ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Determination of secondary metabolites products by *Trichoderma horzianum* and evaluate antimicobial activity

Ali Malik Saad*.

Department of Biology, College of Science for Women, Babylon University, Iraq.

ABSTRACT

Gas chromatography mass spectrum analysis of methanolic extract of the filtrate of *T. harzianum* revealed the presence of nineteen bioactive compounds were identified in the methanolic extract of *Trichoderma horzianum*. GC-MS analysis of *Trichoderma horzianum* revealed the existence of the β-D-Glucopyranose , 1-thio-,1-[N-hydroxy-5-(methylthio)pentanim , 6-Acetyl-β-d-mannose , 17-Octadecynoic acid , Paromomycin ,Imidazole , 2-amino-5-[(2-carboxy)vinyl]- , D-Glucose , 6-O-α-D-galactopyranosyl- ,α-D-Glucopyranoside,O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-, Cyclohexanecarbo-xylic acid , 2-hydroxy-1,6-dimethyl-,methyl ester , 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- , 2H-Pyran,tetrahydro-2-(12pentadecynyloxy)- , 5-Hydroxymethylfurfural , 2-Oxabicyclo[3.3.0]oct-7-en-3-one , 7-(1-hydroxypentyl)- , Dodecanoic acid , 3-hydroxy- , 1-Gala-l-ido-octonic lactone , 1,2,4-trioxolane-2-octanoic acid , 5-octyl-,methyl ester , Acetamide , N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butyn , 2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-8-ol , 5H-Cyclopropa[3,4]benz [1,2-e]azulen-5-one,9-(acetyloxy)-3- and 9,10-Secocholesta-5,7,10(19)-triene3,24, 25-triol,(3β,5Z,7E). *Lycium afrum* was very highly antifungal activity (6.97±0.25) mm. The results of anti-bacterial activity produced by *T. horzianum* showed that the volatile compounds were highly effective to suppress the growth of *E. coli*.

Keywords *Trichoderma horzianum* . Antibacterial. Antifungal. FT-IR . GC/MS . Secondary metabolites.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Fungi produce a wide range of secondary metabolites (SMs), small molecules that are not directly essential for growth yet have important roles in signalling, development and interaction with other organisms (Whipps and Lumsden, 2001; Shalini et al., 2006; Vinale et al., 2008 Poornima, 2011). *Trichoderma* have been successfully applied for control of plant pathogenic fungi (Tronsmo A., and Hjeljord, 1998). The mechanisms underlying their antagonism for plant disease control involve mycoparasitism, antibiosis, competition with other microorganism, promotion of root and plant development, induction of plant disease resistance, inactivation of the pathogen's enzymes (Harman, 2000). Trichoderma spp. produce nonribosomal peptides, for example the epipolythiodioxopiperazines (ETPs) and siderophores. Production of many volatiles like pyrones, sesquiterpenes and non-volatile secondary metabolites like peptaibols have been reported the potential mechanism of *Trichoderma spp*. (Reino et al., 2008).

These metabolites play a key role in biocontrol mechanism and can be studied using mass spectrometry (MS) by which individual volatile metabolite can be identified from complex mixture (Haran et al., 1996; Zhihe 1998; Harman et al., 2006). *Trichoderma* is widely used in agricultural biotechnology and have been already used as biocontrol agents against numerous plant pathogens and quite a few have been developed for commercial use (Harman et al., 2004; Azin et al., 2007; Bae et al., 2011). The objectives of this study are to evaluate antimicrobial efficiency and screening of bioactive chemical compounds from *T. horzianum*.

MATERIALS AND METHODS

Growth conditions of *T. horzianum* and determination of metabolites

T. horzianum was isolated from dried fruit and the pure colonies were selected, isolated and maintained in potato dextrose agar slants (Usha and Masilamani, 2013). Spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25ºC in a shaker for 16 days at 130 rpm. The extraction was performed by adding 25 ml methanol to 100 ml liquid culture in an Erlenmeyer flask after the infiltration of the culture. The mixture was incubated at 4ºC for 10 min and then shook for 10 min at 130 rpm (Ameera *et al.,* 2015; Huda *et al.,* 2015).

Metabolites was separated from the liquid culture and evaporated to dryness with a rotary evaporator at 45ºC. The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4ºC for 24 h before being used for GC-MS. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library as well as on comparison of their retention indices either with those of authentic compounds or with literature values.

Gas chromatography – Mass Spectrum (GC-MS) analysis of the culture filtrate

Extraction of antifungal compounds

The fungus which showed promising activity against the pathogen was cultured in liquid potato dextrose medium at 24°C in darkness for three weeks. After incubation, the culture was filtered twice through Whatman No.1 filter paper and Seitz filter (G.5). To 100 ml of culture filtrate, 10ml of ethyl acetate was added in a separation funnel (250ml), shaken well for 3 min. and the solvent and aqueous layer were separated. The acetonitrile layer of the culture filtrate was used for further analysis.

Gas chromatography – Mass Spectrometry (GC-MS)

Volatile components were identified by GC-MS using GC-MS (Agilent 789A) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250 C^{o}). Ionization voltage was 70 eV and ion source temperature was 230oC. Scan range was 41- 450 amu. The constituents were identified after compared with available data in the GC-MS library in the literatures.

Determination of antibacterial activity

The test pathogens (*Streptococcus pneumonia*, *Pseudomonas eurogenosa, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Streptococcus pyogenes, Staphylococcus aureus, Streptococcus faecalis* and *Klebsiella pneumonia*) were swabbed in Muller Hinton agar plates. 90µl of fungal extracts was loaded on the bored wells. The wells were bored in 0.5cm in diameter. The plates were incubated at 37C° for 24 hrs and examined (Anupama *et al.,* 2007). After the incubation the diameter of inhibition zones around the discs was measured.

Determination of antifungal activity

T. horzianum isolate was suspended in potato dextrose broth and diluted to approximately 105 colony forming unit (CFU) per ml. They were "flood inoculated onto the surface of Potato dextrose agar and then dried. Standard agar well diffusion method was followed (Rajasekar *et al.,* 2012; Tabaraie *et al.,* 2012; Gebreselema *et al.,* 2012; Usha *et al.,* 2013). Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Erygium campestre*, *Allium ampeloprasum*, *Datura stramonium*, *Piper nigrum*, *Cuminum cyminum*, *Laurus nobilis*, *Herniaria hirsute, Malva rotundifolia, Fraxinus excelsior, Antirrhinum majus, Globularia alypum, Lepidium sativum, Chrysanthemum lencanthemum, Caratonia siliqua, Anethum graveolens*, *Lycium afrum*, *Echinops ritro* and *Lonicera caprifolium*) were delivered into the wells. The plates were incubated for 48 h at room temperature.

Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent (Anesini and Perez, 1993; Rukayadi *et al.,* 2006). The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by SPSS software)Version 9.1

RESULTS AND DISCUSSION

The species of *Trichoderma* have been known to produce important secondary metabolites such as antibiotics, plant growth regulators, and mycotoxins, which are mainly used to protect plants from pathogens. Based on morphological characteristics of fungi was isolated in selective media of potato dextrose agar media. Morphological, Microscopical and microscopical characteristics of fungal strains were determined using specific media light and compound microscope **Fig. 1.** The 400ml of fermentation broth (PDA broth) which contain 200µl of the standardized fugal suspensions were used to inoculate the flasks and incubated at 37°C on a shaker at 90 rpm for 7 days. After fermentation, the secondary metabolites were produced by isolated microorganisms.

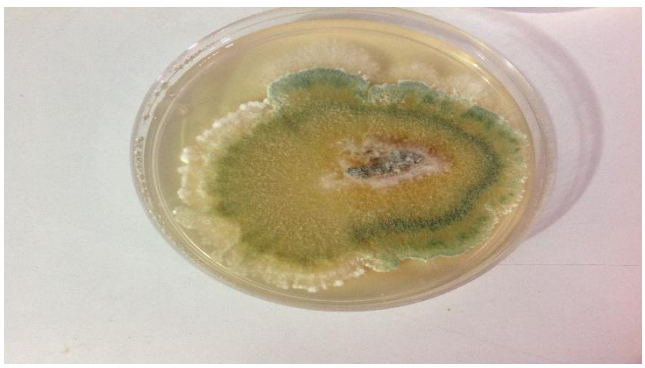
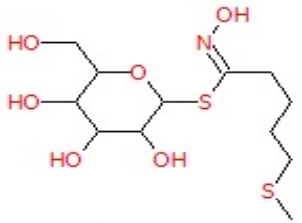
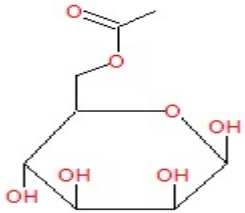
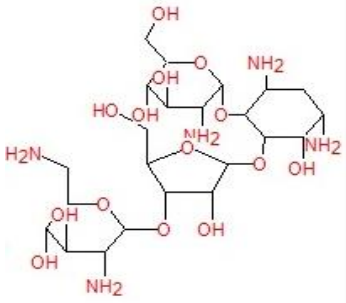
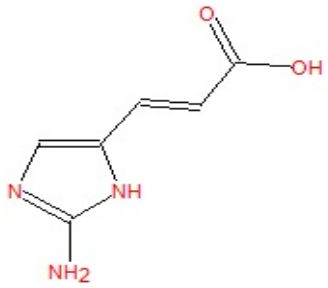
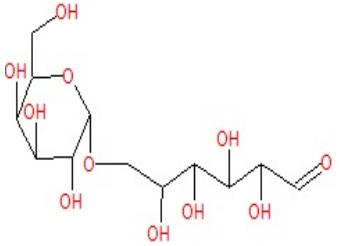
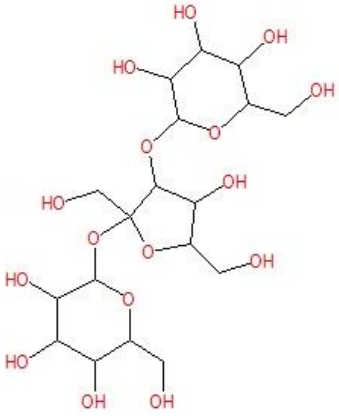


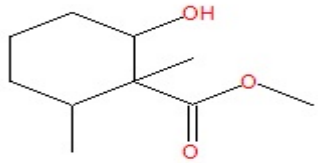
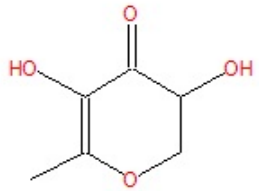
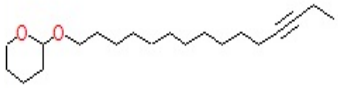
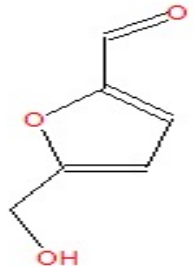
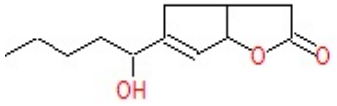
Fig.1: Morphological characterization of *T. horzianum* colony.

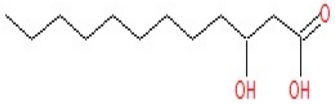
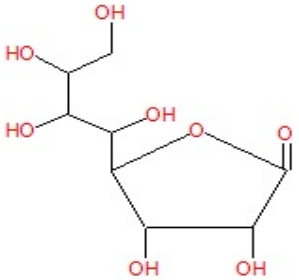
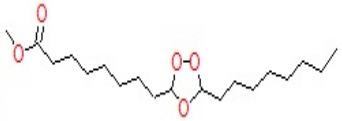
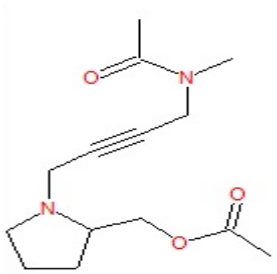
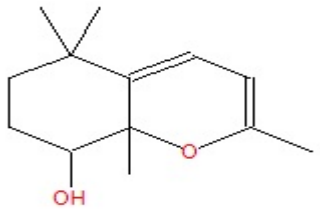
ISSN: 0975-8585

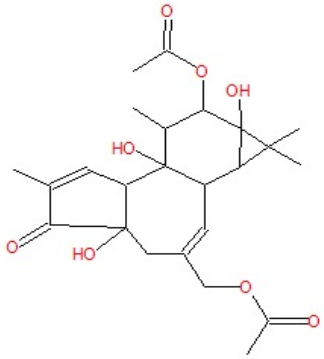
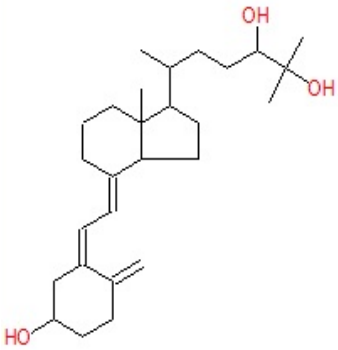
Table 1 Major bioactive chemical compounds identified in methanolic extract of *Trichoderma horzianum* .

