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RESEARCH ARTICLE

A study on the pharmaceutical compounds of *Bacillus thuringiensis* isolated from *Cissus quadrangularis* (L).

Muthuraja K, Saravanan P, Anand D*

Abstract

Medicinal plants can harbor an abundance of beneficial microorganisms such as yeast, fungus, and bacteria. Of them, bacteria are the most promising sources of bioactive compounds, which have enormous implications for a variety of fields including business, agriculture, and medicine. *Cissus quadrangularis*, an aromatic plant is widely known for its medicinal properties. The current study was carried out to screen for the presence of *Bacillus thuringiensis* bacteria in the leaves of *Cissus quadrangularis*. Further investigation was done to explore the contribution of the bacteria in the metabolite content were extracted using chloroform and carbon tetrachloride. The compounds were identified using Gas Chromatography-Mass Spectrometry analysis. The analysis revealed the presence of significant metabolites like α-pinene, 3-carene,o-cymene, Limonene, Eucalyptol, neral, , Isobornyl acetate, 2,4-Di-tert-butylphenol, Dibutyl phthalate, Benzaldehyde, and Phthalic acid, di(2-propylpentyl) ester has medicinal value. This study confirms the presence of bacteria in the *Cissus quadrangularis* leaves and the bacterial isolate from plants could be a potential source of pharmaceutical important compounds.

Keywords: *Cissus quadrangularis, Bacillus thuringiensis,* 16S rRNA, GC-MS, pharmaceutical compounds.

Introduction

The *Cissus quadrangularis* plant is a traditional medicinal herb with a range of potential uses. The Nigathus, an ayurveda text, promotes the therapeutic application of the herb to set bones and cure fractures. In addition to bone remineralization, the plant has ameliorative properties supported by notable phytoconstituents. The pharmacological research conducted on hadjod now validates the plant's old classical references and reaffirms its promise as a remedy for a number of ailments. *Cissus quadrangularis* extracts have anti-oxidant and anti-cancer properties [Mukherjee *et al*,.].

Perennial climber *C. quadrangularis (L)* belongs to the Vitaceae family and is found throughout tropical India. Reported as Nash *et al*. (2019), Sawangjit *et al*. (2017), (Lee *et al.* (2018), Jainu and Devi (2005), it has been used to treat dyspepsia, anorexia, flatulence, colic, seizures, tumors, epistaxis, asthma, irregular menstruation disorders, inflammation, antibacterial infections, and obesity. It is well known to be an age-old medicinal herb that heals the body's white tissue areas (ligaments, tendons, etc.) most effectively (Raj *et al.,* 2011). According to the phytochemical study of *C. quadrangularis* reveals the presence of carotene, phytosterol, terpenoids, β-sitosterol, δ-amyrin, and calcium. (Mishra *et al.* (2011),

Development of novel antimicrobial medications has become increasingly difficult as a result of the emergence of pathogens and antibiotic resistance. Microorganisms have been a natural product source for pharmacological applications for decades. A reported from Food and Drug Administration (FDA) states that microorganisms were responsible for 25% of the 38% of natural materials used as medications (Toghueo 2020; Patridge *et al.,* 2016). The microorganisms that live inside the healthy plant tissues without obviously harming the host interactions, from mildly harmful to symbiotic. (Strobel and Daisy 2003).

There are bacteria in almost all plants on the planet. These organisms inhabit the living tissues of their host plant, where they coexist in a range of symbiotic to harmful associations. The potential to yield novel products and molecules in chemistry and biology that could be used to

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address health issues affecting humans (Karria *et al*., 2012). By generating an abundance of secondary compounds that promote growth, guard against herbivores, and withstand biotic and abiotic challenges, endophytes benefit the host plant (SchulzeMakuch *et al*., 2018). Furthermore, according to they have been a good source of chemical compounds with antibacterial, antifungal, anticancer, anti malarial, antioxidant, antiviral, and immunosuppressive activities that are significant to the pharmaceutical industry. (Joseph and Priya (2011) and Kharwar *et al*. (2011).

Antibiotics are the most significant microbial metabolites that have been isolated till now which inhibit growth of pathogens at low concentrations; diverse bacterial species produce around 2589 pharmaceutical products. They have a significant impact on society's finances, nutrition, and health. *Actinomycetes* produced 8,700 antibiotics and 1,400 other bioactive metabolites, while bacteria produced 2,900 antibiotics and 900 other bioactive metabolites (Berdy 2005).Among them notable antibiotics are quinolones (11%) tetracyclines (6%), macrolides (5%), thienamycin, and cephalosporins (45%), penicillins (15%), quinolones (15%), and tetracyclines (6%). According to natural products or modified natural microbial products make up more than 60% of the authorized small molecule medications. (Newman and Cragg (2007).

