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Isolation and Identification of Bioactive Compounds from *Irpex Lacteus* Wild Fleshy Fungi

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Abstract

Objective:
The objective of this study was to determine the antimicrobial activity of the methanolic extract of wild mushroom (112 sample no.) and identification of bioactive compounds by using GC-MS .Identification of the strain based on DNA Sequencing by ITS1and ITS4.

Methods:
Specimen was tentatively identified on the basis of morphology and culture was obtained in 2% MEA (Malt Extract Agar). The strain was screened by antimicrobial activity. Methanolic extract was prepared by lyophilized the mycelial biomass they were used for antimicrobial activity in concentration of 100 mg/ml, 200mg/ml, 300mg/ml 400mg/ml, 500mg/ml .The resultant fraction of strain (112 samples) was subjected to GC-MS. Finally the strain was identified on the basis of molecular taxonomy.

Results:
The methanolic extract was found to show antimicrobial activity even at the highest concentration of 500 mg/ml for *Klebsiella pneumonia* (MTCC109) 28±0 mm, *Staphylococcus aureus* (MTCC 737)27.33±1.24 mm, *Escherichia coli*(MTCC-739)(19.33±0.47 mm),*Candida albicans*(MTCC-227)(10.33±0.57 mm), *Trichophyton mentagrophyte* (MTCC-8476) (25.66±0.47 mm). 14 bioactive compounds were identified with broad spectra of bioactivities. Through molecular taxonomy sample identifies as *Irpex lacteus.*

Conclusion:
The present study shows that the methanolic extract and ethyl extract of sample were more active fractions. The selected sample has a high inhibitory performance like antimicrobial activity against Gram positive, Gram negative and fungal species. Since bioactive compounds occurring in *Irpex lacteus* have broad spectrum like Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic etc. Moreover help to identify the natural compounds from wild mushroom.

Keywords:
Antimicrobial activity, *Irpex Lacteus*, GC-MS

INTRODUCTION

In 21st century, people are more empowered by the technology like use of artificial synthetic food and they are far away from natural herbal food. Mushroom is not only a food that satisfies hunger but also provides the required nutrients and has medicinal value which prevent diseases. It's surprising that mushrooms are source of biologically active compounds. [1, 2] Mushrooms are not restricted, it has broad spectrum for the bioactivities like antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, immunomodulating. [3, 4] Also mushrooms are attracting International attention as a valuable herb. [5] There are about 140,000 species that have been reported, out which 10% are known and 5% are undiscovered. [6] More than 3000 mushrooms are edible and 100 have commercially use. [7] Both the edible and inedible mushrooms and wild mushroom have biomolecule profile. [8] Mushroom contains variety of bioactive compounds which are useful for human health. [9] As in recent research, different compounds determined by GC-MS from *Pleurotus ostreatus*. [10] Similarly in case of *Pleurotus platypus*. [11] Ganocynin A and B, produced by *Ganoderma pfeifferi*. [12] Most of the species of class-Basidiomycetes (*Agrocybe perfecta*, *Hexagonia hydnoides*, *Irpex lacteus*, *Nothopanus hygrophanus*, *Pycnoporus sanguineus*, and *Tyromyces duracinus*) showed antimicrobial activity against bacteria and yeasts. [13] Antitumor activity detected approximately 80% from several mushroom such as *Flammulina velutipes*, *Clitocybe nebularis*, *Pholiota adipose*. As we know the ratio of well investigated mushroom is low. Therefore, in this study we focused on the awareness for wild mushrooms as biologically active compounds with medicinal potential.

MATERIALS AND METHODS

Collection and isolation of sample:

The wild strain of fleshy fungi was collected from forest of Kasauli (Himachal Pradesh). Sample was placed into plastic bag and cut, cleaned with disinfectants. After that dried at 50 to 60°C and then put in refrigerator for further use. The fruiting body of the mushroom was collected at a young stage with the aid of sterile forceps, wrapped with sterile foil paper and transported to the laboratory, washed thoroughly with several changes of sterile distilled water and 0.001% Mercuric Chloride and aseptically break lengthwise exposing the inner tissue (trama) with the aid of a sterile blade. A small piece of 2 x 2mm of the sterile tissue will aseptically transfer onto plates of (2%) malt extract agar. Three replicates were made and the plates incubated at 25°C to 30°C temperature for 7 days. Sub culturing for pure tissue mycelial production was prepared by transferring a small square of 5 x 5mm from the mother plate onto a fresh solid media plates.

Morphological identification

General characters of fleshy fungi were recorded like locality, date of collection, habitat, altitude etc. As well as macroscopic characters like Pileus, stipe, ring, veil, volva, lamellae etc.

Screening by antimicrobial

Sample preparation:[14] The pure culture of mushrooms was inoculated with mycelial bits (5mm) in 2% Malt extract broth and incubated at 25°C on rotary shaker at 130 rpm for 20 days. Mycelium along with medium was frozen at -10°C for 2 to 3 hrs. [15] After that frozen mycelial was crushed and centrifuged at 15000 rpm for 15 min at-4°C.The supernatant was lyophilized**.** [16] Then the dry powder was extracted with DMSO 100% for further use of antimicrobial activity.

Test organisms:Pathogenic bacterial strains of *Klebsiella pneumonia* (MTCC109),*Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC-739), *Candida albicans*(MTCC-227) and *Trichophyton mentagrophyte* (MTCC-8476)had been obtained from IMTECH Chandigarh.

Antimicrobial activity [17]

Test microorganisms were activated in Nutrient Broth (37°C, 150 rpm, 24h). The standard was used to adjust the turbidity to prepare inoculums from overnight grown bacteria and yeast cultures were used to determine the antimicrobial activity. In this method, the1µl of bacterial suspension (2 $\times 10^{10}$cfu/ml) spreaded on plate of MHA and PDA for fungal culture with 1.5µ (5 $\times 10^{10}$cfu/ml), with agar well borer made wells and inoculated the different concentration 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml and 500mg/ml. The plates was to be incubated at 35°C for 24hrs and in case of yeast culture incubated at 25°C for 72 hrs. Organic solvent served as negative control and antibiotics served as positive control. Percentage of inhibition of sample recorded through this formula. Percentage inhibition= Test /control X 100 **[18]**.

