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Evaluation of Antioxidants, Total Phenolics and Antimicrobial Activities of Ethyl Acetate Extracts From Fungi Grown on Rice Straw

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Abstract: Rice straw (RS) collected from Egyptian agricultural environment (Al-Masied village-Alsharqia Governorate-Egypt) was used as substrate for three fungal isolates namely: 4b, 1Asp*, Pleurotus ostreatus* NRRL 3501 and mixed culture of (4b, 1Asp). Isolates number 4b and 1Asp were identified using the molecular technique (18S rRNA). Isolate 4b and 1Asp, were identified as *Trichoderma saturnisporum* MN1-EGY and *Aspergillus niger* MN2-EGY, respectively. *Trichoderma saturnisporum* MN1-EGY extract exhibited the highest antimicrobial activity compared to the other fungal extracts. Total phenolic, antimicrobial and antioxidant activities were also determined from the ethyl acetate extract of rice straw inoculated by these fungi. The antioxidant scavenging concentration(SC_{50}) values of the tested samples were 88.84, 91.45, 94.65 and 100.0µg/ml, respectively for *Pleurotu*s sp., *Aspergiluus niger*, *Aspergiluus niger* + *Trichoderma* sp., and *Trichoderma* sp., after 15 days incubation, compared to ascorbic acid as positive control with SC_{50} value equal to 8.0 µg/ml. Total phenolic contents (TPCs) were maximum in *Pleurotu*s sp. extract and found to be the most polyphenolic enriched sample (380.64 mg GAE/g dry extract) compared to extracts of *Aspergiluusniger*, *Aspergiluus niger* + *Trichoderma* sp., and *Trichoderma* sp. (261.89, 198.52, and 119.80 mg GAE/g dry extract), respectively. The GC-MS analysis has been also performed for the promising extracts.

Keywords: Rice straw; fungi; molecular identification; antimicrobial; antioxidant; total phenolics

1 Introduction

Lignocelluloses constitutes the major structure of cell wall in woody and non-woody plants and for this reason, they are considered as the major source for renewable organic matter [1,2]. Cellulose, hemicelluloses and lignin associated with each other's, are the major components of lignocellulosic materials [3]. Pretreatment is a vital process in the conversion of lignocellulosic polymers into fermentable sugars [4]. From different known pretreatment methods, biological pretreatment is mentioned for itslow energy, no chemical requirements and mild operating conditions. However, the acceleration is very slow in most biological pretreatment processes [5]. Several fungal strains (brown-, white- and soft-rot fungi) were investigated in the biological pretreatment of lignocellulosic biomass. White rot fungi are the most effective microorganisms in this respect [5]. Solid-state fermentation (SSF) using agriculture lignocellulosic biomass was used for the production of value-added products such as amino acids, organic acids, enzymes, bioactive secondary metabolites, aromatic compounds, in addition to other interesting substances used in the food

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industry [6]. Solid-state fermentation using lignocellulosic biomass has been performed to increase the phenolic contents of certain food products and consequently enhancing their antioxidant activity [7]. Soybean products fermented by *Trichoderma harizanum* using solid-state fermentation showed enhancement antioxidant activity than the unfermented products and this may be due to the higher content of phenolic acids, flavonoids and aglycone isoflavone having more hydroxyl groups achieved by SSF [8]. Numerous biologically active compounds were produced by SSF from diverse lignocellulosic wastes. Gibberellic acid was produced by *Giberella fujikuroi* and *Fusarium moniliforme* when grown on corncobs, while tetracycline and oxy-tetracycline were produced by *Streptomyces rimosus*grown on corncobs as substrate. The production of destrucxins A and B (cyclodepsipeptides) have been accomplished by cultivating *Metarhizium anisopliae*on rice husk. The production of ellagic acid has been found when *Aspergillus niger* was grown on pomegranate peel and creosote bush leaves [9,10]. Akyuz and Kirbag (2009) evaluated the antimicrobial activities of *Pleurotus eryngii* var. *ferulae* by cultivating it on different agro-wastes including wheat straw, cotton stalks and rice bran [11]. Yilmaz et al. studied the possibility of using waste lime plant leaves (*Tiliatomentosa*) for the cultivation of *Pleurotus ostreatus*, they evaluated the total phenolic contents, antioxidants as well as the antimicrobial activity of the fungus and they recommended that this plant residue were good source for mushroom cultivation for the production of bioactive substances [12]. This work represents the dual utilization of rice straw through biological pretreatment and production of value added products includingantioxidant, phenolic and antibacterial compounds.

2 Materials and Methods

2.1 Microorganisms and Culture Conditions

The fungal isolates 4b and 1Asp were isolated from local Egyptian environments [13]. Whereas, *Pleurotus ostreatus* NRRL 3501 was purchased from the Microbiological Resources Centers (MIRCEN), Ain Shams University, Egypt. Rice straw was all set as follow: 1000mL volume bottle containing 33g of rice straw and (50 mL tap water + 50 ml broth) (33% wt/v) were prepared and sterilized at 121^{o}C for 30min. The medium constituents were intended in half of water content (2X) and then 50ml of prepared medium was added to bottles containing rice straw then re-sterilized at 121^{o}C for 1h. The medium consists of the following ingredients (g/L): $(NH_4)_2SO_4$ (2), KH_2PO_4 (3), KCl (0.5), $MgSO_4$ (0.5), yeast extract (0.1), peptone (0.3), $CuSO_4$ $5H_2O$ (0.3) and urea (0.5). Also ingredients (mg/L): $FeSO_4$ (10 mg), $MnSO_4$ (5 mg), $CoCl_2$ (3 mg), $Na_2Mo_4.2H_2O$ (0.1 mg). The pH was adjusted to 5.5. Each bottle was inoculated with 10ml of spore suspension of 7days old fungal culture (OD = 1). Three different fungal strains namely; *Trichoderma saturnisporum* MN1-EGY, *Aspergillus niger* MN2-EGY and *Pleurotus ostreatus* NRRL 3501 in addition to mixed culture of *T. saturnisporum* and *A. niger* were used. The bottles were incubated at 30^{o}C for different periods (15, 45 and 90 days). Weight loss, chemical constituents; cellulase activities, xylanase activity, total phenolics, antimicrobial activity as well as antioxidant activity were measured during the incubation intervals.

2.2 Identification of Fungal Cultures

Fungal cultures (4b and 1Asp) were identified according to a molecular biological protocol by DNA isolation, amplification (PCR) and sequencing of the ITS region. Theprimers ITS2 (GCTGCGTTCTTCATCGATGC) and ITS3 (GCATCGATGAAGAACGCAGC) were used at PCR while ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were used at sequencing. The purification of the PCR products was carried to remove unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore). Sequencing was performed by using Big Dye terminatorcycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA). *Candida* sp. was usedas control.

2.3 DPPH Free Radical Scavenging Activity

The scavenging activity of the stable 2,2'-diphenyl-1-picraylhydrazyl (DPPH) free radical was determined via the method described by Marwah et al. (2007) [14]. Briefly, the reaction medium contained 2 mL of 100 µM DPPH purple solution in methanol and 2 mL of extract, ascorbic acid was used as standard. The reaction mixture was incubated in the dark for 20 min and the absorbance was recorded at 517 nm. The assay was carried out in triplicate. The decrease in absorbance at addition of test samples was used to calculate the antiradical activity, as expressed by the inhibition percentage (%IP) of DPPH radical, following the equation: %IP = [Ac-As]/Ac X 100; where Ac and As are the absorbencies of the control and of the test sample after 20 min, respectively [15,16].

2.4 Determination of Total Phenolic Contents (TPCs)

The total phenolic content of the ethyl acetate extracts of biologically pretreated RS was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth and Gillespie (2007) [17]. Gallic acid wasused as a reference standard for plotting calibration curve. A volume of 0.5 ml ofthe plant extract (100 µg/ml) was mixed with 2 ml of the Folin-Ciocalteu's reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. Theabsorbance of the resulting blue color was measured at 765 nm using double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). The total phenolic contents were determined from the linear equation of a standard curve prepared with gallicacid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract [18,19].