There were 88 genera and 11 orders of bacteria with known relationships to medicinal plants in the literature. With 72.62% of all orders, the most prevalent ones were *Pseudomonadales*, *Enterobacterales*, and *Bacillales*. *Pseudomonas, Pantoea*, and *Bacillus* were the most prevalent genera, accounting for 58.92% of all genera. *Bacillus, Pseudomonas*, and *Paenibacillus* can affect the growth, stress resistance, and metabolism of medicinal plants. Streptomyces is commonly reported to support plant growth and development (Gao *et al*., 2015; Zhao *et al*., 2015; Qi *et al*., 2021). Remarkably, toxic properties against nematodes, mites, and ticks; antagonistic effects against pathogenic bacteria and fungi in plants and animals; plant growth-promoting activities (PGPR); bioremediation of various heavy metals and other pollutants; biosynthesis of metal nanoparticles; production of biopolymer; and anticancer activities are the novel environmental features of *Bacillus thuringiensis* (Jouzani *et al.,* 2017).

To investigate bacteria from indigenous plants as sources of bioactive secondary metabolites, the present study was undertaken to evaluate the pharmaceutical compounds of bacterial secondary metabolite extracts.

Materials and methods

Collection of plant materials

The Fresh and healthy leaves of *Cissus quadrangularis* were collected from Chengattur Village, Chengalpattu District, (Latitude: 12.5268^{O}N and Longitude: 80.0088^{O}E) Tamil Nadu, India, during the month of January and February in a sterile bag and kept at cold and dry conditions for further use. The plant was authenticated by Botanical Survey of India (BSI), Southern Region Tamil Nadu Agricultural University (TNAU), campus Coimbatore. (Ref no: BSI/SRC/5/23/2023/Tech.-593 date:14th Aug 2023).

Surface sterilization

The specimen was washed with tap water and rinsed to remove the external debris and other dirt sticking to the leaf surface. The leaves were bisected into small pieces covering all side. The bisected leaf pieces were then dipped in 70% ethanol for 5 seconds then transferred to sterile Petri dishes containing 4% sodium hypochlorite (NaOCl) and kept for 1 minute. Following that leaf pieces were rinsed in sterile water for 10 seconds and the excess moisture was blotted on a sterile filter paper. It was used for the isolation of bacteria (saini et al,. 2016) .

The Isolation of bacteria

The prepared leaf piece was placed on nutrient agar (NA) in Petri dishes and incubated in the dark at 37^{O}C for overnight. All plates including the control were incubated at room temperature for five days and observed periodically for bacterial growth. Those batches of experiments where the bacterial growth, if any present, in the control plate were completely discarded. The observed colonies were picked on the basis of the morphological features like size, shape, elevation, opacity, surface, texture and margin (Boone *et al.,* 2005). Pure cultures were obtained through the quadrant streak and stored in the refrigerator for molecular analysis. It was sub cultured two to three times to check the purity before DNA extraction and to avoid the mixed profile.

Genomic DNA Isolation from Bacteria

Genomic DNA was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. A part of culture is taken in a micro centrifuge tube. 180 µl of T1 buffer and 25 µl of proteinase K was added and incubated at 56°C in a water bath until it was completely lysed. After lysis, 5 µl of RNase A (100 mg/ml) was added and incubated at room temperature for 5 minutes. 200 µl of B3 buffer was added and incubated at 70°C for 10 minutes. 210 µl of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 x g for 1 minute. The NucleoSpin® Tissue column was transferred to a new 2 ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of B5 buffer. After washing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of BE buffer. The quality and quantity of DNA was checked through 0.8% agarose gel electrophoresis under Gel documentation system (Bio-Rad).

Molecular Identification of Bacteria

The 16S rDNA genes were amplified from purified geneomic DNA of bacteria. The reaction mixture contained 50 mg of template DNA, 10X PCR buffer, 1.5 mM $MgCl_2$, 2.5 mM each dNTPs, 10 pmol of forward and reverse primer and 1.5 U of Taq DNA polymerase. The total mixture volume was 50 µl and the program starts with initial denaturation at 94 °C for 3 min, followed by denaturation at 94 °C for 30 s, annealing at 55 °C for 1.5 min, extension at 72 °C for 2.5 min then the final extension at 72 °C for 5 min. From denaturation to extension step 35 cycles were repeated. The 16S rRNA product was resolved in 1.5% (w/v) agarose gel electrophoresis in 1X TAE buffer using 1 kb ladder as molecular weight marker and visualized by staining with ethidium bromide.

PCR product purification and sequence analysis

ExoSAP-IT Treatment was carried with5 µl of PCR product is mixed using 0.5µl of ExoSAP-IT and incubated at 37°C for 15 minutes. It was followed by enzyme inactivation at 85°C for 5 minutes to remove unwanted primers and dNTPs from PCR product for no interference in downstream applications. Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.,* 2012).