Minimum inhibitory concentration (MIC) determination **[19]**The minimum inhibitory conditions (MIC mg/ml) of the crude extract for respective microbial strains by using dilution method ELISA plate method. Natural products were dissolved in DMSO (500 mg /ml) and further 100 µL natural product and 25 ul broth were proceed for serial dilutions. Each dilution were distributed in 96-well plates, as well as a +ve control. Diluted with culture broth, each test and control well was inoculated with 25 µL of a bacterial suspension (2 $\times 10^{10}$cfu/ml) and fungal suspension (4 $\times 10^{10}$cfu/ml).

Identification of bioactive compound

Extraction procedure and active fractions of extract for GC-MS **[20]**The mycelia of the sample (112) was cultured in Potato Dextrose Broth (PDB) and incubated at 25ºC in a rotator shaker (name of company) at 150 – 200 rpm for 14 to 30 days. Mycelium along with medium was frozen at -10°C for 2 to 3 hrs. After that frozen mycelial was crushed and centrifuged at 15000 rpm for 15 min. Sample soaked in methanol (99.5%) and Ethyl acetate at room temperature separately. The sample covered with foil and allowed to stand for 3 to 4 days on shakers for extraction. The extracted sample was concentrated by lypholisation. **[21]** By using the column fraction samples were prepared .The ethyl acetate extract and methanol extract filtrated through 0.22µm membrane filter and then finally exposed to UV for sterility test. For further use both extracts put in DMSO as a stock 500 mg/ml. Then stored at 4°C to prevent degradation of bioactive compound for further use. To confirmed the fractions are active (F_M and F_E). Bioautographic and TLC on silica gel plate were done for primary investigation.

Primary investigation by Bioautographic study and TLC **[22]:**Prior to GC-MS the two extracts were screened for better antimicrobial activity. Ten µl (10 mg/ml) of each extract were loaded onto TLC plates in a narrow band Eluted using the mobile solvent systems (M3:C1). The developed plates were dried under a stream of fast moving air for 5 days to remove traces of solvent on the plates. Bacterial organism used for *E.coli*, *S. aureus*, *K.pneumonia*2 $\times 10^{10}$cfu/ml, Fungal suspension *C.albicans* and *T. mentagrophyte*5$\times 10^{10}$cfu/ml were approximately used. The prepared chromatograms were sprayed with the bacterial and fungal suspension until wet. This process was carried out in a Laminar flow cabinet .Thereafter, the plates were incubated overnight at 35°C and 100% relative humidity in the dark and then sprayed with a 2 mg/ml solution of p-iodonitrotetrazolium violet (Sigma) (INT) and further incubated overnight or longer in the case of fungal culture.

TLC Chromatography method used for the separation of the components from the two fractions methanol and ethyl acetate (F_M and F_E). The mobile phase ratio 3:1 (Chloroform : Methanol) used in both Bioautography and TLC.

Final identification by Gas Chromatography-mass spectrometry (GC-MS) analysis[23].

GC-MS analysis of this extract was performed using column DB-5(30m x 0.25mm x 1µm) composed of 100% Dimethyl polysilxane. Carrier gas Helium (99.999%) with constant flow rate of 1ml/min and injection volume of 2µl was employed (split ratio of 10:1) ethyl acetate evaporate at 40°C then powder form again extracted with ethyl acetate. 2ul of purely prepared sample was injected into the programme GC-MS instrument and rest of the sample put in deep freeze at -80°C for further study.

Oven temperature Programme: 110° C -2 min hold, Up to 200° C at the rate of 10 ° C/min-No hold, Up to 280 ° C at the rate of 5° C / min-9 min hold, Injector temperature 250° C, Total GC running time 36 min.

MS Programme:MS were taken at 70eV; scan interval 0.5 seconds and fragments from 45 to 450 Da. Total MS running time is 36 min.

Identification of Bioactive Constituents:Interpretation on Mass-Spectrum GCMS was carried out by using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular formula, weight and chemical structure of the Components of the test materials identified.

Molecular Identification of mushroom

Genomic DNA isolated and PCR amplification: Genomic DNA isolated from HIMEDIA kit. Then PCR amplification was performed using a pair of universal primers ITS-1(5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCCTCCGCTTATTGATATGC-3') for the region containing ITS1, ITS4 and 5.8S rDNA. The reaction mixture was followed by the kit (HIMEDIA X-PERT). Amplifications will be performed in 50-µl reactions of PCR buffer and PCR reactions will be consists of an initial denaturation at 94°C for 1 min 25 s, 35 cycles of amplification, and a final extension at 72°C for 10 min; each cycle of amplification will be consisted of denaturation at 95°C for 35 s, annealing for 55 s (at 55°C for reactions with ITS1 and ITS4 and at 60°C for reactions with ITS1 and ITS4), and extension at 72°C for 1 min. Amplification products was electrophoreses in 0.8% agarose gel, stained with ethidium bromide and amplicans will be observed under UV light **[24].**

Sequencing: The PCR product was subjected for sequencing and the DNA sequences was used for. The DNA sequences was used in NCBI(National Center for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) search for bioinformatics analysis, to look for homologus nucleotide sequences .On the basis of this information, species status was assigned .

RESULTS

TABLE 1: General characters data recorded during collection of fungi

Sample no.	Locality	Country/State	Altitude	Habitat	Forest type
1.	Kasauli	India /Himachal Pradesh	1,927 meters (6,322 ft)	Wood	Pine trees

I

II

III

FIGURE 1: (I, II and III): Strain no.112collected from Kasauli (H.P.)