Serial No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment- ions
1.	β-D-Glucopyranose , 1-thio-,1-[N-hydroxy-5-(methylthio)pentanim	3.172	341	341.09668		55,61,73,82,87,100,114,129,145
2.	6-Acetyl-β-d-mannose	3.464	222	222.073953		60,81,97,109,126,144,192
3.	17-Octadecynoic acid	3.779	280	280.24023		55,67,81,95,109,123,137,163,187,211,234,261
4.	Paromomycin	4.385	615	615.296303		57,67,80,94,109,124,145,191,214,237,262,287,323

5.	Imidazole , 2-amino-5-[(2-carboxy)vinyl]-	4.494	153	153.053826		55,69,109,135
6.	D-Glucose , 6-O-α-D-galactopyranosyl-	4.729	342	342.11621		60,73,85,110,126,144,182,212,261
7.	α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-	5.072	504	504.169035		60,73,85,97,113,126,145,187

8.	Cyclohexanecarboxylic acid , 2-hydroxy-1,6-dimethyl-,methyl ester	5.707	186	186.125594		55,83,115,154
9	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	5.839	144	144.042258		55,72,101,144
10.	2H-Pyran,tetrahydro-2-(12pentadecynyloxy)-	6.337	308	308.27153		55,85,101,171,199,227,255
11.	5-Hydroxymethylfurfural	6.525	126	126.031694		53,69,81,97,109,126
12.	2-Oxabicyclo[3.3.0]oct-7-en-3-one , 7-(1-hydroxypentyl)-	8.322	210	210.125594		57,69,85,97,126,153,168,181,210

13.	Dodecanoic acid , 3-hydroxy-	8.608	216	216.1725445		55,69,83,96,112,138,180
14.	1-Gala-l-ido-octonic lactone	10.193	238	238.068868		61,73,84,112,127,142,159,189,220
15.	1,2,4-trioxolane-2-octanoic acid , 5-octyl-,methyl ester	9.747	344	344.256275		56,69,143,185,241,311
16.	Acetamide , N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butyn	12.860	266	266.163042		55,67,82,91,124,141,165,193,251
17.	2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-8-ol	15.160	208	208.14633		57,91,106,134,175,190,208

18.	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9-(acetyloxy)-3-	16.716	448	448.209719		69,83,91,159,179,213,227,282,310,370,388
19.	9,10-Secocholesta-5,7,10(19)-triene3,24,25-triol,(3β,5Z,7E)-	18.221	416	416.329044		55,69,91,118,136,158,176,207,221,253,383,416

ISSN: 0975-8585

Identify the secondary metabolites from *T. horzianum*

Microbial volatile metabolites may be intermediate or end products of metabolic pathways and have been identified as mono- and sesquiterpenes, alcohols, ketones, lactones, esters or C8 compounds (Hynes *et al.,* 2007; Nemcovic *et al.,* 2008). Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *T. horzianum*, shown in **Table 1**. The GC-MS chromatogram of the thirty one peaks of the compounds detected was shown in **Fig. 2**. The First set up peak were determined to be 1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-htdroxy-1-isopropyl)cy, **Fig. 3**. The second peak indicated to be 2-Furancarboxaldehyde,5-methyl, **Fig. 4**. The next peaks considered to be 2(5H)-Furanone, 6-Hydroxymethyl-5-methyl-bicyclo[3.1.0]hexan-2-one, D-Glucose,6-O-α-D-galactopyranosyl, 2-(3-Hydroxy-propyl)-cyclohexane-1,3-dione, 9-Oxa-bicyclo[3.3.1]nonane-1,4-diol, Benzenemethanol,2-(2-aminopropoxy)-3-methyl, 1,2-Cyclopentanedione,3-methyl, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-ß-D-fruc, 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol, Desulphosinigrin, Orcinol, Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide, 2H-Oxecin-2-one.3.4.7.8.9.10-hexahydro-4-hydroxy-10-methyl-.[4, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), Maltol, 2-Tridecyl-5-(acetylamino)tetrahydro-γ-pyrone, Cycloundecanone , oxime, D-Glucose,6-O-α-D-galactopyranosyl, 6-Acetyl-ß-d-mannose, 5-Hydroxymethylfurfural, 1-Gala-l-ido-octonic lactone, Pterin-6-carboxylic acid, Uric acid, Acetamide , N-methyl -N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl], l-(+)-Ascorbic acid 2,6-dihexadecanoate, D-fructose , diethyl mercaptal , pentaacetate, 2-Bromotetradecanoic acid, Octadecanal ,2 –bromo, L-Ascorbic acid , 6-octadecanoate, 18,19-Secoyohimban-19- oic acid,16,17,20,21-tetradehydro-16. (**Fig. 5-22**).

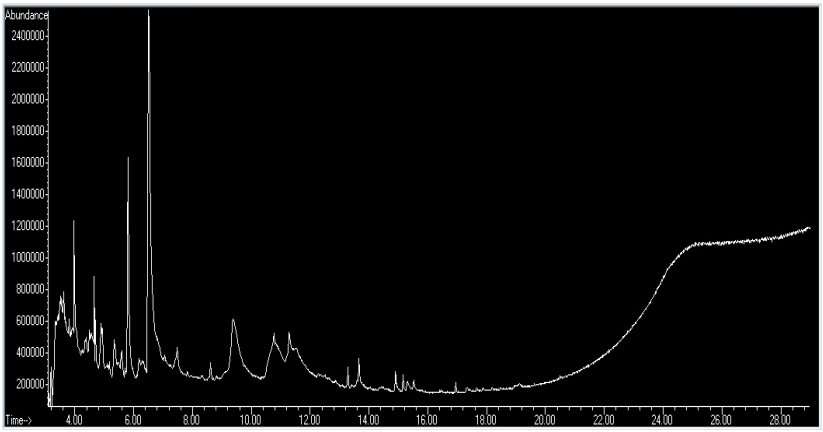


Fig. 2: GC-MS chromatogram of methanolic extract of *T. horzianum*.

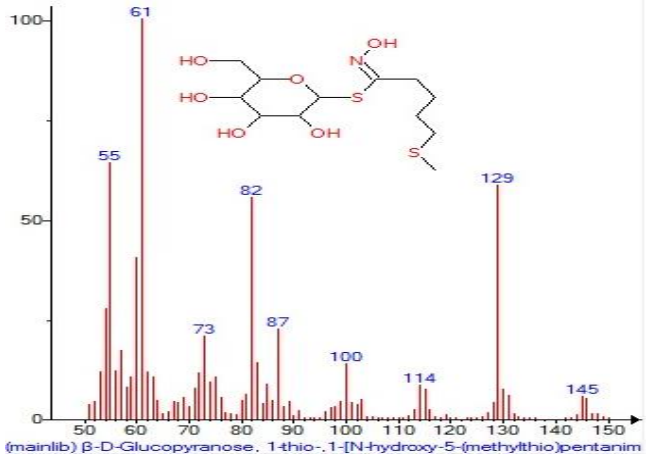


Fig. 3: Mass spectrum of β-D-Glucopyranose , 1-thio-,1-[N-hydroxy-5-(methylthio) pentanim with Retention Time (RT)= 3.172

