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Exopolysaccharides Production and Characterization from Marine-Derived *Penicillium commune* KP942881.1 with Some Medical Potential Applications

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SEVEN common marine-derived fungi were isolated from different collection sites distributed in Eastern and Western harbor of Alexandria, Egypt. The most promising marine-derived fungusproducing exopolysaccharide (EPS) was *Penicillium commune* (KP942881.1) which was identified according to microscopic morphological features and confirmed genetically by 18S rRNA gene. The results of the optimizing conditions for EPS production from marine-derived *P. commune* showed that 40 mg/ml of sucrose, 20 mg/ml of peptone, pH 5 and 3 cm discs of inoculum size and incubation at 30ºC, for 9 days were the optimal conditions with using static condition for all factors. Three main spectroscopic analyses (FTIR, ^{1}H NMR and HPLC) were employed to characterize the EPS extracted from marine-derived *P. commune*.^{1}HNMR analysis of EPS exhibited the presence of β-galatopyranosyl. The HPLC chromatography showed that the EPS consist of two peaks; raffinose and rhamnose. EPS showed antiproliferative activity at dose 10 mg/ml where the percentage inhibition of tumor viability cells of colon was 85%. In breast cell (Mcf-7), EPS inhibited 87% of the tumor cells at dose 10 mg/ml and also the antiviral activity of EPS of *P. commune* exhibited 22.8% inhibition.

Keyword: Marine-Derived *Penicillium commune*, Exopolysaccharides, Characterization, Anti-tumor, Antiviral.

Introduction

There are several groups of microorganisms producing exopolysaccharides (EPSs) with different properties and applications, such as antitumor agents (Chen et al., 2008 and Peng et al., 2003), food packaging materials (Mc Neely et al., 1973), and wound dressing materials (Madla et al., 2005 and Methacanon et al., 2008). In particular, EPS produced by fungi to protect their cells against desiccation, phagocytosis and phage attack, toxic compounds, predation by protozoans, and osmotic stress, as well as in cellular recognition (Vuyst et al., 1999). Marine fungi have proved to be a rich source of new biological natural products. Because of their characteristic properties with reference to temperature, nutrients, competition and salinity, they have developed specific secondary metabolic pathways compared with terrestrial fungi (Liberra & Lindequist, 1995). Marine filamentous fungi have proved to possess tremendous source of new medicines even at low concentrations of their secondary metabolites, as some recent studies prove too (Swathi et al., 2013 and Sharma & Gautam, 2017). Fungal EPSs have been recognized as high value bio-macromolecules for the last two decades.

The purpose of the present researchwas to screening marine EPS producing fungi and evaluates the growth factors that might increase the productivity of EPS. Also characterization of EPS by using HPLC, IR and NMR. Antiviral andanti-tumor activities were analyzed *in vitro*

Materials and Methods

Sample source

Marine water samples were collected from eastern and western port of Alexandria. The water samples were collected in sterile tight bottles and transferred to the laboratory in 24 h of duration.

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Isolation of fungi

The water sample is diluted with different dilution rates. An equal proportion of volume (v/v) is spread on Potato Dextrose agar medium and was incubated for 34- days at 25°C.

Microscopic studies

The selected strain with full loop is placed at the center of Sabouraud dextrose agar and incubated to obtain colony for morphological identification and were identified microscopically according to the keys of Moubasher (1993).

Molecular identification of selected fungi

The study of small subunits of ribosomal RNA has revolutionized the classification of microorganisms, both bacteria and fungi. These techniques, based on the PCR amplification of genes coding for rRNAs and sequence comparison. Rapid identification of filamentous fungi was reached using two specific PCR primers sets (Pedersen et al., 1997 and Turenne et al., 1999).

Firstly, DNA was extracted by use protocol of GeneJet Plant genomic DNA purification Kit (Thermo):K0791. Then PCR was run by using Maxima Hot Start PCR Master Mix (Thermo): K0221. The PCR was cleaned up to the PCR product using GeneJET™ PCR Purification Kit (Thermo): K0701. Finally, sequencing to the PCR product on GATC Company was made by use ABI 3730xl DNA sequencer by using forward and reverse primers.

Physiological factors affecting on EPSs production by P. commune

Different experiments were made to select the most favorable conditions for high production of EPSs dry weight.

Effect of shaking/staticcultures

It was made by using 20g/L glucose and 10 g/L peptone liquid media using static and shaked conditions. The medium was adjusted at initial pH 6. The sterilized flasks were inoculated with fungal disc with 1cm in diameter. After inoculation, the media was incubated at 25°C for 5 days in static and rotary shaker at 90 and120 rpm.