GC-MS sample preparation

The secondary metabolites from the extracted by inoculating 200 µL of bacterial suspension into 250 ml of sterile nutrient broth media. The culture was incubated at 37 °C for 5 days in Orbitol shaker, at 150 rpm. After the incubation period, the culture broth was filtered and filtrates were added with an equal volume of 1:1. (a) Chloroform (250 ml) and (b) Carbon tetrachloride individually. The mix was left overnight at 4 °C. Using a separating funnel, the solvent layer containing the extracted metabolites was collected and evaporated in a rotary evaporator (90 rpm, 40 °C) to obtain the crude metabolites (Kim *et al.,* 2007)

Analysis of secondary metabolites through GC-MS

The bioactive compounds present in the crude extract were analysis using gas chromatography-mass spectrometry (GC-MS). It was performed at a flow rate of 1.0 ml per minute. The evaluation was done thrice. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were confirmed. Then, after evaporation, the purified extract residue was dissolved in 250 µL of acetonitrile and analyzed by GC-MS.

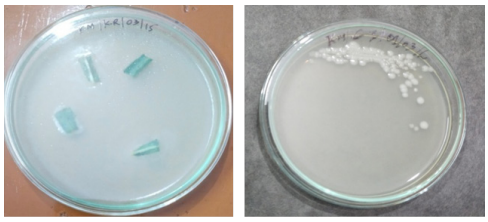
Table 1: Details of Primers used

Target	*Primer Name*	*Direction*	*Sequence (5′ → 3′)*
16S rRNA	16S-RS-F	Forward	CAGGCCTAACACATGCAAGTC
	16S-RS-R	Reverse	GGGCGGWGTGTACAAGGC

Results and Discussion

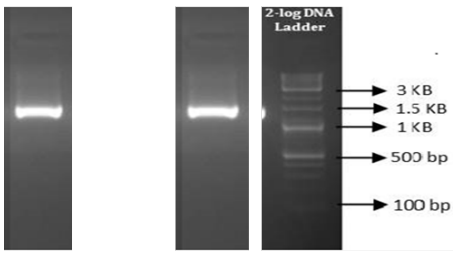
Isolation and identification of bacteria

One bacterial strain was isolated from the leaf segments of *Cissus quadrangularis* (Fig: 1.A&B). 16S rRNA sequencing revealed that the bacteria belongs to the genus *Bacillus*. BLAST search revealed that the isolate is most related to *Bacillus thuringiensis* with 99.6% of sequence identity. The 16S rRNA Gene Sequence (Fig. 2). The sequence is deposited to the National Centre for Biotechnology Information (NCBI) Gene bank with Accession No: OR214936. In order to eradicate the epiphytic germs, those were surface sterilized. The pure cultures were subjected to genomic DNA isolation. The isolated DNA was checked for its quality and quantity to perform PCR. The 16S rDNA genes of the bacterial isolates were amplified and the molecular weight of the amplicon was found to be 1500bp with the above said conditions and they were sequenced to identify the phylotype of the bacterial species. Molecular identification of the isolates was done by sequencing a part of the 16S rDNA. The amplification of the 16S rDNA was confirmed by agarose gel electrophoresis (Fig: 2A&B).



(A) (B)

Fig 1: Isolation of bacteria; (A) the culture initiated from leaf bits, (B) pure culture from isolate.



(A) (B)

Fig. 2: (A) Gel image of isolated DNA (B) Gel image showing Comparison of the isolated DNA with the reference DNA ladder

The PCR product was gel eluted and sequenced. The genomic DNA can then be analyzed using a range of molecular fingerprinting techniques. In order to assess the variety of bacteria, the genomic DNA is typically utilized to amplify a marker gene, typically the 16S rRNA gene (Garbeva *et al*., 2001). The 16S rDNA gene sequence is regarded as a potent tool for the quick identification of bacterial species since it allows for appropriate grouping of organisms even at the subspecies level (Jill and Clarridge 2004). Following BLAST examination of the 16S rDNA sequencing data, *Bacillus thuringiensis* was identified as the organism responsible. It has been demonstrated that many plant species are linked to bacteria such as *Pseudomonas, Bacillus, Azospirillum*, etc. (Chanway *et al*., 1996).

Previously reported as isolated the *Bacillus thuringiensis* from leaf of *Andrographis paniculata* and proved by 16S rRNA sequencing analysis of which similarity it is proved that culture independent of bacteria 16S rRNA is higher significance for the identification for bacterial species (Roy *et al*., 2016).