Morphological Identification: Growth was scattered, The colour of sample was coffee brown and spore print was light brown gills present parallel in shape and smooth, lateral in attachment (with no stem) with pine wood. Fleshy in consistency, hygroscopic (**Figure 1).**

TABLE 2: Biological activity of 100% DMSO solution of mushroom extract against selected bacterial and fungal strains evaluated using well diffusion method, growth inhibition zone (mm) and lack of inhibition (-)

	Zone of inhibition mean ± SD(mm)				
Mushroom Extract (500 mg/ml)	Klebsiella pneumonia **(MTCC109)	Staphylococcus aureus* (MTCC 737)	Escherichia coli **(MTCC-739)	Candida albicans (MTCC-227	Trichophyton mentagrophyte (MTCC-8476)
112	28±0	27.33±1.24	19.33±0.47	10.33±0.57	25.66±0.47
Erythromycin*(20µg/ml)	.-	-	24.33±0.94	-	-
Cephalosporin for**(50 µg/ml)	33±0.8	45±0.8	-	-	-
Flucanazole(50 µg/ml)				22.66±0.94	44.66±0.47

*G (+) bacteria, ** G (-) bacteria.

Biological activity of 100% antibiotic solution against selected bacterial and fungal strains evaluated using well diffusion method, growth inhibition zone (mm).

TABLE 3: Percentage of Inhibition Zones of wild Mushrooms Culture Filtrates against Bacterial and fungal.

Mushroom Extract (100 mg/ml)	Percentage of zone of inhibition (%)				
	Klebsiella pneumonia ** (MTCC109)	Staphylococcus aureus* (MTCC 737)	Escherichia coli ** (MTCC-739)	Candida albicans (MTCC-227)	Trichophyton mentagrophyte (MTCC-8476)
112	84.84	60.72	79.44	45.58	57.45

TABLE 4: Represent the MIC (mg/ml) of the crude extract of wild Mushroom for Microbial strains by using dilution method ELISA plate method (Sagarika .R et al., 2012)

Mushroom Extract (150 mg/ml)	MIC (mg/ml)				
	Klebsiella pneumonia **(MTCC109)	Staphylococcus aureus*(MTCC 737)	Escherichia coli **(MTCC-739)	Candida albicans (MTCC-227)	Trichophyton mentagrophyte (MTCC-8476)
112	4.6	9.3	4.6	18.75	2.32
Erythromycin*(20µg/ml)	0.39	-	0.195	-	-
Cephalosporin for**(50 µg/ml)	-	1.56	-	-	-
Flucanazole(50 µg/ml)	-	-	-	0.312	0.078

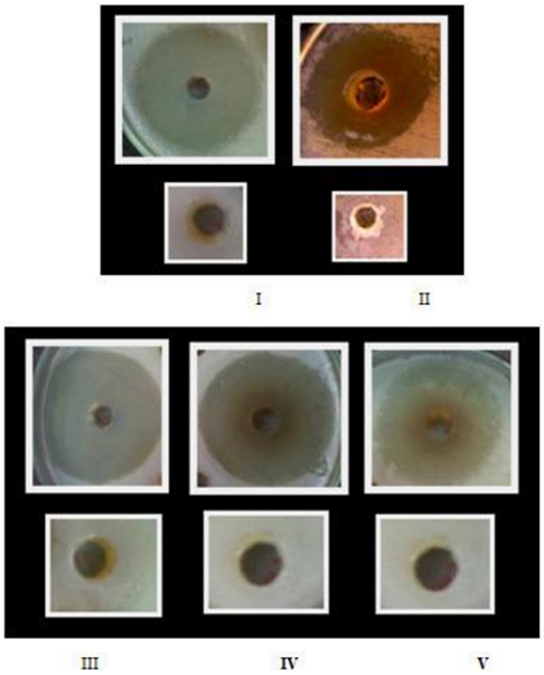

FIGURE 2: Antimicrobial activity test**. (I)** Trichophyton mentagrophyte (Flucanazole) (44.66±0 mm **(II)** Candida albicans (Flucanazole) 22.66±0.94 mm)**(III)** Staphylococcus aureus (Cephalosporin) (45.33±0.8mm), **(IV)** Klebsiella pneumonia **(**Erythromycin) (33 ±0.8) **(V)** Escherichia coli **(**Erythromycin)(24.33.66±0.94mm)),

Antimicrobial activity for screening

The assays were carried out using the DMSO solutions as solvent. The mushrooms extracts showed varying degree of inhibition on the test organisms. The mushroom extract showed the antimicrobial activity for different microorganisms. The result showed that the culture filtrates of 112 of were highly effective against K. pneumonia (28±0 mm),S. aureus (27±1.24 mm), Escherichia coli (19.33±0.47mm) (10.33±0.57 mm), Trichophyton mentagrophyte (25.66±0.47mm) as shown in**(Table 2**) and **(Figure 3)**.Percentage of zone inhibition was recorded in 112 for K. pneumonia (84.84%), for S. aureus (60.72%),Escherichia coli (79.44%), Candida albicans (45.58%), Trichophyton mentagrophyte (57.45%) in reference to +ve control **(Figure 2)**,**(Table3).**

The MIC tests indicated that the extract of 112 exhibited the minimal values of MIC (4.6 mg/ml) against Klebsiella pneumonia, E. coli and for S. aureus (9.3 mg/ml) respectively, as compared with the other two fungal species. Trichophyton mentagrophyte MIC value were (2.32 mg/ml) and Candida albicans MIC (18.75 mg/ml) as shown in **(Table 4)**.