ISSN: 0975-8585

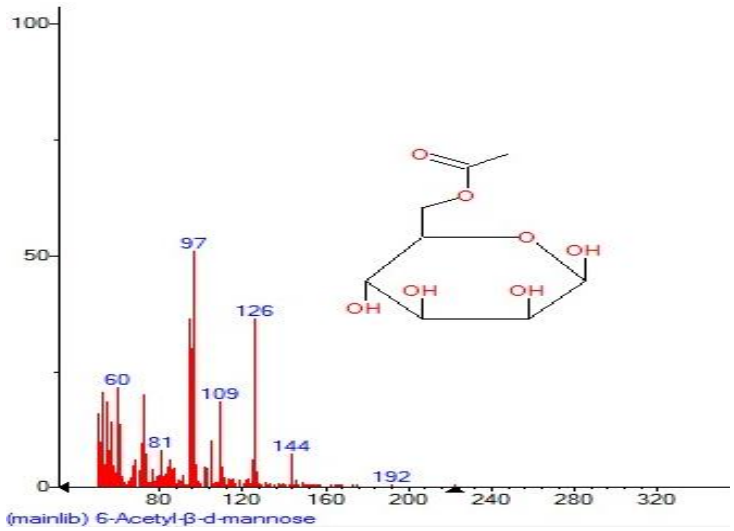


Fig. 4: Mass spectrum of 6-Acetyl-β-d-mannose with Retention Time (RT)= 3.464

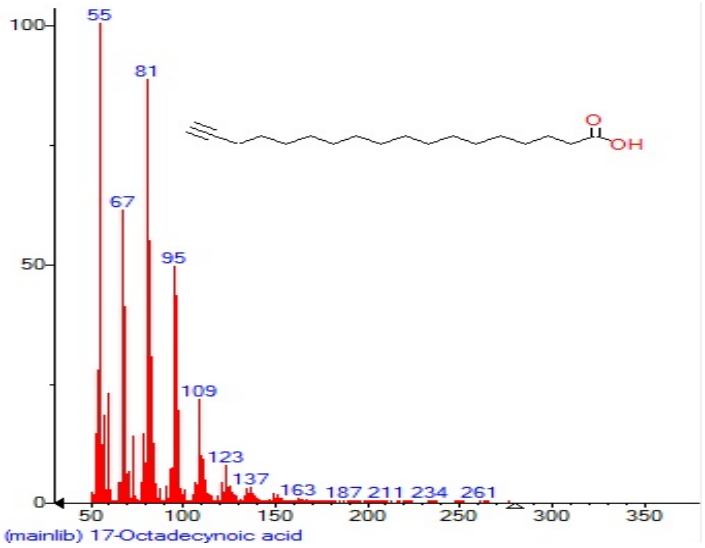


Fig. 5: Mass spectrum of 17-Octadecynoic acid with Retention Time (RT)= 3.779

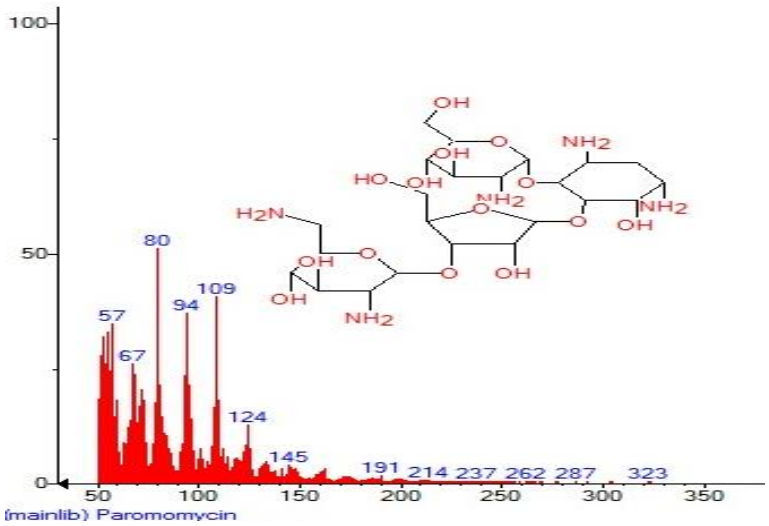


Fig. 6: Mass spectrum of Paromomycin with Retention Time (RT)= 4.385

ISSN: 0975-8585

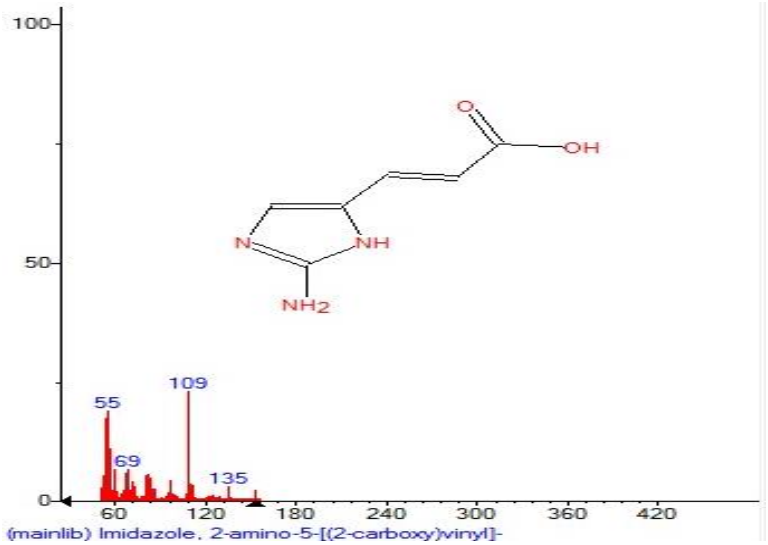


Fig. 7: Mass spectrum of Imidazole , 2-amino-5-[(2-carboxy)vinyl]- with Retention Time (RT)= 4.494

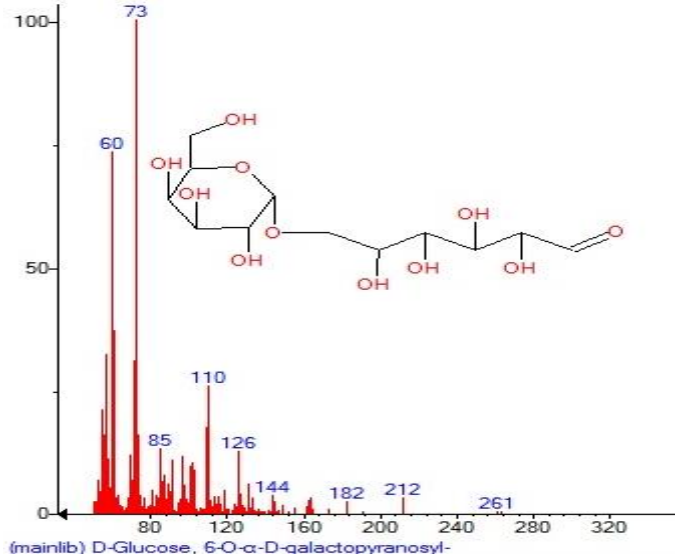


Fig. 8: Mass spectrum of D-Glucose , 6-O-α-D-galactopyranosyl- with Retention Time (RT)= 4.729