2.5 Antimicrobial Activity

Disc agar plate method was used to estimate the antimicrobial activities of ethyl acetate extracts from different fungal strains grown on rice straw for different incubation periods [21,22]. Four different test microbes; *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* were selected to evaluate the antimicrobial activities as representatives of G+ve bacteria, G-ve bacteria, yeast and fungal groups. The bacterial and yeast test microbes were grown on a nutrient agar medium (DSMZ1) of the following components (g/L): Peptone (5.0), Meat extract (3.0), Agar (20.0), distilled water (1000.0 mL) and the pH to 7.0. On the other hand, the fungal test microbe was cultivated on Czapek-Dox medium (DSMZ130) of the following ingredients (g/L): Sucrose (30.00), $NaNO_3$ (3.0), $MgSO_4.7\ H_2O$ (0.50), KCl (0.50), $FeSO_4 \times 7\ H_2O$ (0.01), K_2HPO_4 (1.0), agar (18.0), distilled water (1000 mL) and the pH was adjusted to 7.2. The culture of each test microbe was diluted by sterilized distilled water to about 10power7-10power8 cells/mL, then 1mL of each was used to inoculate 1L-Erlenmeyer flask containing 250 mL of solidified agar media [23]. These media were transferred to sterilized Petri dishes (10 cm diameter having 25 mL of solidified media). The filter paper discs, saturated with 0.2 mg extract, were placed on the surface of agar plates seeded with test microbes and incubated for 24 hrs at the appropriate temperature of each test organism. Antimicrobial activities were recorded as the diameter of the clear zones (including the film itself) that appeared around the films [24].

2.6 GC/MS Analysis

GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS and TGSMS Fused Silica Capillary Column (30 m, 0.251 mm, 0.1 mm Film thickness), National Research Centre, Giza, Egypt. For GC/MS detection, an electron ionization system with ionization energy for 70ev was used as the carrier gas at a constant flow rate of 1 mL/min. The injector and MS transfer line temperature were set at 280°C. The oven temperature was programmed at an initial temperature 40°C (hold 3 min) to 280°C was a final temperature at an increasing rate of 5 °C/min (hold 5 min). The identified components were investigated using a percent relative peak area. A tentative identification of the

compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY Library data of the GC/MS system [25-29].

3 Results and Discussion

3.1 Isolation and Identification of Fungal Strains

Fungal strains (4b and Asp), isolated from Egyptian environments, were identified using the molecular technique (18S rRNA) as an accurate tool for identification. From Blast results, strain 4b exhibited 99% similarity with the previously identified *Trichoderma saturnisporum* strain T-61and then it was submitted to Gene Bank with the name *Trichoderma saturnisporum* MN1-EGY with a unique accession number (KY073254). While strain 1Asp exhibited 98% similarity to with the previously identified *Aspergillus niger* strain according to Blast search. The sequence of strain 1Asp was submitted to Gene Bank with the name *Aspergillus niger* MN2-EGY with the unique accession number (KY305011). Fig. 1 and Fig. 2 represent the phylogenetic tree of *Trichoderma saturnisporum* MN1-EGY and *Aspergillus niger* MN2-EGY, respectively. Molecular identification depending on polymerase chain reaction (PCR) and sequencing was considered as an accurate and quick tool for microbial identification compared to the traditional methods which considered as inaccurate, tired and time consuming [30,31]. Chen et al. investigated an alternative method for microbial identification depending on targeting specific region within RNA gene cluster using common primers through polymerase chain reaction (PCR) amplification [32]. For this purpose, internal transcribed spacer (ITS) region (1-5) of ribosomal DNA (rDNA) is used and the primers used for amplification of ITS's are ITS1 and ITS4 [33]. Several investigations dealing with cellulases production from fungi has been used the molecular technique for their identification. Rathnan et al. isolated from several fungi from soil, compost, etc. These isolates were screened for cellulase production using carboxymethyl cellulose (CMC) containing medium flooded with Congo red [34]. Five isolates NASC1, NASC2, NASC3, NASC 4 and NASC5 exhibited highest clear zone and after secondary screening, isolate NASC3 exhibited the highest activity and molecularly identified using 18Sr RNA technique. The identification was done using ITS4 and ITS5 primers and the fungus was identified as *Penicillium citrinum*. Nine fungal strains of 103 strains screened for xylanase activity were identified based on sequencing ITS ribosomal gene region using ITS and ITS4 as primers. These identified fungi were species of *Fusarium* genus as compared to previous identification [35]. In addition, 16 yeast strains from 390 that showing cellulase as primary screened on solid medium. Also, 5 of these 16 exhibited highest cellulase activity as secondary screened on liquid medium. Isolate AAT6 was potent producer and this strain was molecularly identified by sequencing ITS1 and ITS2 ribosomal DNA. The sequencing results indicating that this strain is yeast-like fungus *Acremonium strictum* [36].

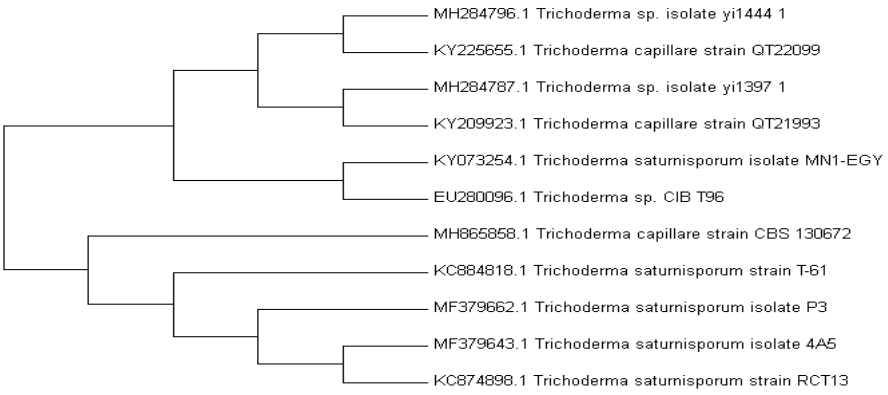

Figure 1: Phylogenetic trees showing relationship of strain *Trichoderma saturnisporum* MN1-EGY with other related fungal species retrieved from Gen Bank based on their sequence homologies of 18S rRNA

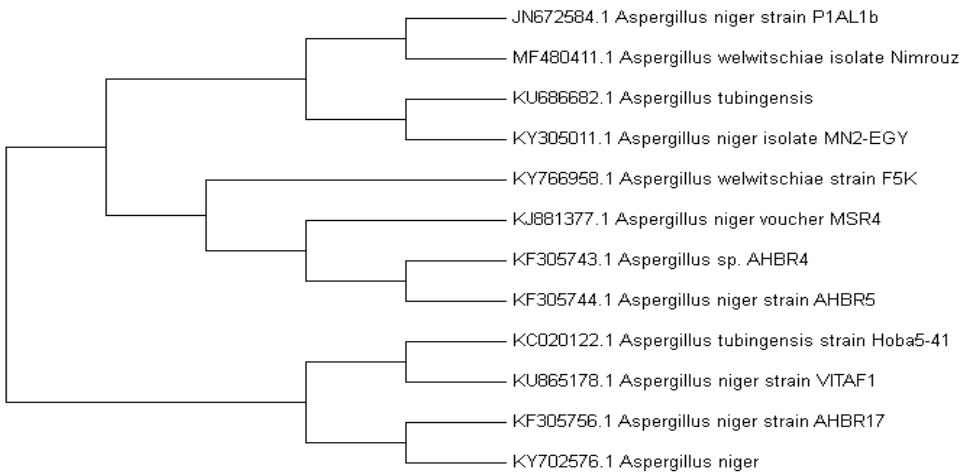

Figure 2: Phylogenetic trees showing relationship of strain *Aspergillus niger* MN2-EGY with other related fungal species retrieved from Gen Bank based on their sequence homologies of 18S rRNA