Effect of different media under static condition

Four different media; potato dextrose medium, malt medium, glucose peptone medium, Capek's medium have been used. They were inoculated with 1 cm fungal disc and then incubated at 25°C in static incubator for 5 days.

Effect of different temperature degrees

Different temperature degrees (20, 25, 30, 35, and 37°C)were tested for 5 days at pH 6 **(**Elshamy & Nehad, 2010).

Effect of different pH values

The prepared liquid glucose peptone media was adjusted at different initial pH values (4, 5, 6, 7, and 8) by using HCL and NaOH to adjust each of pH values for selecting the optimum pH value for high production of EPSs from *P. commune* (Elshamy & Nehad, 2010).

Effect of different incubation periods

In order to select the optimum incubation period for high production of EPSs from *P. commune,* five incubation periods (3, 5, 7, 9 and 11 days) were carried out (Elshamy & Nehad, 2010).

Effect of different carbon sources

In this experiment, the glucose peptone media will be replaced with equimolecular weight concentrations of the following different carbon sources (sucrose, fructose, maltose, CMC and starch). The initial pH was adjusted at 5. After sterilization, the different flasks were inoculated with fungal disc1cm in diameter. Incubate the inoculated flasks at 30°C for 9 days in incubator.

Effect of different nitrogen sources

Peptone nitrogen source of sucrose peptone medium for EPS was replaced with different nitrogen sources (Yeast extract + peptone, sodium nitrite, sodium nitrate, gelatin, and ammonium chloride) at equimolecular weight. The flasks were inoculated with one disc and then were incubated for 9 days at 30°C.

Effect of different inoculum size

The glucose peptone media flasks were adjusted at initial pH 5 for EPSs production. The media were inoculated with different inoculum numbers (one, two, three and four discs) with 1cm diameter size. The inoculated media were incubated at 30°C for 9 days.

Extraction of EPSs

The culture filtrate was separated from the mycelia biomass followed by adding 5% Trichoroacetic acid (TCA) for removing protein (Khalil, 2002) and stored at freezer overnight. The supernatant was mixed with two volumes of 95% of isopropanol, stirred vigorously overnight at 4°C. The resultant precipitate was recovered by centrifugation at 3000 rpm for 20 min (Wu et al., 2008).

Measurement of carbohydrate content

The content of EPS was determined by phenol-sulfuric colorimetric method (Dubois et al., 1956) using glucose as standard and measured spectrophotometrically at 490 nm.

Purification of EPSs

Crude EPS were partially purified by dialysis membrane (Berg et al., 2007).

Characterization of EPSs produced by P. commune

FT-IRanalysis

Fourier transform-infrared (FT-IR) spectroscopy (FT/IR-4100, Japan) was employed using the KBr disc for the analysis and detecting of functional groups (Shen et al., 2013).

NMR analysis

The ^{1}H nuclear magnetic resonance (NMR) spectra of exopolysaccharides in DMSO were obtained with 300 MHz Bruker NMR Spectrometer (Central Lab, Faculty of science, Alex University, Egypt) (Kogan et al., 2002).

HPLC analysis

Exopolysaccharides were hydrolyzed following the method of Chen *et al*. (2005). Analysis of the carbohydrate in the filtrate was performed by using HPLC, Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID-10A Shimadzu detector.

Antitumor activity of EPS from *P. commune*

The human colon (Caco-2) and breast cancer (MCF-7)were kindly provided by the Regional Center for Mycology & Biotechnology at Al-Azhar University Cairo Egypt. They grow on RPMI -1640 medium supplemented with 5% heated Foetal Bovine Serum (FBS), 2mM glutamine and antibiotics (penicillin 100U/ml, streptomycin 100µg/ml), at 37°C in humified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/ml for human colon (Caco-2) andbreast cancer (MCF-*7)*10.75×104 cells/ml followed by 24 h of incubation (Monks et al., 1991). The protein-binding dye sulforhodamineB was used to estimated cells growth. The bound stains were solubilized and the absorbances were measured at 492 nm in plate reader. For each tested compound and cell lines, a dose response curve were obtained and the minimum inhibitory concentration (IC50) cell were calculated as described.