*Bacillu*s and *Pseudomonas* are the most often isolated bacterial genera, with *Burkholderia*, *Microbacterium*, *Micrococcus*, *Pantoea*, and *Stenotrophomonas* being the other two. *Bacillus*, *Burkholderia*, *Micro*date have been identified as antibiotics, anticancer drugs, biological control agents, antivirals, ant*bacterium*, *Micrococcus, Pantoea, Pseudomonas*, and *Stenotrophomonas* are most often isolated bacterial genera; *Pseudomonas* and *Bacillus* are the most common genera (Chaturvedi *et al*., 2016; Hallmann *et al*., 1997).

Many of them have the ability to synthesize bioactive chemicals, some of which have been shown to be valuable in the search for new drugs, and which plants can use to defend themselves against infections. According to their various physiological roles, the majority of natural products derived from endophytes to idiabetic agents, and other bioactive substances (Guo *et al*., 2008).

Leucojum aestivum bulblets were used *in-vitro* to extract the bacteria from the *Bacillus* genus, and their capacity to generate *Amaryllidaceae* alkaloids was investigated. Thus, the *L. aestivum* plant and the isolated *Bacillus sp*. from the bulb lets shared the synthesis of some primary and specialized metabolites. An intriguing new method for manufacturing lycorine and other *Amaryllidaceae* alkaloids is to use these bacteria. According to the obtained data, lycorine, an alkaloid of interest belonging to the *Amaryllidaceae* family, may have originated from the *Bacillus sp*. The bacterium (Spina *et al*., 2021). The bacteria and fungi are thought to be an intriguing source of biomolecules since they can produce specific metabolites similar to those of their host plants (Gouda *et al*., 2016).

It is common practice to isolate *Bacillus* species from plant tissues and seeds. A life sciences business has commercialized *B. pumilus* for its biocontrol capabilities, and various strains of *Bacillus*, including *B. subtilis and B. pumilus*, have been reported to have antagonistic activities against pathogenic fungi (Fongicides *et al*., 2020). Additionally, *B. pumilus* is bacteria that has been identified from various citrus plants (Araujo *et al*., 2002) and *Echinacea* species (Lata *et al*., 2006).

To determine the prevalent organic compounds produced by the most bioactive strain *Bacillus thuringiensi,* chloroform and carbon tetra chloride solvent were used to identify polar and non-polar compounds respectively. Properties were tentatively identified based on comparison of spectra available through the National Institute of Standards and Technology (NIST) database. Majority of the identified bio-active compounds comprise pharmaceutical and antimicrobial activities. Several compounds of identified in the GC-MS analysis are known to have antimicrobial activity Mohamad *et al.,* (2018).

GC-MS Analysis of Bcterial Secondary Metabolites

Chloroform Extract of Bacillus thuringiensis

The following compounds were the solvent chloroform of *B.thuringiensis* like α-Pinene, 3-Carene, o-Cymene, Limonene, Eucalyptol, Cyclohexene, 1-methyl-4-(1-methylethylidene)-, (-)-Isopinocampheol,acetate, Neral, Isobornyl acetate, meso-5,6-Decanediol, 5-Octadecene,(E)-, Dibutylphthalate, Neral, 2,4-Di-tert-butylphenol, Aceticacid,phenylmethylester, Phthalicacid,hept-3-ylisobutylester, Phthalic acid, butyl 2-pentyl ester, Acetaldehyde, (3,3-dimethylcyclohexylidene)-,(E)-, Pentanedioicacid,dimethylester, 2,6-Octadienal, 3,7-dimethyl-,(E)-, Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-,(1S)-, Cyclohexanone, 5-methyl-2-(1-methylethyl)-,(2R-cis)-, Cyclohexanol, 5-methyl-2-(1-methylethyl)-,(1α,2β,5β)-, 3-Cyclohexene-1-methanol,α,α,4-trimethyl-,(R)- 7,9-Di-tert-butyl-1- oxaspiro(4,5)deca-6,9- diene-2,8-dione (Tables 1 and 2; Fig. 3).

Pinenes are hydrocarbons, a type of bicyclic molecule with terpenoid double bonds. Pinenes exhibit a range of medicinal properties, including anti-inflammatory, anti-allergic, antioxidant, anti-schizophrenia, and hypoglycemic actions. Essential medicinal properties of pinenes include

