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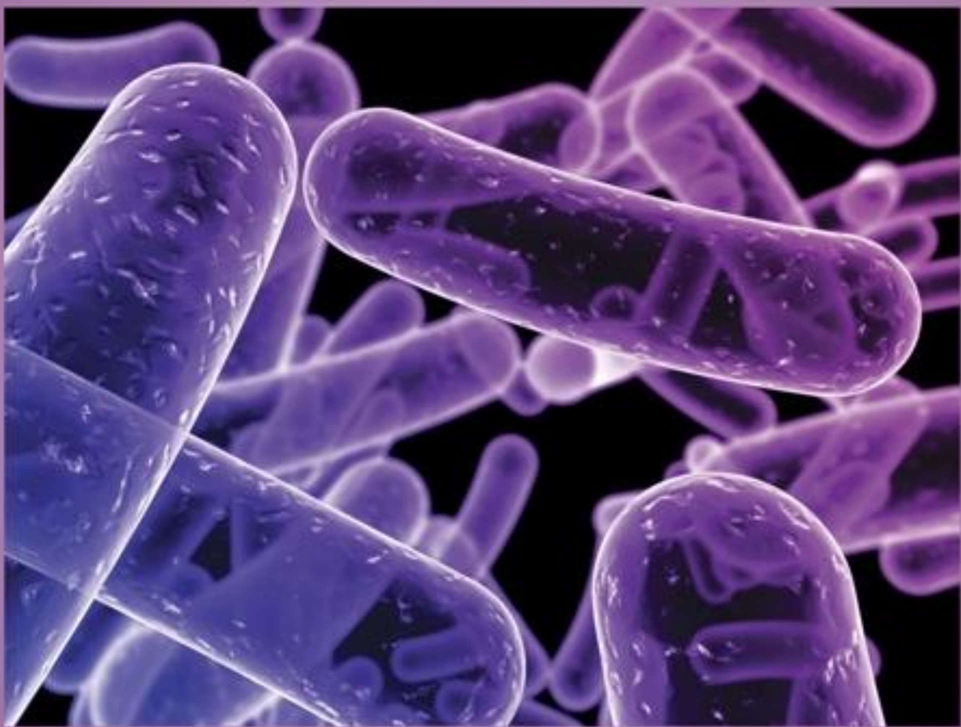

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Analysis of Different Bioactive Compounds Conferring Antimicrobial Activity from *Lactobacillus plantarum* and *Lactobacillus acidophilus* with Gas Chromatography-Mass Spectrometry (GC-MS)

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ABSTRACT

The chemical screening tests are important tools for the detection of bioactive principles. In this study, gas chromatography−mass spectrometry (GC−MS) was successfully used to identify the biochemical constituents produced by *Lactobacillus plantarum* and *Lactobacillus acidophilus* strains. The method is based on liquid-liquid extraction followed by gas chromatography-mass spectrometry (GC-MS) analysis. The GC-MS chromatogram of the extracts of *L. plantarum* and *L. Acidophilus* revealed the presence of twenty-two and seventeen chemical compounds respectively with antimicrobial activities. Bioactive compounds as Pentadecanoic acid, Butanoic acid, Eucalyptol, Isopropyl Myristate possess, and 9-Hexadecenoic acid were detected in the extract of *L. plantarum*. Also, other compounds as 1- Tetradecanol, Isochiapin B, cis-Vaccenic acid, 1,2-Benzenedicarboxylic acid were detected for *L. acidophilus.* Furthermore, different bioactive compounds with antimicrobial activities as Oleic acid, 1-Hexadecanol, 2-methyl, 2,2- Dideutero octadecanal, 9-Octadecenoic acid were detected in both extracts of *L. plantarum* and *L. Acidophilus.* Thus, these probiotics are strong candidates as antimicrobial agents.

INTRODUCTION

Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2002). Several microorganisms live in or on the human body and are beneficial. This biological system is the human microbiome. The relationship between the host and the gut microbiota is symbiotic. Probiotics must survive in the acidic gastric environment and colonize the gastrointestinal tract. They are known to be beneficial not only for adjusting intestinal balance but also for their anti-inflammatory, antioxidant, and anticancer effects (Lee *et al.*, 2015).