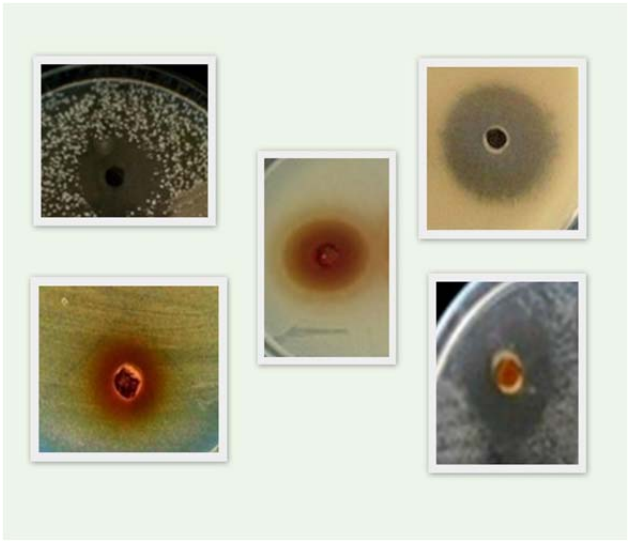

FIGURE 3: Antimicrobial activity test 112 sample**. (I)** *Trichophyton mentagrophyte* (25.66±0.47mm),**(II)***Staphylococcus aureus* (27.33±1.24mm), **(III)***Escherichia coli* (19.33±0.47mm), **(IV)** *Klebsiella pneumonia* (28±0mm) **(V)***Candida albicans* (10.33±0.57 mm)

For rapid identification of active compounds and active fraction by bioautography and TLC:

For rapid identification of active compounds bioautography used. White bands indicate where reduction of INT due to the presence of compounds that inhibited the growth of the bacteria and fungi in **(Figure 4)** .The appearance of white areas on the chromatograms showed inhibition of growth of due to presence of compound that inhibit their growth. Calculated the Rf values of the antimicrobial compounds present in methanol and ethyl extracts against *K. pneumonia*, S. aureus, *E. coli*, *Trichophyton mentagrophyte* and *C. albicans* are presented in **(Tables 5)**. **(Figure 5)** two fractions Ethylacetate (Ee) and Methanol (Me) actively separating the compound. Indicated, that both fractions are good to proceed for GC-MS **(Table 6)**.

Bioautography and TLC results for primary investigation:

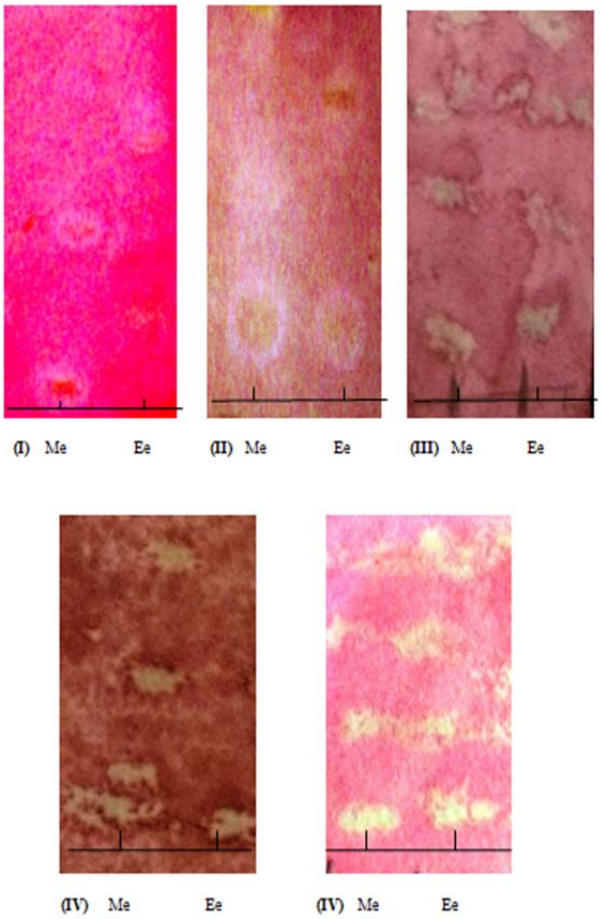

FIGURE 4: Methanol (Me) and Ethylacetate (Ee) fractions of 121separated on TLC plates using and sprayed with (I) *C.albicans*, (II) *S.aureus* , (III) *E.coli* , (IV) *K.pneumonia* and, (V)*T. mentagrophyte* 24 h later by INT. White areas indicate inhibition of microbial growth after 24hrs minutes of incubation at 37°C .

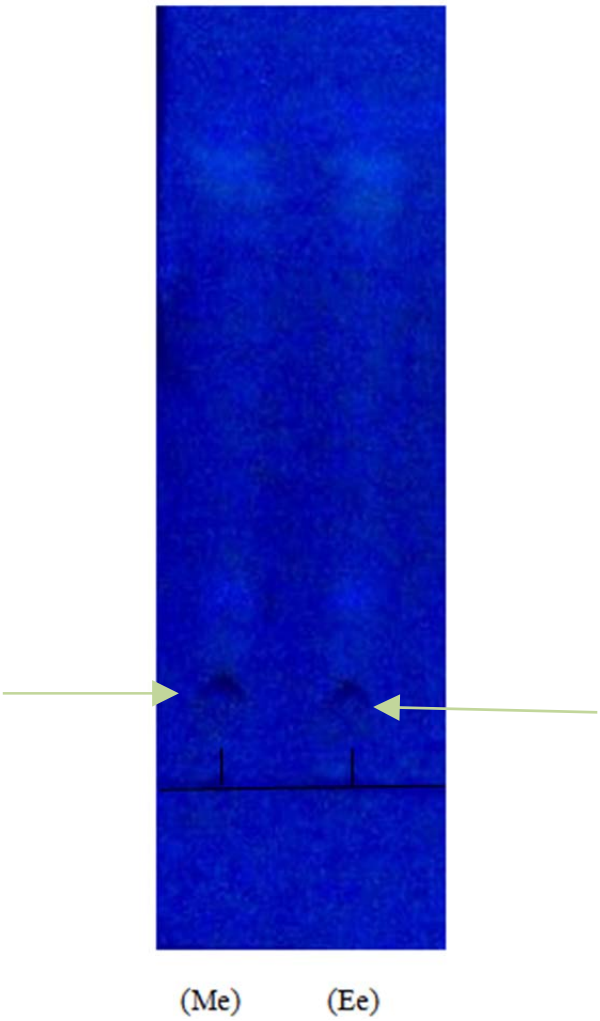

FIGURE 5: Methanol (Me) and Ethylacetate (Ee) fractions of 112separated on TLC plates areas indicated the separation of compound.