3.2 Extraction of Bioactive Metabolites

Rice straw inoculated with different fungal strain has been extracted with ethyl acetate and the extraction has been evaporated till dryness. Several previous investigations reported the use of ethyl acetate for the extraction of bioactive metabolites from fungi. The Endophytic fungus isolated from *0cimum basilicum* has been cultivated on semisolid (agar) and liquid potato-carrot media was extracted with ethyl acetate (37). *Piper coractum* was also used for the isolation of endophytic fungi, these fungi were cultured on potato dextrose broth for 14 days, and their cultures were extracted by the semi-polar solvent ethyl acetate (38)

3.3 Antimicrobial Activities

The antimicrobial activities against Gram-positive (*Staphylococcus aureus*), Gram-negative *(Pseudomonas aeruginosa)*, yeast *(Candida albicans)* and fungal *(Aspergillus niger)* test microbes have been tested for the fungi used in the biological pretreatment during selected incubation periods (15, 45 and 90 days). It has been generally observed that the antimicrobial activities from the fungal strains and the mixed culture did not exhibit an increase with time prolongation (Figs. 3(a)-3(d) & Figs. 4(a)-4(d)). It has been also observed that no antimicrobial activities were found against the fungal test microbe (*Aspergillus niger).* Moreover, antimicrobial activities from *T.saturnisporum* were the highest in comparison to the other fungi and mixed *(T. saturnisporum*and *A. niger)* culture, while the antimicrobial activities from the mixed culture were lower compared to the other fungi. Agro-industrial residues as an abundant and inexpensive sources can be used for the production of bioactive metabolites and introduce environmental and economic impacts [39,40]. *Penicillium chrysogenum* IFL1 was used for the production of bioactive secondary metabolites against bacteria, fungi and amoeba using agro-industrial residues as the sole source for nutrients [41]. Two local strains of *Penicillium* spp. namely: *P. chrysogenum* series UAF R1 and *P.chrysogenum* series UAF R2 were used to maximize their penicillin production using agro-industrial residues [42]. *P. chrysogenum* UAF R1 was found to the best producer of penicillin when corn steep liquor (CSL) was added to the fermentation medium but when CSL was replaced with sugarcane bagasse (SCB), highest yield of penicillin was produced in a value of 62.5% more than CSL. Agriculture residues like apple pomace, cotton-seed meal, soybean powder and wheat bran were taken as basic carbon and nitrogen sources for neomycin production using solid state fermentation technique [43]. *Streptomyces fradaie* NCIM 2418 produced higher yield of Neomycin when grown on apple pomace and low antibiotic production with wheat bran.

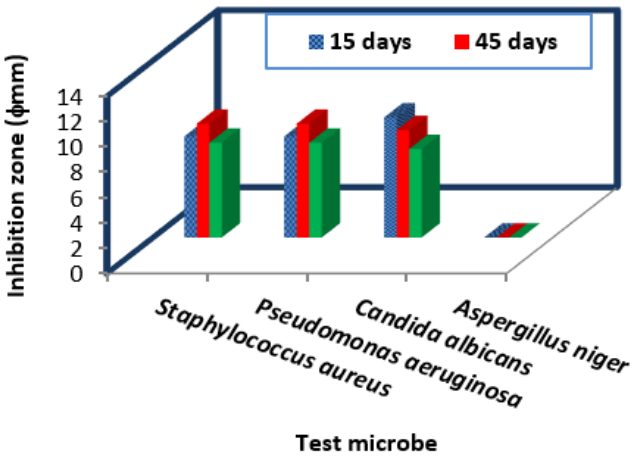

Figure 3(a): Antimicrobial activity of ethyl acetate extract from *Pleurotus ostreatus* NRRL 3501 grown on rice straw

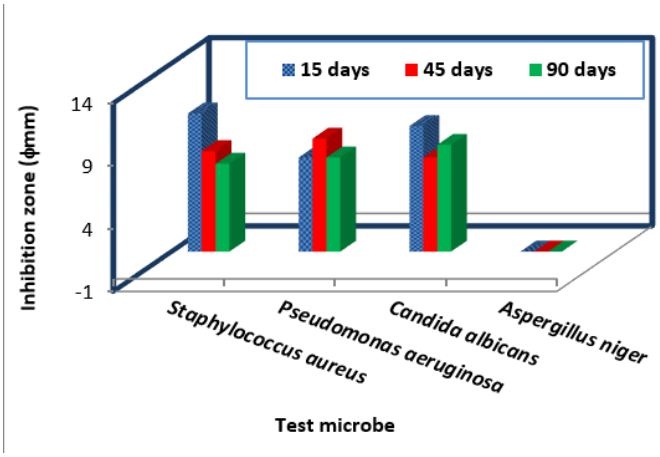

Figure 3(b): Antimicrobial activity of ethyl acetate extract from *Trichoderma saturnisporum* MN1-EGY grown on rice straw

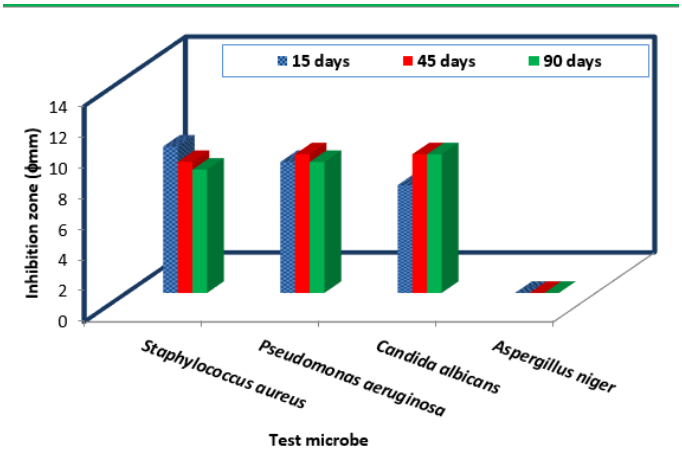

Figure 3(c): Antimicrobial activity of ethyl acetate extract from *Aspergillus niger*MN2-EGY grown on rice straw

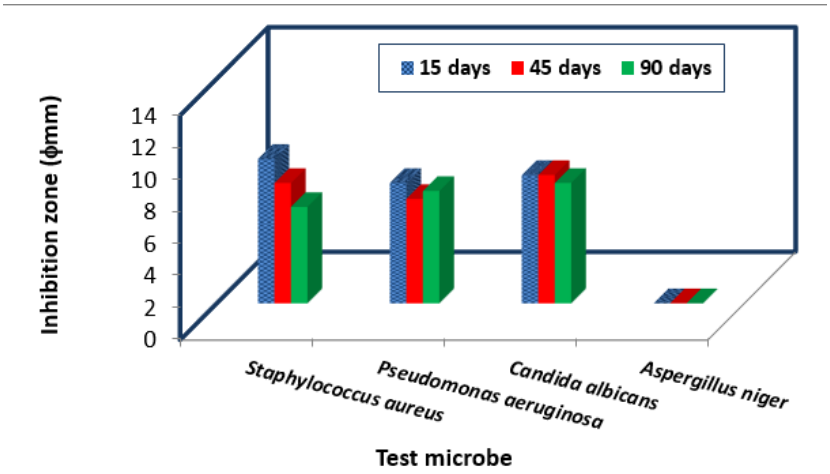

Figure 3(d): Antimicrobial activity of ethyl acetate extract from *the mixed culture* (*Trichoderma* sp., and *Aspergillus* sp.) grown on rice straw