The health benefits of various probiotics are diverse, ranging from complicated host functions such as immune development, metabolic function, or gut-brain interaction.

Another desired attribute is the production of antimicrobial compounds by probiotics. Perturbation of the GIT microbiome plays an important role in the pathophysiology of common gastrointestinal infectious diseases (Papadimitriou *et al.,* 2015). Researchers have proposed that probiotics may prevent gastrointestinal disorders by maintaining homeostasis of the gut microbiome or by competitively inhibiting the growth of pathogens (Hickson, 2011). Thus, Probiotics are available over the counter and represent a low-cost, well-tolerated, safe, nonantibiotic-based strategy that may have efficacy as adjunctive treatment of infections without the attendant risks of promoting antimicrobial resistance (Ratsep, 2014).

One of the most important traits of probiotics is their antagonistic or antimicrobial effect against pathogens. This is achieved by competitive exclusion and/or the production of bacteriocins or bacteriocin-like substances and organic acids, thus regulating the intestinal microbiota (Fijan, 2014). The antagonistic activity of probiotic strains is mostly attributed to the production of metabolites such as organic acids, hydrogen peroxide, ethanol, acetaldehyde, acetoin, carbon dioxide, reuterin, and other bacteriocins as well as competitive exclusion, immune modulation, stimulation of host defenses, and the production of signaling molecules that trigger changes in gene expression (Saxelin *et al.,* 2005).

The *lactobacilli* constitute a major group of the Lactic Acid Bacteria (LAB). They occupy a wide range of niches and are generally found in environments with high levels of carbohydrates, such as food products (dairy products, fermented meat, sourdoughs) as well as (fermenting) plant-derived substrates. In addition, they occupy different niches on and in the human body including the respiratory, gastrointestinal, and urogenital tract. As a consequence, lactobacilli have been studied extensively, initially mainly because of their importance for food production (Siezen & Hylckama., 2011).

Lactobacillus is a gram-positive facultative anaerobic bacterium found widely in fermented food products and can be investigated for the metabolites present in them by GC-MS-based metabolic profiling (Chaudhary *et al.,* 2020). Several microbial species of LAB establish themselves from mouth and gut to large intestine of human beings and thus serve as potential mucosal vaccines (Elagöz *et al.,*1996). From the populous *Lactobacillus plantarum* is the most versatile species/strain with useful properties and is usually found in numerous fermented food products (Guidone *et al.,* 2014). Moreover, *L. plantarum* is widely employed in industrial fermentation and processing of raw foods and is “generally recognized as safe” (GRAS), and has qualified presumption of safety (QPS) status. *L plantarum* strains must have a high ability to survive in the gastrointestinal tract (GI) and adhere to its epithelial cells and most importantly be a safe strain (FAO and WHO) of animals and humans (Behera *et al.,* 2018). *Lactobacillus plantarum* is also used as a probiotic. There have been a growing number of studies about the potential beneficial effects of *L. plantarum* strains on human health (Kim *et al,* 2014).

Lactic acid is the major organic acid produced by *L. plantarum* strain. Other organic acids produced are acetic acid, propionic acid, phenylacetic acid (PLA), formic acid, and succinic acid. The approach of action of organic acids is the reduction of pH in the environment, causing inhibition of several microorganisms (Behera *et al.,* 2018). *Lactobacillus acidophilus* has proven to shorten hospitalization of children with diarrhea, reduce serum cholesterol, be effective as adjuvant therapy for bacterial vaginosis, and exhibit important immunomodulatory effects. (Steinberg *et al.,* 2014).