TABLE 5: Inhibition of growth on Bioauttographic TLC plates by 2 extracts Ethylacetate (Ee) Methanol (Me)of 112 against *C.albicans* , *S.aureus*, *E.coli*, *K.pneumonia* and *T. mentagrophyte.*

Mushroom	Microorganism	Solvent System	Extracts	RF values	Active Bands	Total Bands
Irpex lacteus	C.albicans	C:M	Ethylacetate (Ee)	0.45	-	1
		C:M	Methanol (Me)	0.12, 0.45	2	2
	S.aureus	C:M	Ethylacetate (Ee)	0.2	1	2
		C:M	Methanol (Me)	0.2	1	
	E.coli	C:M	Ethylacetate (Ee)	0.12, 0.62	2	4
		C:M	Methanol (Me)	0.12, 0.37	2	
	K.pneumonia	C:M	Ethylacetate (Ee)	0.12	1	4
		C:M	Methanol (Me)	0.15, 0.37, 0.57	3	
	T. mentagrophyte	C:M	Ethylacetate (Ee)	0.2, 0.3, 0.5	2	2

TABLE 6: The two extracts **Methanol** (Me) and Ethylacetate (Ee) of 112separated on TLC plates with RF value indicated the separation of compound.

Mushroom	Solvent System	Extracts	RF Values	Active Bands	Total Bands
112	C:M	Ethylacetate (Ee)	0.3, 0.8	2	4
	C:M	Methanol (Me)	0.3, 0.8	2	

TABLE: 7 Components identified in the112 sample no.[GC-MS Study]

Sr. No.	Retention Time	Area of percentage	Name of the Compounds	Molecular Formula	Molecular weight	Activity	Name of the Compounds
1	10.83	5.26%	Cycloheptasiloxane, tetradecamethyl-/ Tetradecamethylcycloheptasiloxane (I)	$C_{14}H_{42}O_7Si$	519	Skin-Conditioning Agent, Fragrance, antimicrobial.	**[24, 25, 26]**
2	26.15	47.67%	Azaphenanthrene, 2-methyl-3-(2-phenylethenyl)- (II)	$C_{22}H_{17}N$	295	Antibacterial and antitumor properties.	**[27, 28]**
3	28.13	12.14%	2-[4-(4-Methoxyphenyl)-3-phenylpyrazol-1-yl]-1Hbenzoimidazole (III)	$C_{23}H_{18}N_4O$	366.4	Antimicrobial activities in various benzamidazoles	**[29]**
4	35.82	8.75	2-secoandrosta-1,4,6-triene-17,19-diol,2 cyano-4-methyl-,diacetate (V)	$C_{25}H_{33}NO_4$	411.53	Represses viral replication in cells infected with HIV via binding to the Tat protein, antitumor activity	**[30]**
5	8.28	15.2	Cyclohexasiloxane, dodecamethyl) or (Dodecamethylcyclohexasiloxane)(IX)	$C_{12}H_{36}O_6Si_6$,	444	Antimicrobial, antifouling imunomodulatory and antitumor activities	**[31, 32, 33]**
6	10.48	22.18	Cycloheptasiloxane, tetradecamethyl) or (2,2,4,4,6,6,8,8,10,10,12,12,14,14-Tetradecamethylcycloheptasiloxane)(X)	$C_{14}H_{42}O_7Si_7$	518	Antimicrobial, antifouling imunomodulatory and antitumor activities	**[34, 35, 36]**
7	12.8	9.06	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester or (Gentisic acid (tms)(XI)	$C_{16}H_{30}O_4Si_3$	370	Cosmetic, antifungal activity of the essential oil	**[37, 38]**
8	17.35	19.96	N-Trifluoroacetyl-histidine, methyl ester or Methyl 3-(1H-imidazol5yl)2-[(trifluoroacetyl)amino]propanoate(XI I)	$C_9H_{10}F_3N_3O_3$	265	Not found	
9	18.88	6.90	Phthalic acid, butyl 2-ethylhexyl ester or 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester .(V)	$C_{20}H_{30}O_4$	334	Antibacterial	**[39]**
10	23.05	22.35	9,12-Octadecadienoic acid, methyl ester, (E,E)- or (Linolelaidic acid,methyl ester)(XIII)	$C_{19}H_{34}O_2$	294	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Ant androgenic, Antiarthritic, Anticoronary, Unsaturated fatty acid ester	**[40, 41]**
11	23.49	11.5	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)- or [Methyl (4Z,7Z,10Z,13Z,16Z,19Z)-4,7,10,13,16,19-docosahexaenoate](XIV)	$C_{23}H_{34}O_2$	342	Antidiabetic, Anti asthma Anticancer, Anti heart disease	**[42, 43]**
12	29.55	5.95	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-(VI)	$C_{27}H_{42}O_4$	430	Antiageing, Analgesic, Anti diabetic, Antioxidant, Anti-inflammatory, Anti dermatitic, Antileukemic, . Antitumor, Anticancer, Hepato protective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Anti spasmodic, Anti bronchitic, Anticoronary Vitamin E compound	**[44]**
13	30.83	68.64	Androst-5-en-17-carboxylicacid(VIII)	$C_{30}H_{48}O_4$	472	(On the basis of steroid moiety) Androgenic effects, control estrogen, synthesis, Antitumor activity	**[45, 46]**
14	32.32	4.87	Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester(VII)	$C_{19}H_{21}NOS$	311	Not found	