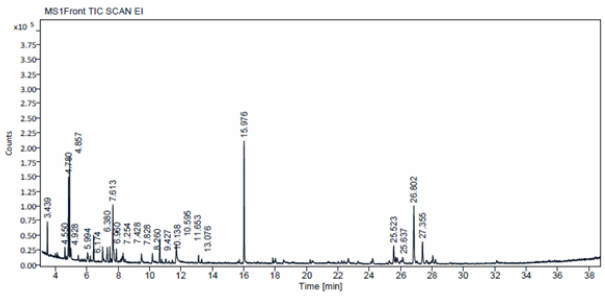
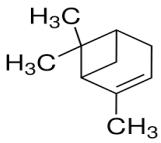
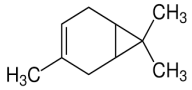
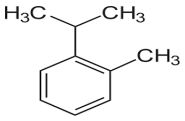
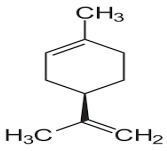
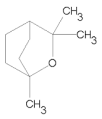
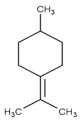
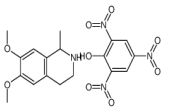
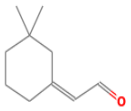
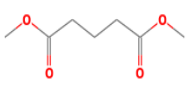
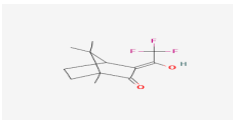
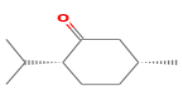
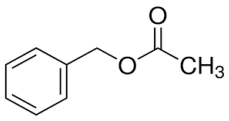
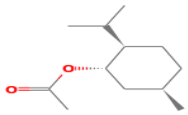
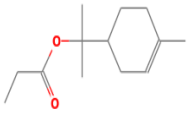
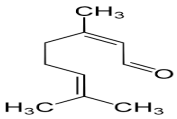
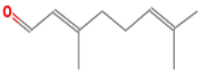
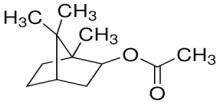
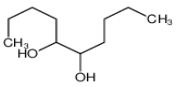
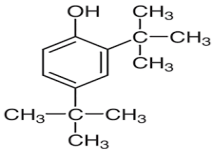
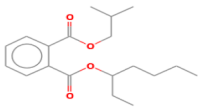
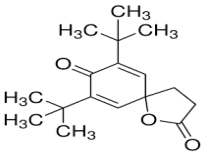
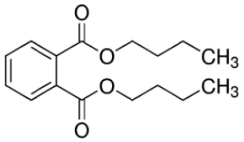
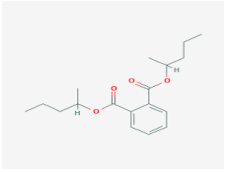


Fig. 3: GC-MS chromatogram depicting chloroform extracts of *Bacillus thuringiensis*

Table 1: Chloroform compounds identified by GC-MS in *B. thuringiensis* extract.

S.No	*Compound name*	*Molecular Formula*	*Molecular Weight*	*Retention time (min)*	*Peak Area*	*Molecular structure*
1	α-Pinene	$C_{10}H_{16}$	136.2340	3.437	3.17	
2	3-Carene	$C_{10}H_{16}$	136.2340	4.550	1.34	
3	o-Cymene	$C_{10}H_{14}$	134.2182	4.780	8.85	
4	Limonene	$C_{10}H_{16}$	136.2340	4.857	10.38	
5	Eucalyptol	$C_{10}H_{18}O$	154.2493	4.928	1.67	
6	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	$C_{10}H_{16}$	136.2340	5.994	1.47	
7	(-)-Isopinocampheol,acetate	$C_{12}H_{20}O_2$	196.2860	6.174	0.76	
8	Acetaldehyde, (3,3-dimethylcyclohexylidene)-,(E)-	$C_{10}H_{16}O$	152.2334	6.380	2.94	
9	Pentanedioicacid,dimethylester	$C_7H_{12}O_4$	160.1678	6.960	2.45	
10	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-,(1S)-	$C_{10}H_{16}O$	152.2334	7.254	2.22	
11	Cyclohexanone, 5-methyl-2-(1-methylethyl)-,(2R-cis)-	$C_{10}H_{18}O$	154.2493	7.428	1.90	
12	Aceticacid,phenylmethylester	$C_9H_{10}O_2$	150.1745	7.613	9.16	

13	Cyclohexanol, 5-methyl-2-(1-methylethyl)-,(1α,2β,5β)-	$C_{10}H_{20}O$	156.2652	7.828	1.86	
14	3-Cyclohexene-1-methanol,α,α,4-trimethyl-,(R)-	$C_{13}H_{22}O_2$	210.3126	8.260	1.69	
15	Neral	$C_{10}H_{16}O$	152.2334	9.427	1.48	
16	2,6-Octadienal, 3,7-dimethyl-,(E)-	$C_{10}H_{16}O$	152.2334	10.138	1.62	
17	Isobornylacetate	$C_{12}H_{20}O_2$	196.2860	10.595	2.89	
18	meso-5,6-Decanediol	$C_{10}H_{22}O_2$	174.28	11.653	5.65	
19	5-Octadecene,(E)-	$C_{18}H_{36}$	252.4784	13.076	1.00	
20	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.3239	15.976	17.05	
21	Phthalicacid,hept-3-ylisobutylester	$C_{19}H_{28}O_4$	320.4232	25.523	3.31	
22	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.3707	25.637	1.05	
23	Dibutylphthalate	$C_{16}H_{22}O_4$	278.3435	26.802	11.47	
24	Phthalicacid,butyl2-pentylester	$C_{20}H_{30}O_4$	334.4498	27.355	4.63	