These metabolic profiles GC-MS has consistently been the most favored analytical technique for the analysis of metabolites present in distinct biological samples. Gas chromatography and mass spectrophotometry (GC-MS) present the high chromatographic resolution ascribed to the high sensitivity and specificity of mass spectrophotometry (Chaudhary *et al.,* 2020). In comparison with other techniques, GC-MS can produce a comparably high reproducibility, high resolution, high-quality sensitivity, and good-throughput analysis, which can be used for analyzing the metabolic products, inclusive of carbohydrates, fatty acids, organic acids, and amino acids (Park *et al.,* 2016). Thus, this study aimed to analyze different metabolites with antimicrobial activity produced by two probiotic strains; *L plantarum* and *L. acidophilus* using GC-MS spectroscopy.

MATERIALS AND METHODS

Collection of Probiotic Strains:

Probiotics *L. Acidophilus* (ATCC 4356) and *L. plantarum* (ATCC 14917) strains were obtained from Microbiological Resources Centre, Faculty of Agriculture, Ain Shams University, Egypt.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

The selected strains were inoculated in MRS broth and incubated at 30°C for 4 days. Equal amounts of ethyl acetate were added to the broth and incubated in a rotary shaker for 24 h. The following day, the upper layer of the broth was separated, collected in a beaker, and allowed to dry. After drying, 2 mL of ethyl acetate was added and the samples were stored at 4°C (Pooja *et al.,* 2017). The extracted secondary metabolites were analyzed by gas chromatography-mass spectrometry using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to 230°C hold for 2 min. increased to the final temperature 290°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250°C, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

RESULTS AND DISCUSSION

GC-MS technique is still considered as one of the best methods for identifying the constituents of volatile matter, long and branched-chain hydrocarbons, alcoholic acids, esters, and other organic compounds. (Abu Zeid *et al.,* 2019). The results of GC-MS analysis led to the identification of several compounds. These compounds were identified through mass spectrometry attached with GC. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of these compounds. Interpretation of mass spectrum GC–MS was conducted using the database of the National Institute of Standards and Technology (NIST). The retention time (RT), relative concentrations (peak areas %), compound name, molecular formula, and molecular weight (MW) of the components of the test materials were ascertained, the components identified by the GC-MS analysis are illustrated in Tables 1 & 2.

Results obtained from the gas chromatography-mass detector showed the presence of a high number of bioactive constituents in all tested fractions. This could give a clue to a wide medicinal activity they may possess. Preliminary investigation of the extract of both *lactobacillus plantarum* and *Lactobacillus Acidophilus* showed the presence of acids, alcohols, aldehydes, aromatic compounds, esters, and ketones (Table 1 & 2). Data showed in (Fig. 1 & Table 1) revealed that 22 bioactive compounds from the extract of *L. plantarum* were detected using GC-Ms spectroscopy. These compounds possess different properties.

Fourteen compounds have an antimicrobial or antibacterial characteristic, such as 2-(4a,8-Dimethyl-6-oxo1,2,3,4,4a,5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde, Oleic acid, Eucalyptol(1,8-cineole), 1,2-Benzenedicarboxylic acid, Quinindoline, Isopropyl myristate, cis-Vaccenic acid, Pentadecanoic acid, 9-Octadecenoic acid, Methyl ester, 1,2,3-propanetriyl ester, (E, E, E)-, 9-hexadecenoic acid.

Our results are in accordance with Mujeeb *et al.* (2014) who showed that Pentadecanoic acid has antibacterial activity. Kennedy *et al.,* (2019) found that Butanoic acid had antibacterial activity. Also, (Safaei-Ghomi & Ahd, 2010; Hendry et al., 2009; Bachir and Benali, 2012) found that Eucalyptol is a strong antimicrobial. Other compounds as Isopropyl Myristate possess antioxidant and antibacterial properties (Faridha Begum *et al*., 2016). Furthermore, Rahman *et al*. (2014) showed that 9-Hexadecenoic acid had antimicrobial properties.Several studies showed that Quinindoline had Antibacterial activity (Sokhanvar and Pordel, 2014; Pordel *et al.*, 2016). Quinindolines are a group of synthetic analogs of the natural alkaloid neocryptolepine. They share many biological properties with this compound, including the ability to interact with DNA as intercalators and to inhibit topoisomerase II reactivity. The quinindoline derivatives also revealed antimicrobial, antimuscarinic, antiviral, and cytotoxic potential. A combination of the quinindoline moiety with the imidazole nucleus may enhance optical and biological properties (Sokhanvar and Pordel., 2014).