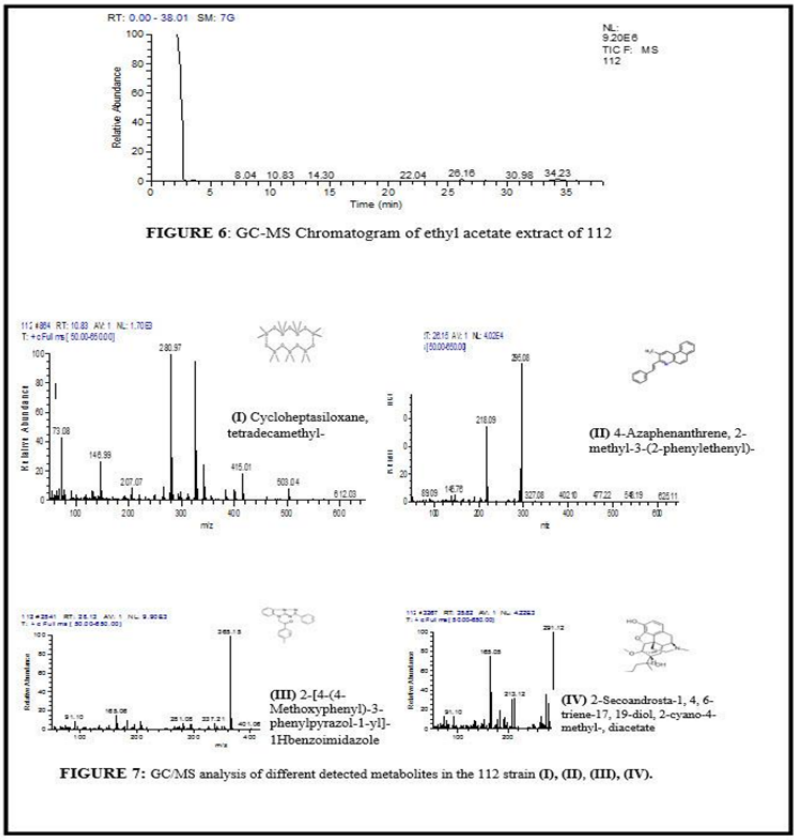

FIGURE 7: GC/MS analysis of different detected metabolites in the 112 strain **(I), (II), (III), (IV).**

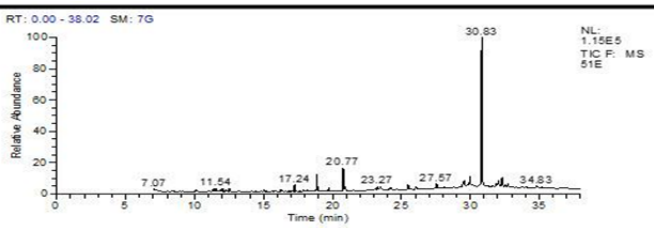

FIGURE 8: GC-MS Chromatogram of ethyl acetate extract of 112

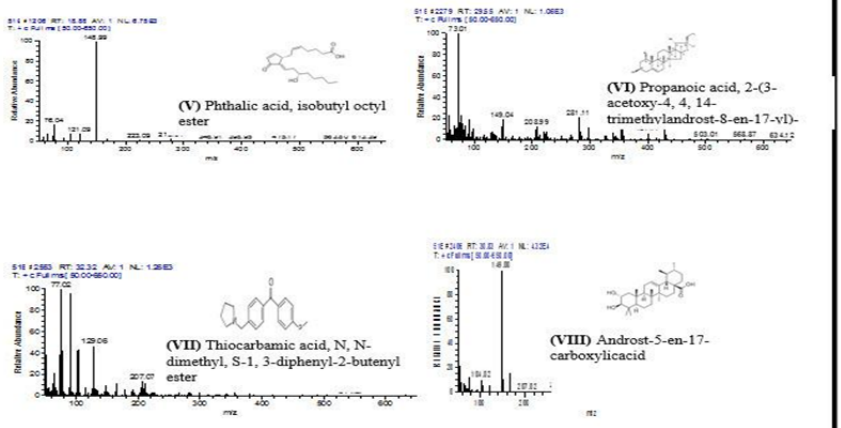

FIGURE 9: GC/MS analysis of different detected metabolites in the 112 strain **((V), (VI), (VII), (VIII).**