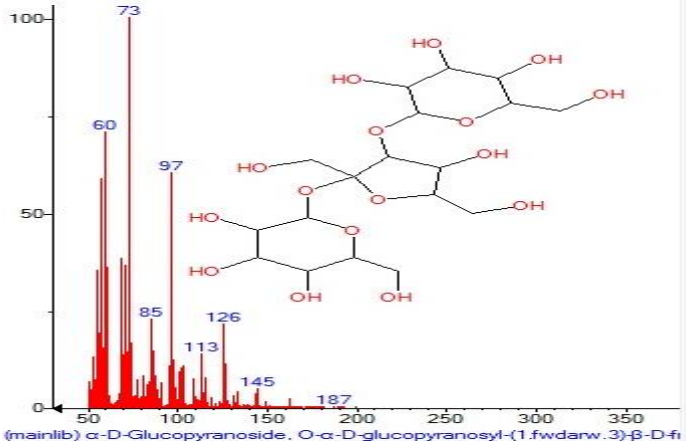


Fig. 9: Mass spectrum of α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D- with Retention Time (RT)= 5.072

ISSN: 0975-8585

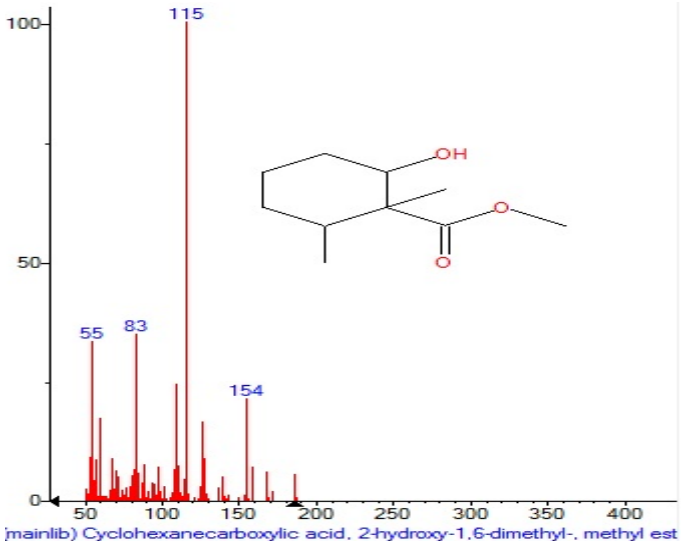


Fig. 10: Mass spectrum of Cyclohexanecarboxylic acid , 2-hydroxy-1,6-dimethyl-,methyl ester with Retention Time (RT)= 5.707

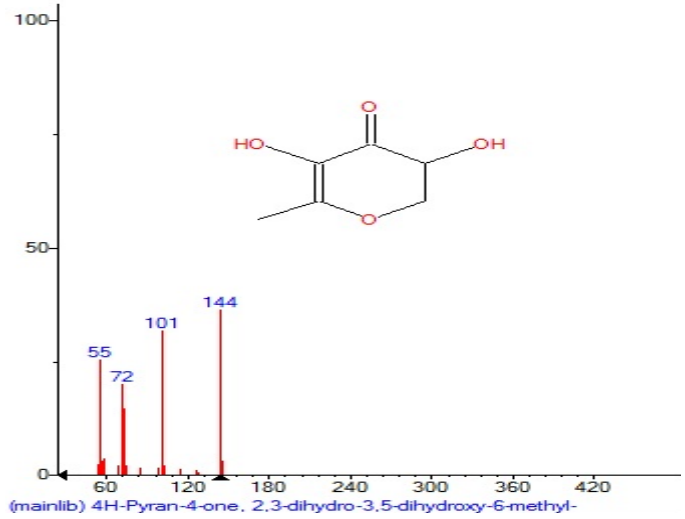


Fig. 11: Mass spectrum of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- with Retention Time (RT)= 5.839

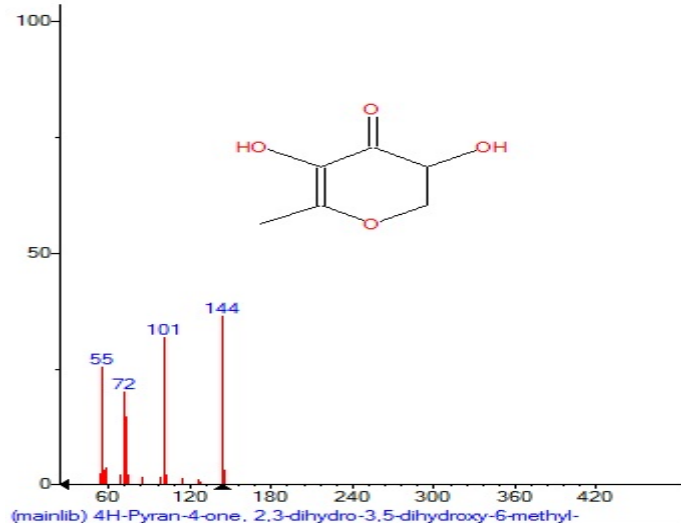


Fig. 12: Mass spectrum of 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- with Retention Time (RT)= 5.839

ISSN: 0975-8585

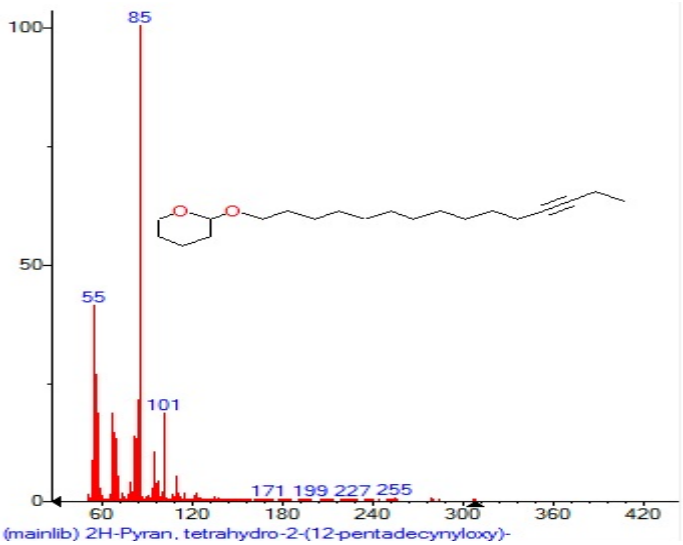


Fig. 13: Mass spectrum of 2H-Pyran,tetrahydro-2-(12pentadecynyloxy)- with Retention Time (RT)= 6.337

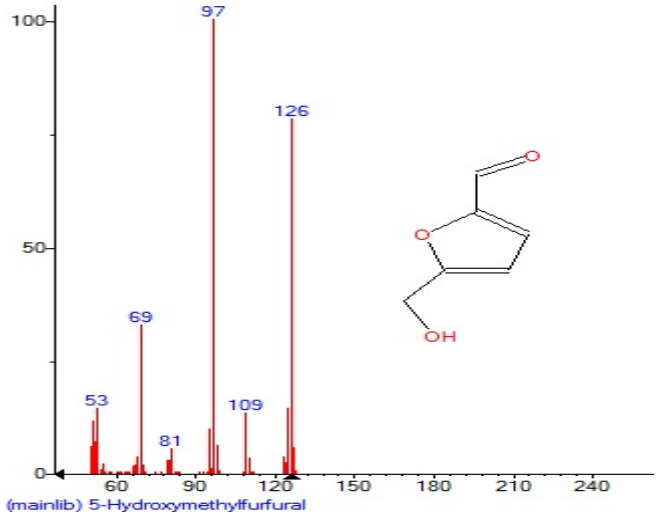


Fig. 14: Mass spectrum of 5-Hydroxymethylfurfural with Retention Time (RT)= 6.525