The GC-MS chromatogram of the extract of the *L. Acidophilus* revealed the presence of 17 chemicals compounds, among these 1,2-Benzenedicarboxylic acids, showed the highest area (22.09 %) followed by 2H-Pyran, 2,2'-[1,10-decanediylbis(oxy)]bis[tetrahydro- (11.78 %), 4H-1-Benzopyran-4-one, 2-(3,4-Dihydroxyphenyl)-6,8-DI-`a-D-Glucopyranosyl-5,7 Dihydroxy- (7.89%), Oleic Acid (5.51%), 9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E, E, E) (5.48%), Ethanaminium, cis-Vaccenic acid (3.56%), Dotriacontane (3.01%) (Table 2 & Fig. 2).

Most of the detected compounds had antimicrobial properties as 1- Tetradecanol that has antibacterial and anti-inflammatory (periodontitis) activity (Mujeeb *et al.*, 2014). Also, Isochiapin B has antimicrobial, antioxidant, and anticancer properties (Marandi, 2017). Other compounds as cis-Vaccenic acid possess antibacterial activity and hypolipidemic effect (Semwal *et al.*, 2018). Moreover, 1,2-Benzenedicarboxylic acid with Antioxidant, antifouling, antimicrobial, cancer enzyme inhibitors in pharmaceutical, cosmetics, and food industries (El-fayoumy *et al.,* 2021).

Data in Tables (1 & 2) showed that some bioactive compounds are produced by both probiotics under study and these compounds possess antimicrobial properties. Among these compounds is Oleic acid. Mudgil *et al.*, (2014) found that Oleic acid is capable of preventing the growth of various ocular pathogenic Gram-negative and Gram-positive bacteria. It can provide an antibacterial defense to tears and can be used to develop lipid-based treatment options for eye infections helping in reducing antibiotic usage. Also, other studies revealed that Oleic acid had different properties as antifungal, Antioxidant, Anti-inflammatory, anti-androgenic, anti-cancer (Liu et al., 2008; Walter *et al.*, 2004, Ragunath *et al.,* 2020, Ganesh and Mohankumar, 2017, Thampy *et al.,* 2014, Awonyemi *et al.,* 2020).

Other compounds are common in both probiotic extracts as 1-Hexadecanol, 2-methyl which has Antimicrobial, anticancer, anti-inflammatory, and antioxidant activities (Ganesh and Mohankumar, 2017). Also, 2,2-Dideutero octadecanal had antimicrobial activity (El-fayoumy *et al.,* 2021). Other compounds as 9-Octadecenoic acid with antibacterial activity (Mujeeb *et al.,* 2014). In conclusion, the extracts of both probiotics tested possess different properties, particularly antimicrobial properties. Thus *L. plantaruum* and *L. acidophilus* are candidates as antimicrobial agents.

Table 1: Different bioactive compounds and their biological activities of *Lactobacillus Plantarum* using GC-MS chromatogram