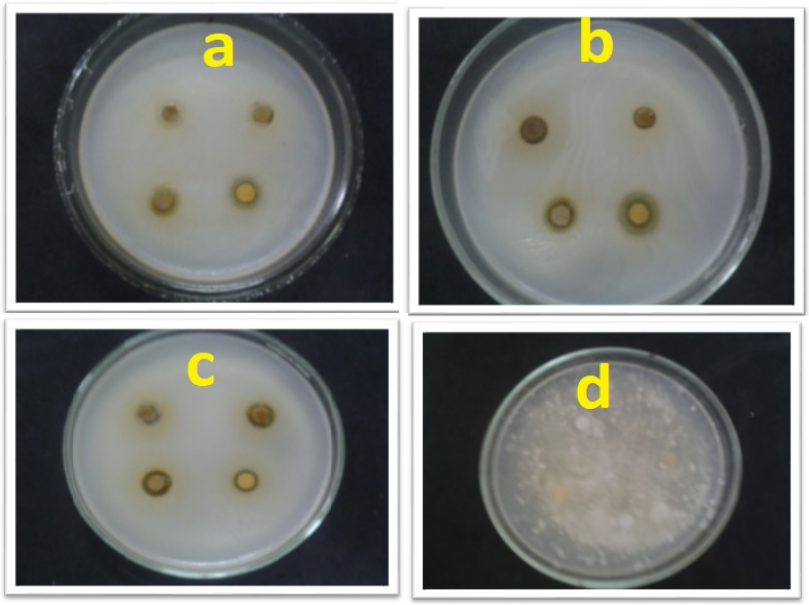

Figure 4: Antimicrobial activity of extracts from different fungal strains grown on rice straw: (a) against *Staphylococcus aureus*, (b) against *Pseudomonas aeruginosa*, (c) against *Candida albicans* and (d) against *Aspergillus niger* test microbes

3.4 Antioxidant Activity

The tested extracts that showed to some extent moderate antioxidant activity was indicated via declining the dark purple color of DPPH radical to yellow and pale purple, and the scavenging activities were directly proportional with the concentrations of the tested samples [44]. In the current study, the SC_{50} values of the tested samples were 88.84, 91.45, 94.65 and 100.0 µg/m L, respectively for *Pleurotus* sp., *Aspergiluus niger*, *Aspergiluus niger* + *Trichoderma* sp., and *Trichoderma* sp. compared to ascorbic acid as positive control with SC_{50} value equal to 8.0 µg/ml (Fig. 5). It had been found thatmost mushroom species showed noticeable DPPH free radical scavenging activities at the selected scope of concentrations [45-47]. In the present investigation the free radical scavenging activity expressed as SC_{50} was found to be 88.84 µg/mL, which was matched with the previous reports, i.e., Wong and Chye reported free radical

scavenging activity of 83.04% in petroleum ether extract of *P. porrigens* [48], also the free radical scavenging of the *P. sajor-caju* was found 88.07% [47], the hot water extract of *P. florida* showed DPPH scavenging effect (87.03%) [49]. On the other hand, Yadav et al. (2014) reported that the ethyl acetate extract of *A. niger* showed DPPH scavenging activity with 71% [50], Kandasamy et al. were reported on the antioxidant activity of *Trichoderma* sp. [51]. Also, the ethyl acetate extract of *Trichoderma* sp. showed DPPH scavenging activity with SC_{50} equal to 47 µg/mL [52].

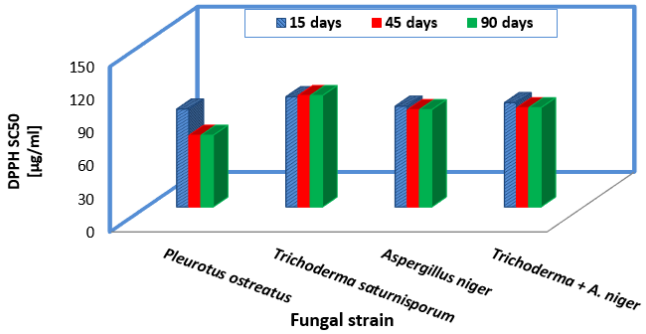

Figure 5: The antioxidant activity of different extracts from rice straw pretreated with different fungal strains for different incubation periods

3.5 Total Phenolics

Fig. 6 revealed that the TPCs were maximum in *Pleurotus ostreatus* extract and was found to be the most polyphenolic enriched sample of TPC (380.64 mg GAE/g dry extract) compared to extracts of *Aspergiluus niger*, *Aspergiluus niger* + *Trichoderma* sp., and *Trichoderma* sp., of TPCs (261.89, 198.52, and 119.80 mg GAE/g dry extract, respectively. Kaur *et al*. reported that the TPCs of three Indian *Pleurotus* species namely; *P. florida*, *P. ostreatus*a and *P. sajor-caju* were 85.19, 89.61 and 77.27 µg GAE/g dry weight, respectively [53]. In addition, the TPCs in the 1st and 2nd primordial phases from ethanol and aqueous extracts of *P. djamor* were 14.36 & 11.47 and 19.02 & 14.82 mg GAE/g dry weight, respectively [54]. Moreover, our current results to some extent were matched with the earlier report of Mohamed et al. [55]. It has been investigated that low values of TPCs in other *Pleurotus* sp. such as; *P. sajor-caju* (52.20 mg/g) [47], *P. cystidiosus* (10.24 mg/g), and *P. ostreatus* (15.7 mg/g) have been observed [56]. On the other hand, TPC of the ethyl acetate extract of *A. niger* was 58.46 mg of GAE/g of extract [50]. Ruma et al. reported that the TPC of the ethyl acetate extract of *Trichoderma* sp. was 94.53 mg/mL [52].

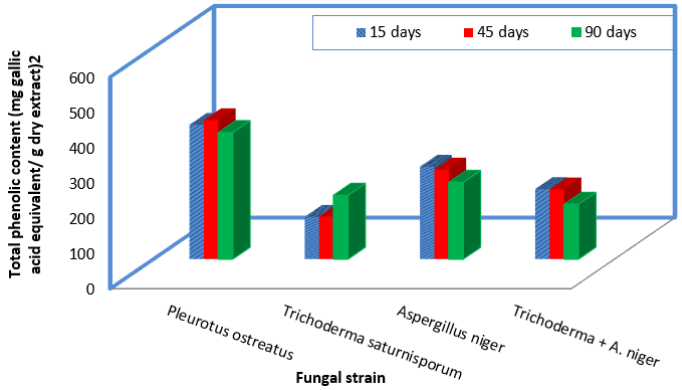

Figure 6: The total phenolic contents of different extracts from rice straw pretreated with different fungal strains for different incubation periods

3.6 Gas Chromatography/Mass Spectroscopy Studies of Extracts from Different Biologically Treated Rice Straw

The GC-MS analysis of the *Pleurotuso streatus* NRRL3501 extract revealed the presence of nine compounds (Tab. 1 and Fig. 7), representing (92.08%) of the total composition. *n*-Eicosane (40%), 8-Nonynoic acid, methyl ester (27.26%), and cyclopentaneundecanoic acid, methyl ester (16.03%) were themajor identified components. But, The GC-MS analysis of the *Trichoderma saturnisporum* MN1-EGY extract showed the presence of twenty-nine compounds (Tab. 2 and Fig. 8), representing (92.29%) of the total composition. 2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R[R*,R*(E)]] (78.27%), Methyl-2-2hydroxyethyl)-1-hexyldiester of phthalic acid (6.69%), Tetracosane (1.89%), Pentacosane (1.62%), Tricosane (1.32%), and Tetratetracontane (1.14%) were the major identified components. In addition, the GC-MS analysis of the *Aspergillus niger* extract exhibited the presence of thirteen compounds (Tab. 3 and Fig. 9), representing (95.79%) of the total composition. *n*-Eicosane (85.74%), Dimethyl 2,c-5-diphenyl-r-2, c-4-pyrrolidinedicarboxylate (1.79%), and 13,16-Octadecadienoicacid, methyl ester (1.17%) were the major identified components. Moreover, The GC-MS analysis of the mixed (*T. saturnisporum* and *A. niger*) culture extract revealed the presence of thirteen compounds (Tab. 4 and Fig. 10), representing (83.25%) of the total composition. *n*-Eicosane (52.54%), 9-Octadecenoic acid, methyl ester (3.48%), and eupatoriochromene (3.10%) were the major identified components. Regarding the major specific compounds; eicosane was particular in *Streptomyces* strain KX852460 by GC-MS [57], 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl was identified in the ethanolic extract of *Hypericum mysorense* leaf part [58] and 9-Octadecenoic acid, methyl ester was identified in the petroleum ether extract (oil) of *Iris germanica* [59].