modulation of antibiotic resistance, genomic instability, cytogenic and oxidative effects, anti-leishmania activity, allergic rhinitis, scavenging of free radicals, anti-inflammatory and hypoglycemic properties, and anti-schizophrenia properties. Furthermore, as various studies have examined α-pinene'santiapoptotic, anticancer, and antimetastatic activities (Chen *et al*., 2014, 2015; Hou *et al*., 2019; Karthikeyan *et al*., 2018; Khoshnazar *et al*., 2020; Matsuo *et al*., 2011), new drug discovery initiatives. In-vitro and In-vivo, α-pinene had anticancer action on hepatocellular carcinoma BEL-7402 cells and suppressed proliferation of liver cancer cells (Allenspach and Steuer, 2021).Numerous investigations examined α-pinene's neuro protective properties. There is an emphasis on developing novel medications since many of the medications currently available for the treatment of neurodegenerative illnesses (such as Parkinson's disease, epilepsy, and Alzheimer's disease) have serious adverse effects. According to several studies (Goudarzi and Rafieirad, 2017; Khoshnazar *et al*., 2020; Lee *et al*., 2017; Zamyad *et al*., 2019), α-pinene able to lessen neurodegenerative disease symptoms.

A member of the monoterpene class, α-pinene is widely found in conifers, Juniper ssp., and Cannabis ssp. higher plants. For ages, respiratory tract infections have been treated using α-pinene. According to Allenspach and Steuer (2021) it possesses pharmacological, herbicidal, insecticidal, and antibacterial properties. Biological evaluation of 5-phenyl-3-isoxazolecarboxylic acid methyl ester-chalcone hybrids displayed potent *in-vitro* activity against *Mycobacterium tuberculosis* (Sahoo *et al.,* 2021).

3-carene compounds pharmaceutically used previously reported in antimicrobial activity and proposed action mechanism of 3-carene against *Brochothrix thermosphacta* and *Pseudomonas fluorescens* (Huizhen Shu et al., 2019). One of the main ingredients in extracts derived and essential oils that are used as antimicrobial agents in traditional medicine is p-cymene. However, due to the paucity of information regarding the substance's safety and efficacy in vivo, more research is necessary before a firm recommendation can be made regarding the application of p-cymene in medical care for humans and biomedical applications, where it is an exciting possibility to functionalize biological materials and nanotechnology.

O-Cymene, reported also known as p-cymene, is a naturally occurring aromatic compound found in various essential oils such as cumin, thyme, and oregano. It has been studied for its pharmacological properties and potential therapeutic uses. Some of its pharmacological uses include in Antimicrobial Properties O-Cymene exhibits significant antimicrobial activity against bacteria, fungi, and viruses. Studies have demonstrated its efficacy against various pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and herpes simplex virus. (Borges *et al*., 2013; Nostro *et al*., 2007).

Limonene compounds is reported as naturally occurring compound found in the peels of citrus fruits like oranges, lemons, and limes. It belongs to a class of compounds called terpenes. Research has shown that limonene possesses various biological activities, including anticancer properties. Several studies have investigated the potential anticancer effects of limonene. limonene inhibited the growth of mammary tumors in rats and skin cancer in mice. However, it's important to note that while these preclinical studies show promising results, more research is needed to fully understand the anticancer effects of limonene in humans. Additionally, the mechanisms by which limonene exerts its anticancer effects are not yet fully understood. (Haag, J.D *et al*., 1994 and Crowell, P.L., & Gould, M.N. 2009).

Associated with medicinal plants are abundant in secondary metabolites possessing antimicrobial properties. Their whole life cycle is spent within plant tissues, devoid of any illness-causing agents or symptoms (Bacon and White, 2000; Saikkonen *et a*l., 2004). Furthermore, research has shown that endophytes connected to medicinal plants have the ability to create same metabolites both in host plant tissue and in vitro (Kusari *et al*., 2013; Dos Santos *et al*., 2016). The *Bacillus* genus is widely recognized for its inherent ability to produce secondary metabolites that include antibacterial and antifungal properties, and it has great promise in managing plant diseases (Radhakrishnan *et al*., 2017).