No.	R.T.	Area %	Compound name	Formula	Molecular Weight	Biological activity
1	5.19	2.74	PENTADECANOIC ACID	C15H30O2	242	Antibacterial activity (Mujeeb *et al.,* 2014)
2	5.88	0.81	Butanoic acid	C5H11NO3S	165	antibacterial (Kennedy *et al.,*2019)
3	5.95	0.81	1-Hexadecanol, 2-methyl	C17H36O	256	Antimicrobial, anticancer, anti-inflammatory, and antioxidant activities (Ganesh & Mohankumar, 2017)
4	7.54	8.49	Eucalyptol	C10H18O	154	strong antimicrobial, (Safaei-Ghomi & Ahd, 2010), (Hendry *et al.,* 2009), (Bachir and Benali, 2012)
5	10.99	1.18	9,12,15-OCTADECATRIENOIC ACID,2-[(TRIMETHYLSILYL)OXY]-1-[[(TRIMETHYLSILYL)OXY] MET HYL] ETHYL ESTER, (Z, Z, Z)-	C27H52O4Si2	496	Anticancer, anti-inflammatory (al Bratty *et al.,* 2020)
6	19.46	3.10	Limonen-6-ol, pivalate	C15H24O2	236	Antioxidant and anti-inflammatory (Hadi *et al.,* 2016)
7	23.65	0.53	1-(4- ISOPROPYL PHENYL) -2-METHYL PROPYL ACETATE	C15H22O2	358	Anti-inflammatory, antileishmanial, and antitrypanosomal (Elsayed *et al.,* 2020)
8	23.86	18.54	4)-2a,8-Dimethyl-6-oxo-1,2,3,4,4a,5 ,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde	C15H22O2	234	antibacterial and antifungal activity (Rokade and Sayyed, 2009)
9	24.60	2.31	Propionic acid-1)- ,hydroxy-2-isopropyl-5-3methyl cyclohexyl)	C13H20O3	224	Antiangiogenic, activity against solid tumor growth (Hussein *et al.,* 2016)
10	25.25	1.94	2,2-DIDEUTERO OCTADECANAL	C18H34D2O	270	Antimicrobial activity (El-fayoumy *et al.,* 2021)
11	27.32	4.12	ISOPROPYL MYRISTATE	C17H34O2	270	Antioxidant & antibacterial (Faridha Begum *et al.,* 2016)
12	27.53	1.06	9-OCTADECENOIC ACID (Z)-	C18H34O2	282	Antibacterial (Mujeeb *et al.,* 2014)
13	27.98	6.39	QUININDOLINE	C18H14N2	258	Antibacterial activity (Sokhanvar and Pordel, 2014), (Pordel *et al.,* 2016)
14	29.47	1.65	10-OCTADECENOIC ACID, METHYL ESTER	C19H36O2	296	Antibacterial, antifungal, antioxidant, decrease blood cholesterol (Belakhdar *et al.,* 2015)Enhances the immunity (Asghar *et al.,* 2011)
15	32.80	3.90	cis-Vaccenic acid	C18H34O2	282	antibacterial activity and hypolipidemic effect (Semwal *et al.,* 2018) Anticancer (Ragunath *et al.,* 2020), Anti hypercholesterolemic, anti-inflammatory (Awonyemi et al.,2020), (Malathi *et al.,* 2016)
16	34.48	14.95	Oleic Acid	C18H34O2	282	Antibacterial (Mudgil *et al.,* 2014), antifungal (Liu *et al.,* 2008), (Walter *et al.,* 2004) Antioxidant (Ragunath *et al.,* 2020)), Anti-inflammatory preservative and hypocholesterolemic anti-androgenic, anti-cancer (Ganesh and Mohankumar, 2017), (Thampy *et al.,* 2014) (Awonyemi *et al.,* 2020)
17	38.69	1.09	9-HEXADECENOIC ACID	C16H30O2	254	antimicrobial properties (Rahman *et al.,* 2014)
18	40.45	7.05	1,2-BENZENEDICARBOXYLICACID	C24H38O4	390	Antibacterial., Antioxidant, antifouling, antimicrobial, cancer enzyme
19	43.49	0.35	9-Octadecenoic acid,1,2,3-propanetriyl ester, (E,E,E)-	C57H104O6	884	Antibacterial, anticancer (al Bratty *et al.,* 2020)
20	44.20	4.92	Ethyl iso-allocholate	C26H44O5	436	Antimicrobial activity (Malathi *et al.,* 2016) Anti-inflammatory activity (Hussein *et al.,* 2016)
21	44.30	2.47	FLAVONE 4'-OH,5-OH, 7-DI-O-GLUCOSIDE	C27H30O15	594	antioxidant activity. (Semwal *et al.,* 2018), (El-fayoumy *et al.,* 2021)
22	44.71	0.72	4H-1-BENZOPYRAN-4-ONE, 2-(3,4-DIHYDROXY PHENYL)-6,8 -DI-á-D-GLUCOPYRANOSYL-5, 7-DIHYDROXY	C27H30O16	610	Antioxidant, antimicrobial, cancer enzyme inhibitors in pharmaceutical, cosmetics, and food industries (El-fayoumy *et al.,* 2021)