Table 1: Chemical compositions of the ethyl acetate extract from *Pleurotus austeratus* NRRL3501 grown on rice straw

No.	R_t.	Area %	M.W.	M.F.	Identified compounds[1]
1	16.43	1.17	638	$C_{34}H_{42}N_{210}$	t-butyl-5'-benzyloxycarbony-l3,4'-bis-(2-methoxycarbonylethyl)-3'-methoxycarbonylmethyl-4-methylpyrromethane-5-carboxylate
2	27.37	1.90	612	$C_{28}H_{32}N_6O_6S_2$	Trp-CysS-SCys-Trp
3	32.38	1.52	523	$C_{25}H_{33}N_9O_2S$	2,8-Bis-(dimethylamino)-4,6-bis-(propylamino)-5-(4nitrophenyl)-5-Hthiopyrano-[2,3d:6,5d']-dipyrimidine
4	34.87	1.62	612	$C_{40}H_{40}N_2O_4$	2,9-Bis-[2,6-bis-(but-3-enoxy)phenyl]-1,10-phenanthroline
5	37.0	1.34	154	$C_{10}H_{18}O$	*Cis*-Piperitol
6	42.28	40.0	282	$C_{17}H_{34}O_2$	*n*-Eicosane
7	45.47	1.24	156	$C_{10}H_{20}O$	3,3,5,5-Tetramethylcyclohexanol
8	46.26	27.26	168	$C_{10}H_{16}O_2$	8-Nonynoic acid, methyl ester
9	46.39	16.03	268	$C_{17}H_{32}O_2$	Cyclopentaneundecanoic acid, methyl ester
Total %	**92.08%**				

[1] Compounds identified via comparison its mass spectrum with NIST library, Adams, 2001 and literature. M.F.: Molecular formula; M.W.: Molecular weight; R_t.: Retention time.

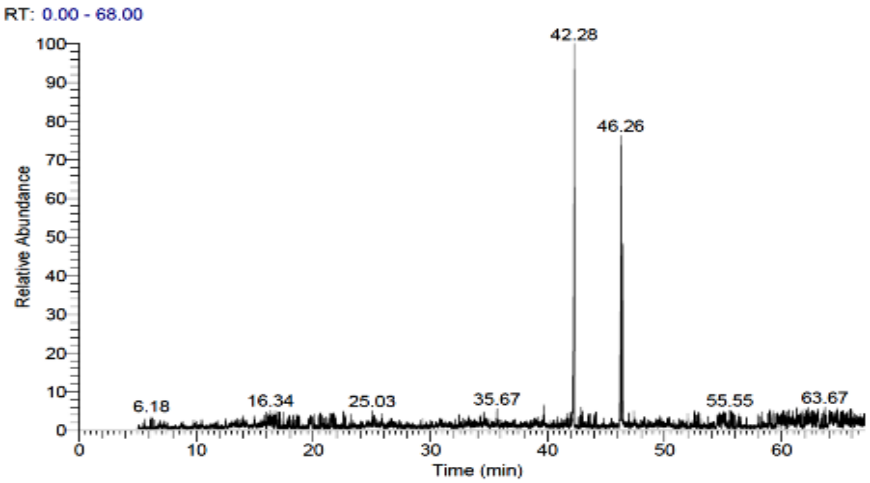

Figure 7: Gas chromatography-mass spectrometry (GC-MS) chromatogram of the ethyl acetate extract from *Pleurotus austeratus* NRRL3501 grown on rice straw

Table 2: Chemical compositions of the ethyl acetate extract from *Trichoderma saturnisporum* MN1-EGY grown on rice straw

No.	*Rt.*	Area %	M.W.	M.F.	Identified compounds[1]
1	5.71	0.12	586	$C_{36}H_{58}O_2S_2$	di(dodecylhydroxyphenyl)disulfide
2	6.87	0.31	118	$C_4H_6O_4$	Ethanedioic acid, dimethyl ester
3	14.39	0.16	860	$C_{55}H_{104}O_6$	9-Octadecenoic acid (Z),-3[(1oxohexadecyl) oxy]-2-[(1-oxooctadecyl)oxy]propyl ester
4	25.32	0.14	196	$C_{10}H_{12}O_4$	Dimethyl-1,4 cyclohexadiene-1,2-dicarboxylate
5	25.52	0.32	234	$C_9H_{14}O_7$	Trimethyl citrate
6	26.37	0.56	246	$C_{14}H_{18}N_2O_2$	1-(-4Methoxyphenyl)-2-pentene-1,4-dione-4-dimethylhydrazone
7	30.55	0.14	198	$C_{14}H_{30}$	Tetradecane
8	30.75	0.13	568	$C_{40}H_{56}O_2$	Zeaxanthin
9	31.54	0.10	488	$C_{35}H_{52}O$	17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren2one
10	33.59	0.21	386	$C_{28}H_{50}$	Benzene,(2decyldodecyl)
11	34.48	0.15	406	$C_{22}H_{14}O_8$	6,6'-Dimethyl-5,5',8,8-'tetrahydroxy-2,2'-binaphthalene-1,1',4,4'-tetrone
12	34.86	0.14	270	$C_{17}H_{34}O_2$	Pentadecanoic acid,14methyl,methylester
13	34.98	0.18	366	$C_{26}H_{54}$	Octadecane,3-ethyl-5-(2-ethylbutyl)
14	35.61	0.83	270	$C_{17}H_{34}O_2$	Hexadecanoicacid,methyl ester
15	36.94	0.19	550	$C_{40}H_{54}O$	Anhydrolutein II
16	37.73	**6.69**	**308**	**$C_{17}H_{24}O_5$**	**Methyl-2-(2-hydroxyethyl)-1-hexyl diester of phthalic acid**
17	38.71	0.19	404	$C_{25}H_{40}O_4$	Phthalic acid, ethyl pentadecyl ester
18	38.82	0.34	310	$C_{22}H_{46}$	Docosane
19	39.43	0.39	298	$C_{19}H_{38}O_2$	Heptadecanoic acid,16-methyl,methyl ester
20	40.62	0.81	324	$C_{23}H_{48}$	Heptadecane, 9-hexyl
21	41.63	0.49	402	$C_{20}H_{34}O_8$	Tributyl acetyl citrate
22	42.35	**1.32**	**324**	**$C_{23}H_{48}$**	**Tricosane**
23	44.01	**1.89**	**338**	**$C_{24}H_{50}$**	**Tetracosane**
24	45.60	**1.62**	**352**	**$C_{25}H_{52}$**	**Pentacosane**

25	46.38	**78.27**	**296**	**$C_{20}H_{40}O$**	**2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R[R*,R*(E)]]**
26	47.14	**1.14**	**618**	**$C_{44}H_{90}$**	**Tetratetracontane**
27	48.61	0.81	380	$C_{27}H_{56}$	Heptacosane
28	50.04	0.55	408	$C_{29}H_{60}$	Nonacosane
29	50.24	0.10	536	$C_{40}H_{56}$	Carotene
Total %		**92.29 %**			

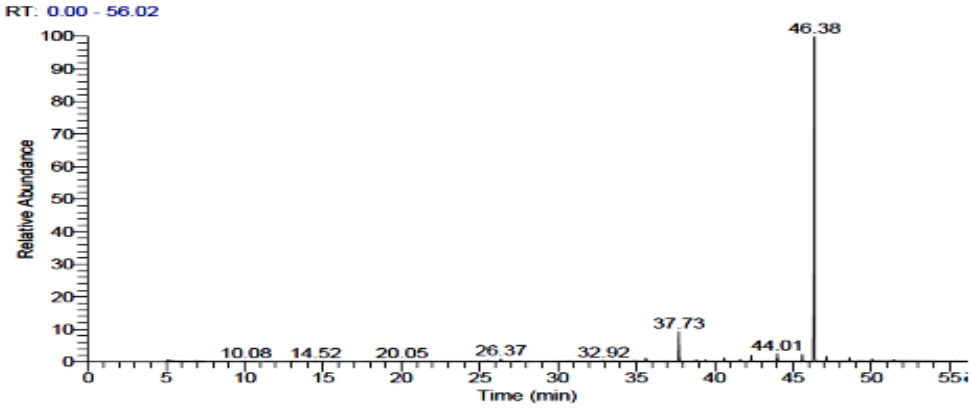

Figure 8: Gas chromatography-mass spectrometry (GC-MS) chromatogram of the ethyl acetate extract from *Trichoderma saturnisporum* MN1-EGY grown on rice straw