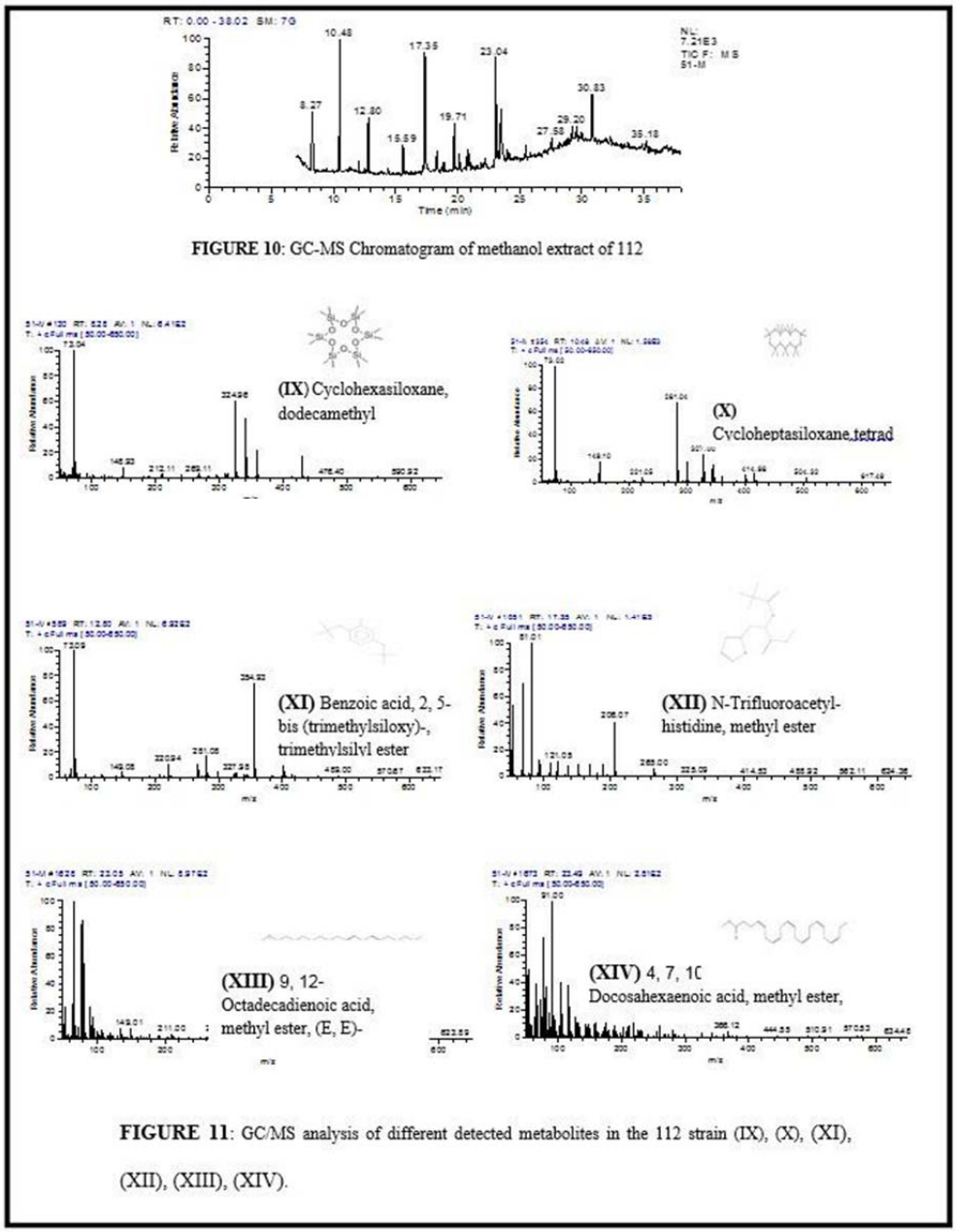

FIGURE 10: GC-MS Chromatogram of methanol extract of 112

FIGURE 11: GC/MS analysis of different detected metabolites in the 112 strain (IX), (X), (XI), (XII), (XIII), (XIV).

GC-MS identification of bioactive compound

Prior to GC-MS. The sample was subjected for TLC to make it evident for the complete separation of compound in two extracts methanol and ethylacetate extract as in Table 6. After the separation of the components the compounds were identified. The Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the Unknown component was compared with the spectrum of the known components stored in the NIST library. Software adopted to handle mass spectra and chromatograms was Turbo Mass 5.2.0. The GC-MS chromatograph of methanol extract and ethyl acetate was presented in Figure 5. The retention time (RT), molecular formula, molecular weight (MW) and bioactivity in the methanol extract and ethylacetate extract are presented in (**Table 7)**. Compounds were identified in the methanol extract were 6 and 8 compounds were identified in the ethylacetate extract (**Figure 6, 7, 8, 9, 10, 11)** The compound present in considerable concentration included (Azaphenanthrene, 2-methyl-3-(2-phenylethenyl) (47.67%), Cycloheptasiloxane, tetradecamethyl) (22.18%), (Linolelaidic acid, methyl ester) (22.35 %), Androst-5-en-17-carboxylicacid (20.14%), (68.64%) Androst-5-en-17-carboxylicacid. N-Trifluoroacetyl-histidine, methyl ester or Methyl 3-(1H-imidazol5yl)2-[(trifluoroacetyl) amino] propanoate and Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester except these two all compounds were bioactivity.

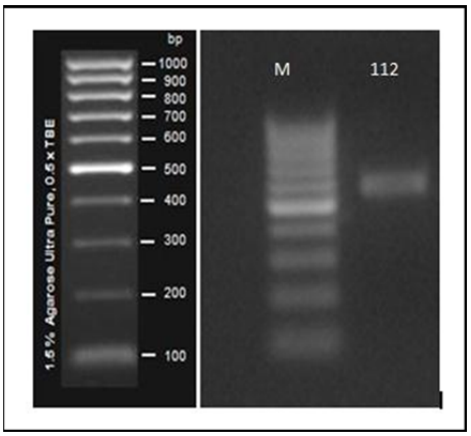

FIGURE: 12 Gel purified PCR product of the wild mushroom samples (112) at 650 bp (M marker lane, 100 bp plus ladder).

MOLECULAR STUDY RESULTS

Molecular Identification

The DNA was isolated. The electrophoresis universal primer of ITS1 /ITS4. The PCR purified product amplified at 650 bp band showed in **(Figure 12)**.The PCR products were gel purified, run in1% agarose gel, and processed for nucleotide sequencing. The nucleotide sequences as shown in (**Table 8)** of 112 samples were obtained and analyzed for Basic Local Alignment Search Tool (BLAST) search program such as (National Center for Biotechnology Information (NCBI) site) for identification as shown in **(Table 8), (Figure 13)**

TABLE 8: Nucleotide sequences of the field isolated mushroom samples.

Sample number	Sequences
112/12	AACCTGCGGAAGGATCATTATCGAGTTTTGAACGGGTTGTAGCTGGCCTCTCACGAGGCATGTGCACGCCTGGCTCATCCA CTCTTAACCTCTGTGCACTTTATGTAAGAGAAAAAAATGGTGGAAGCTTCCAGGATCTCGCGAGAGGTCTTCGGTTGAACA AGCCGTTTTTCTTTCTTATGTTTTACTACAAACGCTTCAGTTATAGAATGTCAACTGTGTATAACACATTTATATACAACTTT CAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTCTCATGGTATTCTCAACC CCTAAATTTTTGTAATGAAGGTTTAGCGGGCTTGGACTTGGAGGTTGTGTCGGCCCTCGCTGGTCGACTCCTCTGAAATGCA TTAGCGTGAATCTTACGGATCGCCTTCAGTGTGATAATTATCTGCGCTGTGGTGTTGAAGTATTTATGGTGTTCATGCTTCG AACCGTCTCCTTGCCGAGACAATCATTTGACAATCTGAGCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATC

TABLE 9: Summary of the BLAST result

Samples	Sequence length blasted (bp)	Highest coverage	% identity (Accession no.)	Identified name of samples
112	650	100%	100% JX290579.1	*Irpex lacteus*