Carbon tetrachloride extracts of Bacillus thuringiensis

The following compounds were the solvent carbon tetrachloride of *B.thuringiensis* Benzaldehyde, 1-Propene,1,1-dichloro-, 1-Hexanol,2-ethyl-, Benzylalcohol, Hexane, 1-chloro-5-methyl-, Propiohydrazide, 2- benzylthio-N2- benzylideno, Butane, 1,2-dichloro-2-methyl-, 2-Bromopropionic acid,2-ethylhexylester, Butane, 1,4-dichloro-2-methyl-, Pentanedioicacid anti bacteial (Parveen *et al*., 2018),dimethylester, Aceticacid, phenylmethylester, Cyclohexene, 1-chloro-4-(1-chloroethenyl)-, Benzaldehyde,4-propyl-, Bicyclo[2.2.2] oct-2-ene,2-chloro-, 1,5-Cyclooctadiene, 1,6- dichloro-,

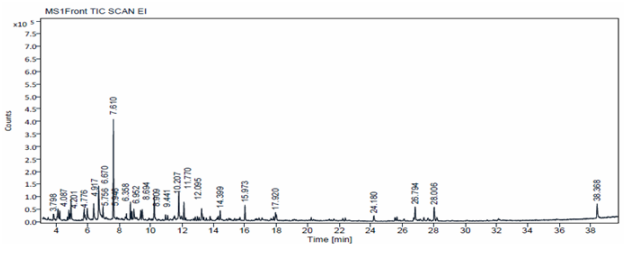
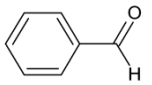
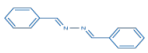
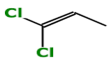
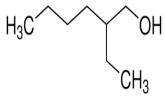
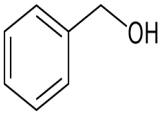
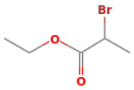
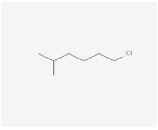
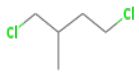
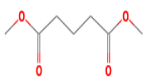
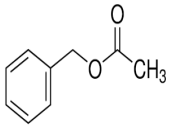
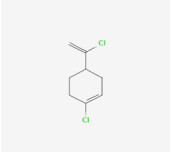
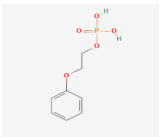
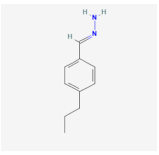
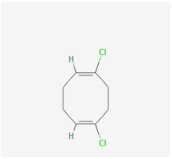
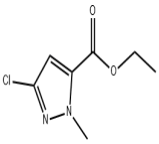
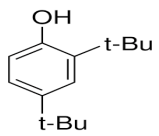
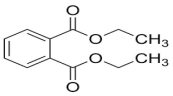
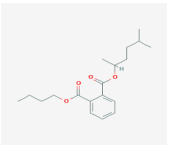
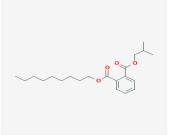
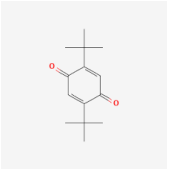
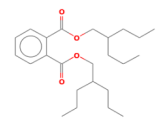


Fig. 4: GC-MS chromatogram depicting carbon tetrachloride extracts of *Bacillus thuringiensis*

Table 2: Carbon tetrachloride compounds identified by GC-MS in *B.thuringiensis* extract.

S.No	*Compound name*	*Molecular Formula*	*Molecular Weight*	*Retention time (min)*	*Peak Area*	*Molecular structure*
1	Benzaldehyde	C_7H_6O	106.1219	3.798	1.49	
2	Propiohydrazide, 2- benzylthio-N2-benzylideno	$C_7H_8N_2O$	136.1512	4.087	1.84	
3	1-Propene,1,1-dichloro-	$C_3H_4Cl_2$	110.970	4.201	1.19	
4	1-Hexanol,2-ethyl-	$C_8H_{18}O$	130.2279	4.775	1.63	
5	Benzylalcohol	C_7H_8O	108.1378	4.919	4.94	
6	2-Bromopropionic acid,2-ethylhexylester	$C_5H_9BrO_2$	181.028	5.756	2.17	
7	Hexane, 1-chloro-5-methyl-	$C_7H_{15}Cl$	134.647	5.944	2.39	
8	Butane, 1,2-dichloro-2-methyl-	$C_5H_{10}Cl_2$	141.039	6.356	4.20	
9	Butane, 1,4-dichloro-2-methyl-	$C_5H_{10}Cl_2$	141.039	6.669	13.63	
10	Pentanedioicacid,dimethylester	$C_7H_{12}O_4$	160.1678	6.950	3.67	
11	Aceticacid,phenylmethylester	$C_9H_{10}O_2$	150.1745	7.607	17.76	
12	Cyclohexene, 1-chloro-4-(1-chloroethenyl)-	$C_8H_{10}Cl_2$	177.071	8.694	3.14	