Table 3: Chemical compositions of the ethyl acetate extract from *Aspergillus niger*MN2-EGY grown on rice straw

No.	R_t.	Area %	M.W.	M.F.	Identified compounds[1]
1	15.84	0.57	646	$C_{40}H_{54}O_7$	(25R)3á(4'OBenzoyl2',3'didehydro2',3'dideoxyà,Lrhamnopyranosyl oxy)5àspirostan2one
2	16.37	0.63	184	$C_{11}H_{20}O_2$	Hexyl Tiglate (2-butenoic acid, 2-methyl-, hexyl ester)
3	23.41	0.67	648	$C_{44}H_{56}O_4$	5,11,17,23-Tetratbutyl-25,26,27,28-tetrahydroxycalix-4-arene
4	25.29	0.66	645	$C_{35}H_{51}NO_{10}$	8-*O*-Methyl-Falconerine
5	27.20	0.60	601	$C_{35}H_{53}O_8$	Tetronasin
6	42.29	85.74	282	$C_{17}H_{34}O_2$	*n*-Eicosane
7	45.91	0.68	442	$C_{28}H_{42}O_4$	Dimethyl1,1':4',1"-tercyclo[2.2.2]octane-4,4"-dicarboxylate
8	46.13	-	-	-	Unknown
9	46.45	1.17	294	$C_{19}H_{34}O_2$	13,16-Octadecadienoicacid, methyl ester
10	46.55	0.60	644	$C_{44}H_{32}N_6$	5,10-bis(3-aminophenyl)-15,20-diphenylporphyrin
11	46.85	0.94	616	$C_{44}H_{32}N_4$	2,3-Dihydro-5,10,15,20-tetraphenyl-22H,24H-prophyrin
12	49.95	1.79	339	$C_{20}H_{21}NO_4$	Dimethyl 2,c-5-diphenyl-r-2,c-4-pyrrolidinedicarboxylate
13	50.07	-	-	-	Unknown
14	52.21	0.76	484	$C_{27}H_{32}O_8$	Bis[1,2-(bis(methoxycarbonyl)-3,5,6-trimethylphenyl]methane
15	52.34	0.98	560	$C_{41}H_{40}N_2$	Diethyl{3-[2-(3,5,3",5"-tetramethyl[1,1';3',1"]terphenyl-5'-yl)naphthalene-1-yl]pyridine-4-yl}mine
16	54.55	-	-	-	Unknown
Total %		**95.79%**			

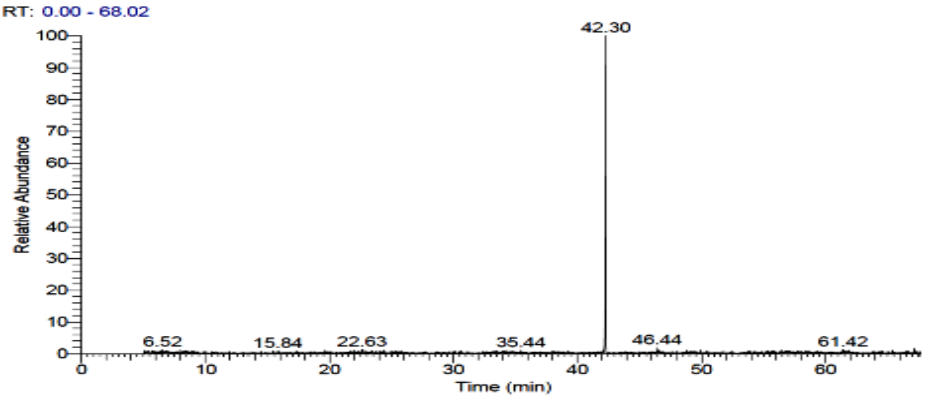

Figure 9: Gas chromatography-mass spectrometry (GC-MS) chromatogram of the ethyl acetate extract from *Aspergillus niger*MN2-EGY grown on rice straw

Table 4: Chemical compositions of the ethyl acetate extract of the mixed culture of *Trichoderma saturnisporum* MN1-EGY and *Aspergillus niger*MN2-EGY grown on rice straw

No.	R_t.	Area %	M.W.	M.F.	Identified compounds[1]
1	18.46	-	-	-	Unknown
2	26.43	2.34	166	$C_9H_{10}O_3$	Ethyl-vanillin
3	27.20	2.28	131	C_9H_9N	Skatole (1H-indole, 3-methyl)
4	27.60	2.13	208	$C_{12}H_{16}O_3$	*Cis*-Isoelemicin
5	28.08	2.50	594	$C_{36}H_{38}N_2O_6$	2,2'-bis-(Nor)-Guattaguianine
6	31.24	-	-	-	Unknown
7	31.72	2.19	512	$C_{38}H_{24}O_2$	5',5"-diphenyldibenzo[2.2]paracyclophane-1,9-diene-4',4"-dicarbaldehyde
8	33.07	2.58	262	$C_{16}H_{22}O_3$	*O*-methyl-*α*-Pipitzol
9	35.20	3.10	218	$C_{13}H_{14}O_3$	Eupatoriochromene (Ketone, 7-hydroxy-2,2-dimethyl-2H-1-benzopyran-6-yl-methyl)
10	38.69	-	-	-	Unknown
11	42.25	52.54	282	$C_{17}H_{34}O_2$	*n*-Eicosane
12	46.46	3.48	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester
13	48.29	2.09	594	$C_{44}H_{34}O_2$	Dimethoxy-para-Bis-[benzocycloheptenobenzocycloheptenо]benzene
14	49.49	2.81	556	$C_{36}H_{32}N_2O_4$	5,10-Dihydro-1,3,9,11-tetramethoxy-6,12-di(4'-methylphenyl)indolo[2,3 b]carbazole
15	51.52	3.01	466	$C_{31}H_{22}N_4O$	N-(6-Methylpyridin-2-yl)-4-(9-phenyl-1,10-phenanthrolin-2-yl)benzamide
16	59.31	2.11	640	$C_{42}H_{40}O_6$	15,11-metheno-11H-tribenzo[c,g,n][1,6]dioxacyclopentadecin-7 carboxaldehyde,22-ethoxy-19-(2-ethoxy-3-formyl-5-methylphenyl)-5,21-dihydro-9,13,17-trimethyl
Total %		**83.25 %**			

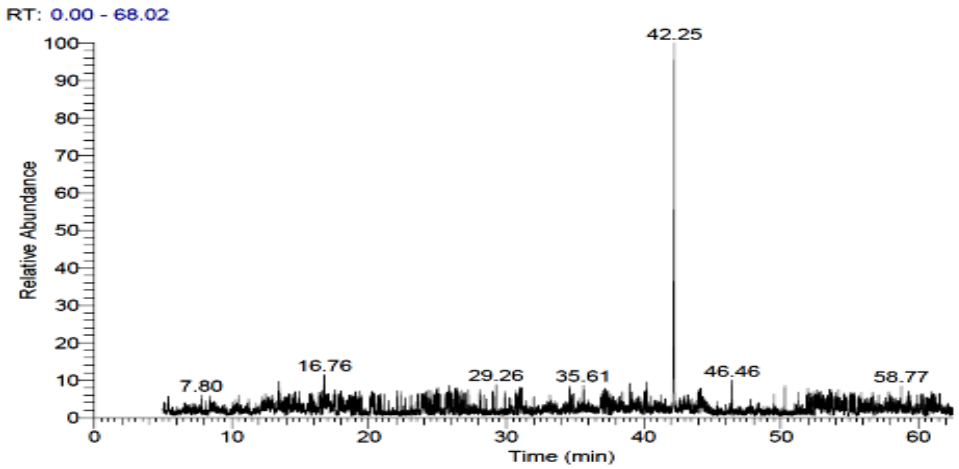

Figure 10: Gas chromatography-mass spectrometry (GC-MS) chromatogram of the ethyl acetate extract of the mixed culture of *Trichoderma saturnisporum* MN1-EGY and *Aspergillus niger*MN2-EGY grown on rice straw