Sequence ID: gb|JX290579.1| Length: 1254 Number of Matches: 1

Range 1: 24 to 673 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1201 bits(650)	0.0	650/650(100%)	0/650(0%)	Plus/Plus

```
Query  1    AACCTGCGGAAGGATCATTATCGAGTTTTGAACGGGTTGTAGCTGGCCTCTCACGAGGCA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  24   AACCTGCGGAAGGATCATTATCGAGTTTTGAACGGGTTGTAGCTGGCCTCTCACGAGGCA  83

Query  61   TGTGCACGCCTGGCTCATCCACTCTTAACCTCTGTGCACTTTATGTAAGAGaaaaaaaTG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  84   TGTGCACGCCTGGCTCATCCACTCTTAACCTCTGTGCACTTTATGTAAGAGAAAAAAATG  143

Query  121  GTGGAAGCTTCCAGGATCTCGCGAGAGGTCTTCGGTTGAACAAGCCGTTTTTCTTTCTTA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  144  GTGGAAGCTTCCAGGATCTCGCGAGAGGTCTTCGGTTGAACAAGCCGTTTTTCTTTCTTA  203

Query  181  TGTTTTACTACAAACGCTTCAGTTATAGAATGTCAACTGTGTATAACACATTTATATACA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204  TGTTTTACTACAAACGCTTCAGTTATAGAATGTCAACTGTGTATAACACATTTATATACA  263

Query  241  ACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  264  ACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAA  323

Query  301  GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  324  GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTT  383

Query  361  GGTATTCCGAGGAGTATGCCTGTTTGAGTCTCATGGTATTCTCAACCCCTAAATTTTTGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  384  GGTATTCCGAGGAGTATGCCTGTTTGAGTCTCATGGTATTCTCAACCCCTAAATTTTTGT  443

Query  421  AATGAAGGTTTAGCGGGCTTGGACTTGGAGGTTGTGTCGGCCCTCGCTGGTCGACTCCTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  444  AATGAAGGTTTAGCGGGCTTGGACTTGGAGGTTGTGTCGGCCCTCGCTGGTCGACTCCTC  503

Query  481  TGAAATGCATTAGCGTGAATCTTACGGATCGCCTTCAGTGTGATAATTATCTGCGCTGTG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  504  TGAAATGCATTAGCGTGAATCTTACGGATCGCCTTCAGTGTGATAATTATCTGCGCTGTG  563

Query  541  GTGTTGAAGTATTTATGGTGTTCATGCTTCGAACCGTCTCCTTGCCGAGACAATCATTTG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  564  GTGTTGAAGTATTTATGGTGTTCATGCTTCGAACCGTCTCCTTGCCGAGACAATCATTTG  623

Query  601  ACAATCTGAGCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATC  650
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  624  ACAATCTGAGCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATC  673
```

FIGURE: 13 BLAST result of sample no.112 showing the 100 %match with (*Irpex lacteus).*

DISCUSSION

112 exhibited moderate to good antibacterial activity against the bacterial and fungal pathogens tested. Similar results were reported in of *Pleurotus eryngii* ,*P. ostreatus*, *P.sajor-caju*, *L. squarrosulus* and *Agaricus bisporus* extract against bacteria *Klebsiella pneumoniae*, *Staphylococcus aureus* and fungal strains *Candida albicans Trichophyton* spp **[47, 48]**. The filtrates of wild mushrooms (112) under study showed a wide range of antibacterial and antifungal activity. The antimicrobial compounds were detected by using two extracts (F_E) and (F_M). Actively growing microorganisms have the ability to reduce INT to a purple-red colour **[49]**.As Antimicrobial Compounds detected by the same method of Bioautography Leaves extracts from South African **[50]**.In the presence of active mushroom compounds on the chromatograms, the growth of the organism is inhibited .In some cases, no inhibition of microbial growth was observed. The absence of activity could be due to evaporation of the active compounds, due to presence of little amount of the active compound **[51]**.Through TLC isolation of active bioactive fraction ethyl acetate (F_E) and methanol extract (F_M). The 14 compounds were identified by GC-MS. As table illustrated, most bioactive compound with different medicinal properties through GC-MS., tetradecamethyl Cycloheptasiloxane-10.83, Azaphenanthrene, 2-methyl-3-(2-phenylethenyl)-26.15, 2-[4-(4-methoxyphenyl)-3-phenyl-pyrazol-1-yl]-1H-benzimidazole-28.13, Androst-5-en-17-carboxylic acid, 3-acetoxy-33.83, -octyl, 2-secoandrosta-1,4,6-triene-17,19-diol,2 cyano-4-methyl-,diacetate-35.82. The study show tetradecamethyl Cycloheptasiloxane were characterized from the mycelia of Ophiocordyceps sinensis having anti-tumor activity **[52]**. Antibacterial effect of Some Azaphenanthrene Compounds investigated and antitumoral activity in Azaphenanthrene Alkaloids from Anaxagorea *dolichocarpa*, *Sprague* and *Annonaceae*. Novel benzimidazole derivatives investigated in vitro antibacterial and antifungal properties against some human pathogenic microorganisms **[53, 54]**. As compairing the past studies Linolelaidic acid were found in plants e.g. *Cocculus Hirsutus*, *Sonerila Tinnevelliensis* (1.08%) (6.09%) well as in medicinal mushroom (1.77%) **[55, 56]**. Therefore, similarly the other compound isolated from 112 sample. In the present study, GC-MS analysis and anti-microbial activity showed *Irpex Lacteus* 100 % success as a medicinal wild mushroom. The identification of bioactive compounds responsible for different medicinal value. The nobility of this study is that most the compound had been isolated from plants. Whole extract of *Irpex Lactus* showed broad spectrum of antibacterial activity. The bioactive compound has been isolated from mycelial biomass not from the fruiting body and the sample (112) show 100% identity with *Irpex lacteus*. Therefore here new sample of *Irpex lacteus* with gills has been isolated.

Conclusion:

From the above study, it is concluded that *Irpex lacteus* has shown a broad spectrum of bioactivity. This has proved antimicrobial, anti-tumor, and anticancer effects. Additionally, this is the first time that these compounds have been identified from *Irpex lacteus* found in wild. As the growth rate is very fast therefore, if commercialized can prove to be a very convenient source of bioactive compounds. Also, in the above method samples have been cultured from Mycelia, which is a very reliable source of growing the sample in abundance. Most importantly, though this sample matches 100% with *Irpex lacteus*, but as it has gills, therefore a new variety has been identified.

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