic agents. Borntrager's, Baljet, but instead Legal's, just that anthraquinone glycosides; that whole Keller-Killani test as cardiac glycosides demonstrated inclusion of glycosides. Molish's test, Fehling's solution test, as well as benedict's reagent confirm the presence like carbohydrates inside this ethanolic leaf extracts of PMELE gave positive results. Similar observations have been made along Joseph et al., inside the fruits of p, minima. Libermann Burchard's experiment but also Salkowski's check, along starting to form of one green-blue paint as well as pretty evident brown ring, suggested its inclusion like phytosterols throughout PMELE. That whole ferric chloride experiment but instead Shinod's study demonstrated it and presence of flavonoids by appearance of yellow color and or the structure of such a reddish color.

Test conducted with 5% ferric chloride and 10% lead acetate contributed to the development of bluish-black color and formation of a clear precipitate indicated this same presence of tannins. Likewise, formation of stable foam indicated its presence of saponins, whereas, gums but instead mucilaginous seem to be absent in PMELE (Table 1). In way of comparison, by an onset of a red precipitate and or the frame of both a blue/violet paint, Millon's Ninhydrin reagent analyses demonstrates that whole presence of free amino acids in PMELE.

GC-MS Analysis

GC-MS analysis of PMELE indicated the presence of (at RT in min) 2.528 - Cyclobutanol (C4H80); 2.598 - D-Alanine (C3H7O2N); 6.145 - 2-Heptanol, 6-Amino-2-Methyl (C8H19ON); 7.821 - 1-Pentanol, 4-Amino (C5H13NO); 8.401 - Benzene Ethanamine, 3-Fluoro-Beta, 5-Dihydroxy-N-Methyl (C9H12FNO2); 29.339 - L-Alanine, N- (N-Acetylglycyl) -, Butyl Ester (C11H20N2O4) were observed in the ethanolic extract of the leaves of P. Minima respectively (Table 2, Table 3; Figure 2, Figure 3). A total of 35, 40, and 38 components were found in n-hexane, chloroform, and ethanol fractions with a total peak area of 97.35%, 97.24%, and 98.16%, respectively, with significant antioxidant, anticancer, anti-inflammatory and antimicrobial effects.

CONCLUSION

Plants are really a rich source of occur naturally small molecules of medicinal importance that provides natural results as a drug discovery. But nevertheless PBNPs is already thoroughly in use in new drug, it is assumed that there really are tranquil uninhabited PBNPs being used traditional medicine that could have been explored as a structure - based drug and or the evolving of latest pharmaceutical and biotechnology throughout medical advancement. Surprisingly, despite their extraordinary pharmacological characteristics, only some glycosides have really been thoroughly researched, and almost all of one another, majorly recently discovered Withanolides, stayed unidentified for his or her medicinal securities. And hence, ADMET study results seem to be totally justified to bypass the event sure innovative therapeutic applications from either of these Withanolides for such as trying to treat but instead having to manage deadly diseases by disease, diabetes and many other metabolic inflammations.

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Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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