Table 2: Different bioactive compounds and their biological activities of *Lactobacillus acidophilus using* GC-MS chromatogram

No.	R.T.	Area%	Compound name	Molecular Formula	Molecular weight	Biological activity
1	5.20	11.78	2H-PYRAN, 2,2'- [1,10-DECANEDIYLBIS (OX Y)] BIS [TETRAHYDRO	C20H38O4	342	Anti-bacterial and anti-fungal effects (Mohammed *et al.,* 2016)
2	6.18	1.51	1-Hexadecanol, 2-methyl	C17H36O	256	Antimicrobial, anticancer, anti-inflammatory, and antioxidant activities. (Ganesh and Mohankumar, 2017)
3	8.11	0.56	1-TETRADECANOL	C14H30O	214	antibacterial and anti-inflammatory (periodontitis) activity (Mujeeb et al., 2014)
4	18.80	1.42	2,2,3,3,4,4 HEXADEUTERO OCTADECANAL	C18H30D6O	274	Anticancer activity (G, L. et al., 2019)
5	19.57	3.01	DOTRIACONTANE	C32H66	450	Anticancer, antimicrobial, antioxidant activity (El-fayoumy *et al.,* 2021),(Kawuri and Darmayasa., 2019)
6	20.70	0.94	ISOCHIAPIN B	C19H22O6	346	Anti-insect, antimicrobial, antioxidant, anticancer (Marandi, 2017)
7	24.62	1.29	2,2-DIDEUTERO OCTADECANAL	C18H34D2O	270	Antimicrobial activity (El-fayoumy *et al.,* 2021)
8	26.98	0.82	Oleic Acid	C18H34O2	282	Antibacterial (Mudgil *et al.,* 2014), antifungal (Liu et al., 2008), (Walters et al., 2004) Antioxidant (Ragunath *et al.,* 2020), Anti-inflammatory preservative and hypocholesterolemic anti-androgenic, anti-cancer (Ganesh and Mohankumar, 2017); Thampy *et al.,* 2014; Awonyemi *et al.,* 2020)
9	31.41	2.23	9-OCTADECENOIC ACID (Z)-	C18H34O2	282	Antibacterial (Mujeeb *et al.,* 2014)
10	31.50	1.20	Estra-1,3,5(10)-tries-17á-ol	C18H24O	256	Antibacterial, Antitumor, anti-inflammatory and antioxidant activities (Mohammed *et al.,* 2016)
11	34.48	3.56	cis-Vaccenic acid	C18H34O2	282	antibacterial activity and hypolipidemic effect (Semwal *et al.,* 2018)Anticancer (Ragunath *et al.,* 2020), Anti hypercholesterolemic, anti-inflammatory (Awonyemi., 2020Malathi *et al.,* 2016)
12	36.61	1.39	trans-13-Octadecenoic acid	C18H34O2	282	Anti-inflammatory, antiandrogenic, dermatitigenic, anaemiagenic, insecticides, flavor (Awonyemi *et al.,* 2020)
13	40.45	22.09	1,2-Benzenedicarboxylic acid	C24H38O4	390	Antioxidant, antifouling, antimicrobial, cancer enzyme inhibitors in pharmaceutical, cosmetics, and food industries (El-fayoumy *et al.,* 2021)
14	43.54	5.47	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	C57H104O6	884	Antibacterial, anticancer (al Bratty *et al.,* 2020)
15	44.24	7.89	4H-1-BENZOPYRAN-4-ONE, 2-(3,4-DIHYDROXY PHENYL)-6,8 -DI-á-D-GLUCOPYRANOSYL-5, 7-DIHYDROXY	C27H30O16	610	Antioxidant, antimicrobial, cancer enzyme inhibitors in pharmaceutical, cosmetics, and food industries (El-fayoumy *et al.,* 2021)
16	44.44	1.71	Ethyl iso-allocholate	C26H44O5	436	Antimicrobial activity (Malathi *et al.,* 2016) Anti-inflammatory activity (Hussein *et al.,*2016)
17	44.74	1.42	FLAVONE 4'-OH,5-OH,7-DI-O-GLUCOSIDE	C27H30O15	594	antioxidant activity. (Semwal *et al.,* 2018), (El-fayoumy *et al.,* 2021)