13	Ethanol,2-phenoxy-	$C_8H_{10}O_2$	138.1638	8.907	1.93	
14	Benzaldehyde,4-propyl-	$C_{10}H_{12}O_2$	164.2011	10.207	6.22	
15	Bicyclo[2.2.2]oct-2-ene,2-chloro-	$C_8H_{13}Cl$	144.642	11.770	6.33	
16	1,5-Cyclooctadiene, 1,6-dichloro-	C_8H_{12}	108.1809	12.095	3.58	
17	1H-Pyrazole, 5-chloro-1-methyl-3-(1-methylethyl)-	$C_6H_{10}N_2$	110.1570	14.399	1.59	
18	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.3239	15.971	3.64	
19	DiethylPhthalate	$C_{12}H_{14}O_4$	222.2372	17.921	2.53	
20	Phthalic acid, 5-methylhex-2-ylbutylester	$C_{19}H_{28}O_4$	320.4	24.178	1.56	
21	Phthalicacid,butylnonylester	$C_{23}H_{36}O_4$	376.5295	26.791	4.39	
22	2,5-di-tert-Butyl-1,4-benzoquinone	$C_{14}H_{20}O_2$	220.3074	28.004	3.89	
23	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.5561	38.368	4.00	

1H-Pyrazole, 5-chloro-1-methyl-3-(1-methylethyl)-, 2,4-Di-tert-butylphenol, DiethylPhthalate, Phthalic acid, 5-methylhex-2-ylbutylester, Phthalicacid,butylnonylester, 2,5-di-tert-Butyl-1,4-benzoquinone, Phthalic acid, di(2-propylpentyl) ester (Table 2; Fig. 4).

Numerous biological activities, including antimicrobial, antifungal, antitubercular, anti-inflammatory, anti-convulsant, anticancer, antiviral, angiotensin-converting enzyme inhibitory, neuroprotective, cholecystokinin-1 receptor antagonistic, and estrogen receptor (ER) ligand activity, have been documented for pyrazoles (Katz *et al*., 1965). Numerous pyrazole derivatives have already been used clinically as nonsteroidal anti-inflammatory drugs. These include anti-pyrine or phenazone, which is both analgesic and antipyretic; metamizole or dipyrone, which is both analgesic and antipyretic; aminopyrine or aminophenazone, which is both anti-inflammatory and antipyretic; phenylbutazone, which is anti-inflammatory and antipyretic and is primarily used in the treatment of osteoarthritis, rheumatoid arthritis, spondylitis, and Reiter's disease; sulfinpyrazone, which is used for chronic gout; and oxyphenbutazone, which is antipyretic, analgesic, anti-inflammatory, mild uricosuric (Ilango and Valentina, 2007). Allelopathic, antibacterial, insecticidal, and other biological actions have been described for phthalic acid esters (PAEs), improve the ability of plants, algae, and microbes to withstand biotic and abiotic stress (Huang *et al*., 2021). Numerous biological effects, including antibacterial, antiseptic, antioxidant, anti-inflammatory, and anticancer properties, are exhibited by eucalyptol (1,8-cineole) (Salehi *et al*., 2019).

Non-polar compounds demonstrated a greater reaction against Candida species (MIC = 0.04–0.63 mg/mL) in comparison to polar compounds. According to Neiva *et al.* (2020), several compounds exhibited distinct antibacterial action, indicating that compounds can be modified to target particular infections based on species and polarities. According to Tepe *et al.* (2004), antioxidant activity was demonstrated by essential oils and the non-polar subfractions of leaf extracts to scavenge free radicals and prevent the growth of pathogens. *P. curatellifolia* nonpolar extracts exhibit higher antifungal properties but lower antibacterial activity. *P. curatellifolia* possesses anti-Candida properties due to its nonpolar extract components (Mawire *et al.,* 2021). Since the *C. krusei* cell membrane has great hydrophobicity allows for a larger affinity to nonpolar molecules, the interaction between *C. krusei* cells and nonpolar substances is likely the cause of the hexane extract's strong activity against the bacteria (Samaranayake *et al.,* 1994).

Conclusion

The identification of new drugs for existing diseases is a new thrust area. Due to existing lifestyle, unhygienic practices, and environmental impacts, there will be an increasing number of diseases for existing drugs. The identification of drugs from various sources will be helpful for human beings. Drugs from naturals sources will be less toxic to human health and reduce complications. The present study was focused on the pharmaceutical compounds of *Bacillus thuringiensis* bacteria isolated from *Cissus quadrangularis* leaf concluded that the compounds of bacterium promising medicinal compounds. These properties could potentially be utilized in the development of novel pharmaceutical products for various applications, such as antimicrobial agents or therapeutic compounds. This study identified broad array of polar and non-polar compounds through GC-MS analysis of the crude extract depicted presence of important bioactive compounds with medicinal properties. Further research is recommended to explore the complete potential of these compounds and their possible applications in the pharmaceutical industry.

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