4 Conclusion

Rice straw is considered as valuable agricultural residue that could be used for several interestingvalue added products. Manyinterestingapplications of rice straw inbioethanolproduction as well as the production of valuable antimicrobial compounds have been previously studied. In this study rice straw was used as sole carbon source for fungi in solid state fermentation. The ethyl acetate extract of the fermentation medium was subjected to different biological pretreatment studies. It has been found that the most extracts exhibited antimicrobial, antioxidant and total phenolic activities. GC/MS studies revealed that these extracts contain many interesting compounds. In future work, scale up fermentation will be done leading to the isolation of pure bioactive compounds.

References

1. Bidlack, J., Malone, M., Benson, R. (1992). Molecular structure and component integration of secondary cell walls in plants. *Proceedings of Oklahoma Academy of Science, 72,* 51-56.
2. Howard, R. L., Abotsi, E., Jansen van, R. E. L., Howard, S. (2003). Lignocellulose biotechnology, issues of bioconversion and enzyme production. *African Journal of Biotechnology, 2(12),* 602-619.
3. Fengel, D., Wegener, G. (1984). *Wood: chemistry, ultrastructure, reactions*. Walter de Gruyter, Berlin and New York.
4. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y. et al. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology, 96(6),* 673-686.
5. Sun, Y., Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production-A review. *Bioresource Technology, 83(1),* 1-11.
6. Mussatto, S. I., Aguilar, C. N., Rodrigues, L. R., Teixeira, J. A. (2009). Fructooligosaccharides and β-fructofuranosidase production by *Aspergillus japonicas* immobilized on lignocellulosic materials. *Journal of Molecular Catalysis B: Enzyme, 59,* 76-81.
7. Choung, M. G., Baek, I. Y., Kang, S. T., Han, W. Y., Shin, D. C. et al. (2001). Isolation and determination of anthocyanins in seed coats of black soybean (Glycine max (L.) Merr.). *Journal of Agriculture and Food Chemistry, 49,* 5848-5851.
8. Singh, H. B., Singh, B. N., Singh, S. P., Nautiyal, C. S. (2010). Solid-state cultivation of *Trichoderma harzianum* NBRI-1055 for modulating natural antioxidants in soybean seed matrix. *Bioresource Technology, 101,* 6444-6453.

9. Aguilar, C. N., Aguilera-Carbo, A., Robledo, A., Ventura, J., Belmares, R. et al. (2008). Production of antioxidant nutraceuticals by solid-state cultures of pomegranate (*Punica granatum*) peel and creosote bush (*Larrea tridentata*) leaves. *Food Technology and Biotechnol*ogy*, 46,* 218-222.
10. Pandey, A., Soccol, C. R., Mitchell, D. (2000). New developments in solid state fermentation, I-bioprocesses and products. *Process Biochemistry, 35(10),* 1153-1169.
11. Akyuz, M., Kirbag, S. (2009). Antimicrobial activity of *Pleurotus eryngii* var. ferulae grown on various agro-wastes*. EurAsia Journal BioSciences, 3(8),* 58-63.
12. Yilmaz, A., Yildiz, S., Tabbouche, S., Kilic, A. O., Can, Z. (2016). Total phenolic content, antioxidant and antimicrobial properties of *Pleurotus ostreatus* grown on lime (*Tilia tomentosa*) leaves. *Journal of Biological Chemistry, 44(2),* 119-124.
13. Nagah, M., Abdel-Aziz, M. S., El-Sherbiny, G. M., Saad, A. M., Moghannem, S. A. M. et al. (2016). Qualitative and quantitative screening of cellulases from different local Egyptian fungal Strains. *Middle East Journal of Applied Sciences, 6(3),* 579-587.
14. Marwah, R. G., Fatope, M. O., Mahrooq, R. A., Varma, G. B., Abadi, H. A. et al. (2007). Antioxidant capacity of some edible and wound healing plants in Oman. *Food Chemistry, 101(2),* 465-470.
15. Ghareeb, M. A., Shoeb, H. A., Madkour, M. F., Refaey, L. A. et al. (2014) Antioxidant and cytotoxic activities of *Tectona grandis* Linn leaves. *Intenational Journal of Phytopharmacology, 5(2),* 143-157.
16. Ghareeb, M. A., Shoeb, H. A., Madkour, M. F., Refaey, L. A., Mohamed, M. A. (2014). Antioxidant and cytotoxic activities of flavonoidal compounds from *Gmelina arborea* (Roxb.). *Global Journal of Pharmacology, 8(1),* 87-97.
17. Ainsworth, E., Gillespie, K. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocol*s*, 2(4),* 875-877.
18. El-Sayed, M. M., Maher, M. E., Eman, A. E., Ghareeb, M. A. (2009). Total phenolic contents and antioxidant activities of *Ficus sycomorus* and *Azadirachta indica*. *Pharmacology Online, 3,* 590-602.
19. Ghareeb, M. A., Shoeb, H. A., Madkour, H. M. F., Refahy, L. A., Mohamed, M. A. et al. (2013). Radical scavenging potential and cytotoxic activity of phenolic compounds from *Tectona grandis* (Linn.). *Global Journal of Pharmacology, 7(4),* 486-497.
20. Shoeb, H. A., Madkour, H. M. F., Refahy, L. A., Mohamed, M. A., Saad, A. M. et al. (2014). Antioxidant and cytotoxic activities of *Gmelina arborea* ROXB. leaves. *British Journal of Pharmaceutical Research, 4(1),* 125-144.
21. Collins, C. H., Lyne, P. M. (1984). *Microbiological Methods* (5th ed.) Butterworths Co., Ltd., London.
22. Abdel-Aziz, M. S., Abou-El-Sherbini, K. S., Hamzawy, E. M. A., Amr, M. H. A., El-Dafrawy, S. (2015). Green synthesis of silver nano-particles by *Macrococcus bovicus* and its immobilization onto montmorillonite clay for antimicrobial functionality. *Applied Biochemistry and Biotechnology, 176(8),* 2225-2241.
23. Ghareeb, M. A., Hamed, M. M., Saad, A. M., Abdel-Aziz, M. S., Hamed, A. A. et al. (2019). Bioactive secondary metabolites from the locally isolated terrestrial fungus. Penicillium sp. SAM16-EGY. *Pharmacognosy Research, 11,* 162-170.
24. Abdel-Aziz, M. S., Ghareeb, M. A., Saad, A. M., Refahy, L. A., Hamed, A. A. (2018). Chromatographic isolation and structural elucidation of secondary metabolites from the soil-inhabiting fungus *Aspergillus fumigatus* 3T-EGY. *Acta Chromatographica, 30(4),* 243-249.
25. Adams, R. P. (2001). *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy.* Allured Publishing, Carol Stream, IL, USA.
26. Ghareeb, M. A., Refahy, L. A., Saad, A. M., Ahmed, W. S. (2016). Chemical composition, antioxidant and anticancer activities of the essential oil from *Eucalyptus citriodora* (Hook.) leaves. *Der Pharma Chemica, 8(1),* 192-200.
27. Madkour, H. M. F., Ghareeb, M. A., Abdel-Aziz, M. S., Khalaf, O. M., Saad, A. M. et al. (2017). Gas chromatography-mass spectrometry analysis, antimicrobial, anticancer and antioxidant activities of *n*-hexane and methylene chloride extracts from *Senna italica*. *Journal of Applied Pharmaceutical Science, 7(6),* 23-32.
28. Mossalem, H. S., Ghareeb, M. A., Refahy, L. A., Mohamed, A. S., Habib, M. R. (2017). Gas chromatography-mass spectrometry analysis and antioxidant activity of *Punica granatum* L. peels and its role as immunostimulant against *Schistosoma mansoni* infection in *Biomphalaria alexandrina*. *Asian Journal of Pharmaceutical and Clinical Research, 10(1),* 1-7.