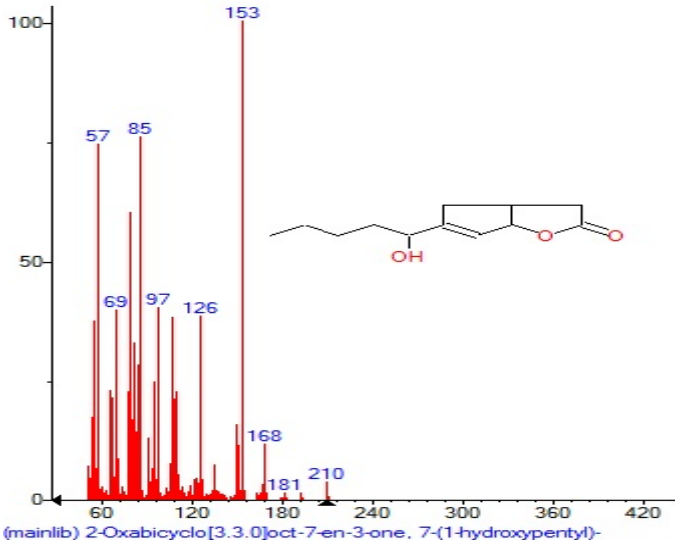


Fig. 15: Mass spectrum of 2-Oxabicyclo[3.3.0]oct-7-en-3-one , 7-(1-hydroxypentyl)- with Retention Time (RT)= 8.322

ISSN: 0975-8585

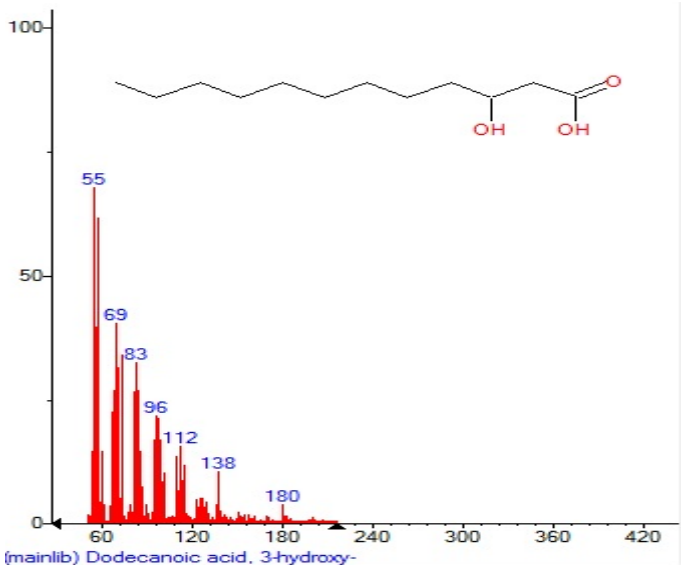


Fig. 16: Mass spectrum of Dodecanoic acid , 3-hydroxy- with Retention Time (RT)= 8.608

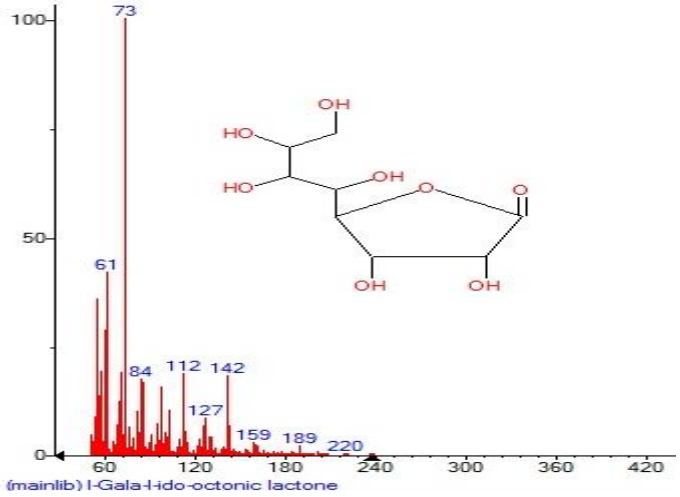


Fig. 17: Mass spectrum of 1-Gala-l-ido-octonic lactone with Retention Time (RT)= 10.193

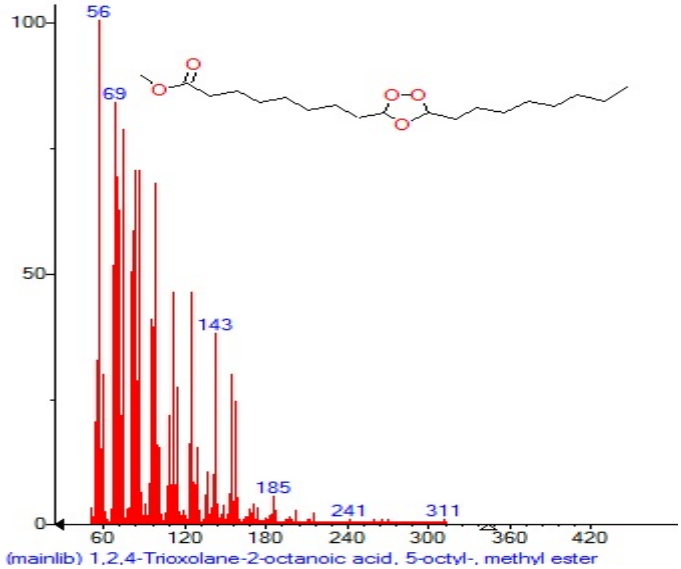


Fig. 18: Mass spectrum of 1,2,4-trioxolane-2-octanoic acid , 5-octyl-,methyl ester with Retention Time (RT)= 9.747

ISSN: 0975-8585

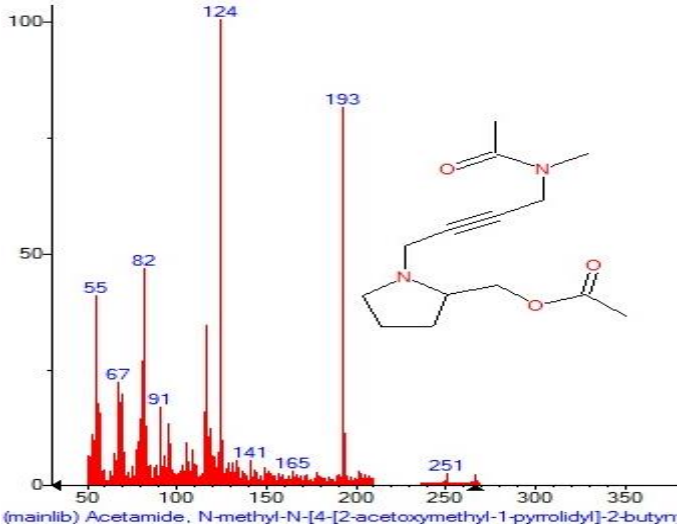


Fig. 19: Mass spectrum of Acetamide , N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butyn with Retention Time (RT)= 12.860