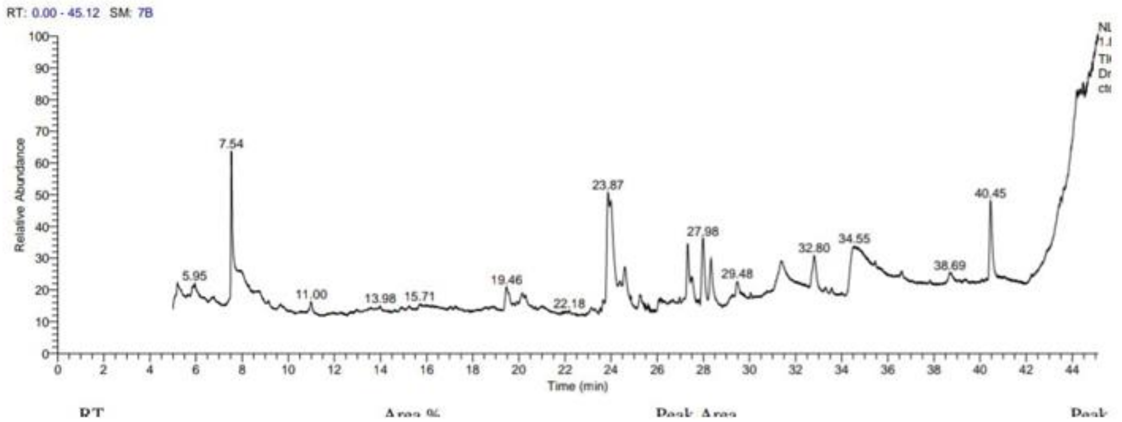

Fig.1.GC-MS chromatogram of metabolites secreted by *L. plantarum*.

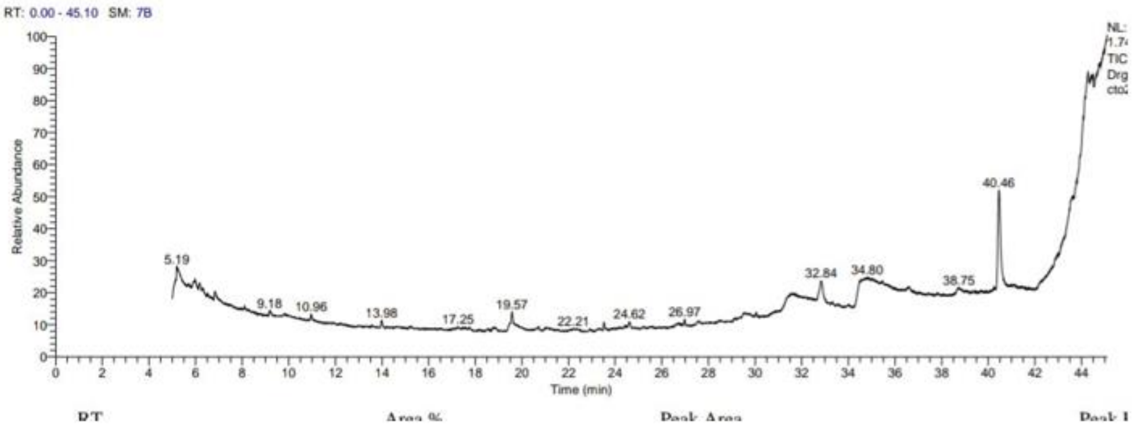

Fig.2.GC-MS chromatogram of metabolites secreted by L. Acidophilus.

Conclusion:

This research investigated that the antimicrobial efficiency of MPPE and JE of the *C. sinensis* related to the synergistic action of alkaloids, flavonoids, tannins, and phenol. According to the findings of this investigation, MPPE is an effective antibacterial agent.

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