29. Saad, A. M., Ghareeb, M. A., Abdel-Aziz, M. S., Madkour, H. M. F., Khalaf, O. M. et al. (2017). Chemical constituents and biological activities of different solvent extracts of *Prosopis farcta* growing in Egypt. *Journal Pharmacognosy Phytotherapy, 9(5),* 67-76.
30. Raper, K. B., Fennell, D. I. (1965). *The Genus Aspergillus*, pp. 686. Baltimore, Williams & Wilkins Company.
31. Zhao, J., Kong, F., Li, R., Wan, X,, Wang, D. (2001). Identification of *Aspergillus fumigatus* and related species by nested PCR targeting ribosomal DNA internal transcribed spacer regions. *Journal of Clinical Microbiology*, *39(6),* 2261-2266.
32. Chen, Y., Eisner, J. D., Kattar, M., Rassoulian-Barrett, S. L., Lafe, K. et al. (2001). Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. *Journal of Clinical Microbiology, 39(11),* 4042-4051.
33. White, N. A., Dehal, P. K., Duncan, J. M., Williams, N. A., Gartland, J. S. et al. (2001). Molecular analysis of intraspecific variation between building and "wild" isolates of *Serpula lacrymans* and their relatedness to *S. himantioides*. *Mycology Research, 105,* 447-452.
34. Rathnan, R. K., Nair, P., Balasaravauan, T. (2012). Isolation, identification and characterization of efficient cellulolytic fungi from natural resources. *International Journal of Microbial Resource Technology, 1(4),* 379-387.
35. Alvarez-Navarrete, M., Reyna-López, G. E., Flores-García, A., López-Gómez, R., Martínez-Pacheco, M. M. (2015). Selection and molecular identification of fungal isolates that produce xylanolytic enzymes. *Genetics and Molecular Research, 14(3),* 8100-8116.
36. Goldbeck, R. (2012). Screening and identification of cellulase producing yeast-like microorganisms from Brazilian biomes. *African Journal of Biotechnology, 11(53),* 11595-11603.
37. Haque, M. A., Hossain, M. S., Rahman, M. Z., Rahman, M. R. et al. (2005). Isolation of bioactive secondary metabolites from the endophytic fungus of 0cimum basilicum. *Dhaka University Journal of Pharmaceutical Sciences, 4(2),* 127-130.
38. Astuti, P., Wahyono, Nababan, O. A. (2014). Antintimicrobial and cytotoxic activities of endophytic fungi isolated from Piper crocatum Ruiz & Pav. Asian *Pacific Journal of Tropical Biomedicine, 4(Suppl 2),* 592-596.
39. Nigam, P. S. (2009). Production of bioactive secondary metabolites. *Biotechnology for Agro-Industrial Residues Utilisation, Utilisation of Agro-Residues,* 129-145.
40. Nigam, P. S., Gupta, N., Anthwal, A. (2009). *Pre-treatment of agro-industrial residues. Biotechnology of agro-industrial residue utilization13-33.* Springer.
41. Lopes, F. C., Tichota, D. M., Sauter, I. P., Meira, S. M. M., Segalin, J. et al. (2013). Active metabolites produced by *Penicillium chrysogenum* IFL1 growing on agro-industrial residues. *Annales of Microbiology, 63(2),* 771-778.
42. Sajjad-Ur-Rahman, S., Rasool, M. H., Rafi, M. (2012). Penicillin production by wild isolates of *Penicillium chrysogenum* in Pakistan. *Brazilian Journal of Microbiology, 43(2),* 476-481.
43. Vastrad, B. M., Neelagund, S. E. (2011). Optimization and production of neomycin from different agro-Industrial wastes in solid state fermentation. *International Journal of Pharmaceutical Sciences and Drug Research, 3(2),* 104-111.
44. Arbaayah, H., Umi Kalsom, Y. (2013). Antioxidant properties in the oyster mushrooms (*Pleurotus* spp.) and split gill mushroom (*Schizophyllum commune*) ethanolic extracts. *Mycosphere, 4(4),* 661-673.
45. Jayakumar, T., Thomas, P. A., Geraldine, P. (2009). *In-vitro* antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*. *Innovative Food Science Emerging Technologies, 10(2),* 228-234.
46. Wong, K. H., Naidu, M., David, R. P., Abdulla, M. A., Kuppusamy, U. R. (2009). 0Functional recovery enhancement following injury to rodent peroneal nerve by lion's mane mushroom, *Hericium erinaceus* (Bull., Fr.) Pers. (Aphyllophoromycetideae). *International Journal of Medicinal Mushrooms, 11,* 225-236.
47. Gogavekar, S. S., Rokade, S. A., Ranveer, R. C., Ghosh, J. S., Kalyani, D. C. (2014). Important nutritional constituents, flavour components, antioxidant and antibacterial properties of *Pleurotus sajor-caju. Journal of Food Science and Technology, 51(8),* 1483-1491.
48. Wong, J. Y., Chye, F. Y. (2009). Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis, 22(4),* 269-277.
49. Panjwani, R., Ashish, S., Pandey, A. K. (2015). Antioxidative property of *Pleurotus florida*, an edible oyster mushroom in M.P. *World Journal of Pharmaceutical Sciences, 5(1),* 1383-1392.

50. Yadav, M., Yadav, A., Yadav, J. P. (2014). *In vitro* antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. *Asian Pacific Journal of Tropical Medicine, 7(S1),* 256-261.
51. Kandasamy, S., Kandasamy, K., Saravanakumar, K. (2014). Antioxident activity of the mangrove endophytic fungus (*Trichoderma* sp.). *Journal of Coastal Life Medicine, 2(7),* 566-570.
52. Ruma, K., Sunil, K., Prakash, H. S. (2013). Antioxidant, anti-inflammatory, antimicrobial and cytotoxic properties of fungal endophytes from *Garcinia* species. *International Journal Pharmcy Pharmaceutical Sciences, 5(3),* 889-897.
53. Kaur, G., Kalia, A., Sodhi, H. S. (2015). Antioxidant potential and anti-bacterial activities of *Pleurotus* extracts. *Indian Journal of Fundamental and Applied Life Sciences, 5(1),* 1-6.
54. Nattoh, G., Musieba, F., Gatebe, E., Mathara, J. (2016). Towards profiling differential distribution of bioactive molecules across four phenologies in *Pleurotus djamor* R22. *Asian Pacific Journal of Tropical Disease, 6(6),* 472-480.
55. Mohamed, I. M., Mohamed, M. R. M., Abdul, B. J., Asarudeen, A. (2011). Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. *International Food Research Journal, 18,* 579-582.
56. Yang, J. H., Lin, H. C., Mau, J. L. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry, 77(2),* 229-235.
57. Ahsan, T., Chen, J., Zhao, X., Irfan, M., Wu, Y. (2017). Extraction and identification of bioactive compounds (Eicosane and Dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. *AMB Express, 7(54),* 1-9.
58. Gopinath, S., Sakthidevi, G., Muthukumaraswamy, S., Mohan, V. R. (2013). GC-MS analysis of bioactive constituents of *Hypericum mysorense* (Hypericaceae). *Journal of Current Chemical and Pharmaceutical Sciences, 3(1),* 6-15.
59. Asghar, S. F., Habib-ur-Rehman, Choudahry, M. I., Atta-ur-Rahman (2011). Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bio-assays of crude extract of *Iris germanica*. *International journal of Genetics and Molecular Biology, 3(7),* 95-100.