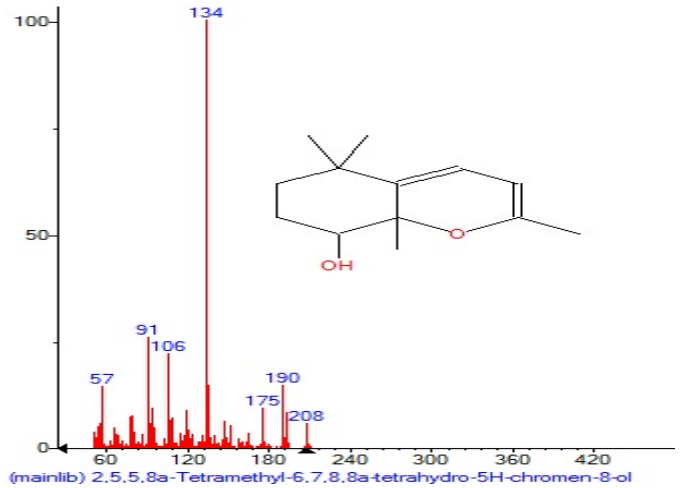


Fig. 20: Mass spectrum of 2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-8-ol with Retention Time (RT)= 15.160

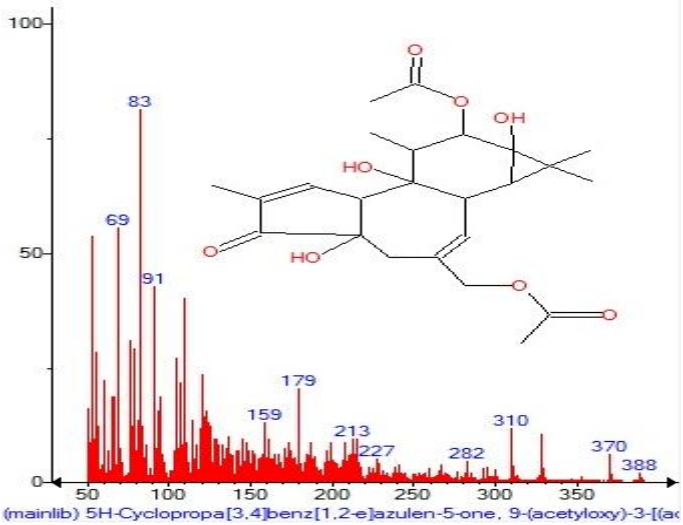


Fig. 21: Mass spectrum of 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9-(acetyloxy)-3- with Retention Time (RT)= 16.716

ISSN: 0975-8585

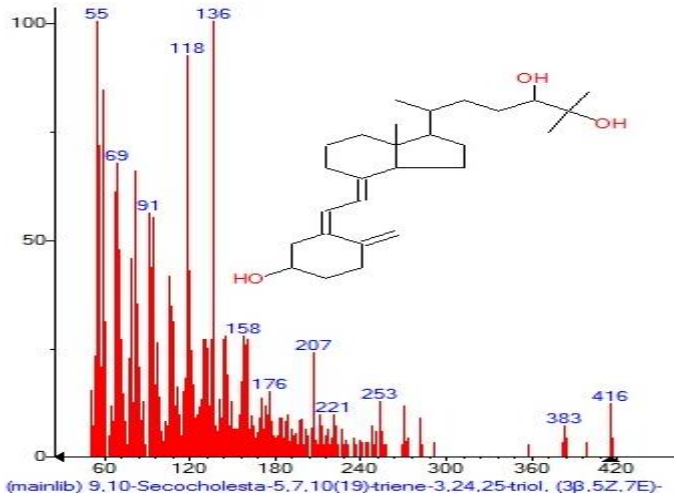


Fig. 22: Mass spectrum of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- with Retention Time (RT)= 18.221

Many compounds are identified in the present study. Some of them are biological compounds with antimicrobial activities. *Trichoderma* sp. are opportunistic, avirulent plant symbionts invader, confer with fast growing nature, strong spore producer and acts as a source of various cell wall degrading enzymes and secondary metabolites (Vinale, 2008). Based upon analytical reports, Trichoderma spp. Are prolific producers of SMs (natural products), with the structures of more than 100 compounds reported (Reino et al., 2008).

Antibacterial and antifungal activity

Clinical pathogens selected for antibacterial activity namely, *Streptococcus pneumonia*, *Pseudomonas eurogenosa, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Streptococcus pyogenes, Staphylococcus aureus, Streptococcus faecalis* and *Klebsiella pneumonia.* Maximum zone formation against *E. coli* (5.67±0.13) mm, **Table 2**. In agar well diffusion method the selected medicinal plants (*Erygium campestre*, *Allium ampeloprasum*, *Datura stramonium*, *Piper nigrum*, *Cuminum cyminum*, *Laurus nobilis*, *Herniaria hirsute, Malva rotundifolia, Fraxinus excelsior, Antirrhinum majus, Globularia alypum, Lepidium sativum, Chrysanthemum lencanthemum, Caratonia siliqua, Anethum graveolens*, *Lycium afrum*, *Echinops ritro* and *Lonicera caprifolium*) were effective against *Trichoderma horzianum*, Table 3. *Lycium afrum* was very highly antifungal activity (6.97±0.25) mm against *T. horzianum*. *T. horzianum* was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug Amphotericin B and fluconazole to some extent. Strains of *Trichoderma* like *T. harzianum, T. hamatum*, *T. asperellum* and *T. atroviride* are applied for the control of phytopathogens and also as plant growth promoters in agriculture (Verma et al., 2007; Vinale et al., 2008; Korpi et al., 2009). It is able to secrete 40 different secondary metabolites that may contribute to their mycoparasitism and antibiotic action. These volatile and nonvolatile toxic metabolites hinder the colonization of pathogen (Reino *et al.,* 2008; Poornima, 2011), induce resistance and promote the growth of plants to large extent (Shalini *et al.,* 2006; Siddiquee *et al.,* 2012).

Table 2: Zone of inhibition (mm) of test bacterial strains to *Trichoderma horzianum* bioactive compounds and standard antibiotics.

Bacteria	Fungal products /Antibiotics			
	Fungal metabolites	Cefotoxime	Kanamycin	Streptomycin
Streptococcus pneumonia	**5.00±0.18**	**0.99±0.19**	**0.88±0.17**	**1.22±0.17**
Pseudomonas eurogenosa	4.07±0.29	1.00±0.10	0.73±0.12	0.81±0.22
Staphylococcus epidermidis	4.50±0.20	1.05±0.14	1.00±0.20	1.00±0.11
Escherichia coli	5.67±0.13	0.98±0.23	0.63±0.10	1.03±0.27
Proteus mirabilis	5.41±0.10	1.65±0.18	0.98±0.20	0.75±0.19
Streptococcus pyogenes	5.05±0.11	0.95±0.14	1.10±0.14	0.96±0.10

Staphylococcus aureus	3.90±0.26	1.58±0.27	1.72±0.11	1.09±0.17
Streptococcus faecalis	4.99±0.10	0.25±0.20	1.39±0.18	0.63±0.19
Klebsiella pneumonia	3.74±0.21	0.99±0.19	0.96±0.27	0.99±0.15

Table 3 Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of plants to *Trichoderma horzianum*.

S. No.	Plant	Zone of inhibition (mm)
1.	*Erygium campestre (L)*	6.09±0.25
2.	*Allium ampeloprasum(L)*	4.00±0.23
3.	*Datura stramonium (Alkaloids)*	4.38±0.29
4.	*Piper nigrum (Crude)*	5.11±0.17
5.	*Cuminum cyminum(L)*	5.26±0.18
6.	*Laurus nobilis(L)*	5.17±0.25
7.	*Herniaria hirsuta (L)*	5.94±0.17
8.	*Malva rotundifolia(L)*	5.71±0.15
9.	*Fraxinus excelsior(L)*	5.94±0.20
10.	*Antirrhinum majus (L)*	5.16±0.28
11.	*Globularia alypum(L)*	3.55±0.18
12.	*Lepidium sativum(L)*	5.29±0.16
13.	*Chrysanthemum lencanthemum(L)*	4.97±0.18
14.	*Caratonia siliqua (L)*	4.37±0.23
15.	*Anethum graveolens (L)*	5.62±0.28
16.	*Lycium afrum (L)*	6.97±0.25
17.	*Echinops ritro (L)*	5.37±0.11
18.	*Lonicera caprifolium(L)*	5.52±0.18
19.	Amphotericin B	5.35±0.22
20.	Fluconazol	6.28±0.29
21.	Control	0.00

CONCLUSION

The results of this study showed that *T. horzianum* species produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *T. horzianum* species can be useful.

ACKNOWLEDGMENT

I sincerely wish to thank Dr. Ali Al-Marzuqi for providing me the opportunity to work on this project. I am thankful to you for helping me through the various analysis stages, and for providing helpful criticism and feedback throughout the writing process. I also would also like to thank Zainab Al-Habubi from the Department Biology for her guidance and help in the laboratory work.

REFERENCES

[1] Anesini C, Perez C (1993) Screening of plants used in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol 39:119-128.

[2] Anupama M, Narayana KJ, Vijayalakshmi M (2007) Screening of streptomyces perpuofucus for antimicrobial metabolites. Res journal of microbiology 2:992-994.

[3] Azin M, Moravej R, Zareh D (2007) Self-directing optimization of parameters for extracellular chitinase production by *Trichoderma harzianum* in batch mode. Proc Biochem 34(6–7):563–566

[4] Bae H, Roberts DP, Lim HS, Strem MD, Park SC, Ryu CM, Melnick RL and Bailey BA (2011) Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms 24(3):336-51

[5] Gebreselema G, Feleke M, Samuel S, Nagappan R (2013) Isolation and characterization of potencial antibiotic producing actinomycetes from water and sediments of lake Tana, Ethiopa. Asian pacific journal of Tropical biomedicine 3(6):426-435.

[6] Haran S, Schinckler H, Cheet I (1996) Molecular Mechanism of lytic enzymes involved in the biological activity of *Trichoderma harzianum*. *Microbiol*, 142 2312- 2331.
[7] Harman GE (2000) Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Diseas 84:377.
[8] Harman GE (2006) Overview of mechanisms and uses of *Trichoderma* spp. Phytopathol 96(2):190–194.
[9] Harman GE, Howell CR, Viterbo A, Chet I and Lorito M (2004) *Trichoderma* species-opportunistic avirulent plant symbionts. Nat Rev Microbiol 2(1):43–56
[10] Hynes J, Muller CT, Jones TH, Boddy L (2007) Changes in volatile production during the course of fungal mycelial interactions between *Hypholoma fasciculare* and *Resinicium bicolor*. J. Chem. Ecol 33:43.
[11] Korpi A, Jarnberg J, Pasanen AL (2009) Microbial volatile organic compounds, Crit. Rev. Toxicol., 39, 139.
[12] Nemcovic M, Jakubıova L, Ven I, Vladim F (2008) Induction of conidiation by endogenousvolatile compounds in *Trichoderma spp*, *FEMS Microbiol. Lett.* 284:231.
[13] Poornima S (2011) Evaluation of disease control and plant growth promotion potential of biocontrol agents on *Pisum sativum* and comparison of their activity with popular chemical control agent-carbendazim. J. Toxicol. Environ. Health Sci 3:127-138.
[14] Rajasekar T, Balaji S, Kumaran S (2012) Isolation and characterization of marine fungi metabolites against clinical pathogens. Asian. Pacific journal of tropical disease S387-S392.
[15] Reino JL, Guerriero RF, Hernandez-Gala R, Collado IG (2008) Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem. Rev 7:89.
[16] Shalini S, Narayan KP, Lata, Kotasthane AS (2006) Genetic relatedness among Trichoderma isolates inhibiting a pathogenic fungi *Rhizoctonia solani*. Afr. J. Biotechnol 5:580-584.
[17] Siddiquee S, Cheong BE, Taslima K, Kausar H and Hasan MM (2012) Separation and identification of volatile compounds from liquid cultures of *Trichoderma harzianum* by GC-MS using three different capillary columns. J Chromatogr Sci 50(4):358-367
[18] Tabaraie B, Ghasemian E, Tabaraie T (2012) Comparitive evolution of cephalosphrinc production in solid state fermentation and submerged liquid culture. Journal of microbial biotechnology food science 2(1):83-94.
[19] Tronsmo A, Hjeljord L, Plant microbe interactions and biological control, in *Biological control with Trichoderma species*, New York, Marcel Dekker, 111 (1998)
[20] Usha NS and Masilamani SM (2013) Bioactive compound produced by streptomycin strain. International journal of pharmacy and pharmaceutical science 5(1):0975-14.
[21] Verma M, Brar SK, Tyagi RD, Surampalli RY, Valero JR (2007) Antagonistic fungi, *Trichoderma spp*: panoply of biological control, Biochem. Eng. J. 37:1.
[22] Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008) *Trichoderma*–plant–pathogen interactions. Soil Biol. Biochem 40:1-10.
[23] Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, Woo SL, Lorito M (2008) A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiol. Mol. Plant Pathol. 72: 80.
[24] Whipps JM, Lumsden RD (2001) Commercial use of fungi as plant disease biological control agents: status and prospects In: Fungal Biocontrol Agents: Progress, Problems and Potential. Wallingford, T.B., Jackson C, Magan N (ed.). CABI Publishing, pp. 9-22.
[25] Zhihe C, Quingping W, Linong X, Xiaogan Z, Junei Z (1998) Advance of biocontrol of *Trichoderma* and *Gliocladium*. J Microbiol 25(5):284-286.