Antiviral activity of EPS from P. commune

Vero cells (derived from the kidney of normal African green monkey) were obtained from American Type Culture Collection (ATCC) and kindly provided by the Regional Center for Mycology & Biotechnology at Al-Azhar University Cairo, Egypt**.** Continuous cell line was established by Yasumura & Kawakita (1963). Vero cells were grown as monolayer in media supplemented with 10% inactivated fetal bovine serumand the monolayers of (10,000) cells were plated (10^4 cells/ well) in 96-well tissue culture plate and incubated for 24 h at 37°C in a humidified incubator with 5% CO_2 before treatment with the extracts. Different concentrations of Endotoxin (10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 mg/ml) were added to the cell monolayer and the plates were incubated for 48h into CO_2 incubator at 37°C and 5% CO_2**.** Fifty microliters of MTT reagent was added to each welland after addition of MTT reagent the plates were incubated in dark for 4 h for the reduction of MTT to form azan**.** One hundred microliters of DMSO was added to each well to solubilize the purple crystals of formazan and absorbance was measured at (570 nm). The percentage of cell survival was calculated by the following equation:

$$\text{Survival rate \%} = \frac{A\ \text{sample} - A_b}{A_c - A_b} \times 100$$

A_c=Negative control

A_b=Blank

Results and Discussion

Isolation of marine-derived fungi

Seven common marine-derived fungi were isolated from different collection sites distributed in Eastern and Western harbor of Alexandria, Egypt. They were coded as; TE1, TE2, TE3, TW1, TW2, TW3, and TW4. They were sub-cultured on potato dextrose medium, and then the isolated colonies were grown on potato dextrose agar plates at 25°C and incubated for 5 days. After that time, the plates were maintained at 4°C until used. However, they were transferred once every two weeks to maintain availability and stability for extracellular polysaccharides production. It was found that fungus TE2 is the most promising one for EPS production and mycelial growth.

Microscopic investigation for the most promising marine-derived fungus (TE2)

This fungus was identified at Faculty of science, Botany Department; Asyut University and was identified according to the keys of Moubasher (1993). Based on the mycelium and spore morphology studies, this isolate was identified as; *Penicillium commune* and confirmed by 18S rRNA gene.

Genotypic identification of the most promising marine-derived fungus (TE2)

The most promising marine-derived fungus TE2 which identified previously by microscopic investigation as; *Penicillium commune* was subjected, moreover, to genotypic identification by 18S rRNA gene. The molecular characterization confirmed the same obtained identification and the results are shown in Table 1 after multiple sequence alignment between the obtained sequences. Sequencing data were aligned against the 18S rRNA sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi). It has been found that the fungal isolates TE2 had a 99% identical counterpart with respect to its 18S rRNA sequence and take the accession no. KP942881.1. The most closely related species and the percentages of identity are presented in Table 1.

TABLE 1. The most closely related species and their percentages of identity.

Description	Max score	Total score	Query cover	E value	Identity (%)	Accession no.
Penicillium commune EECC-651	839	839	86%	0.0	99	KP942881.1
Eupenicillium sp. CS-P/F09	758	758	84%	0.0	97	KF528000.1
Penicillium expansum isolate 2534	749	749	88%	0.0	95	FJ008994.1
Penicillium sp. DY31-W1304-MS15	715	715	86%	0.0	95	KM274132.1
Penicillium sp. 386	647	647	89%	0.0	91	FJ008987.1
Penicillium expansum strain Fi-7	630	630	83%	6e-180	92	GU004268.1
Penicillium sp. PSF24	466	466	81%	2e-130	86	HQ850347.1
Penicillium paneum JCM 28412	438	438	81%	4e-122	85	LC133845.1
Penicillium paneum JCM 28498	359	359	73%	3e-98	83	LC133871.1
Penicillium chrysogenum	350	350	80%	2e-95	82	KT963794.1

Physiological factors affecting EPS production from marine-derived P. commune KP942881.1

Eight factors (static and shaking conditions, media,temperatures, pH level, incubation period, carbon sources, nitrogen sources and inoculum size) were examined independently on the EPS production from marine-derived *P. commune*.

Effect of static and shaking conditions

The effect of static and shaking conditions (90 and 120 rpm) on the mycelial growth and EPS production of *P. commune* KP942881.1 was investigated. From the data presented in Fig. 1, static condition was the suitable condition for high mycelial growth production and EPS of *P. commune* KP942881.1. The EPS concentration in static condition was 1.250.134± mg/ml and mycelial dry weight was 0.3290.014± g/100 ml. So, static condition was selected as the most suitable condition for EPS production in the subsequent experiments. This result agreed with Rupérez et al. (1984) who precipitated an extracellular polysaccharide from *Penicillium allahabadense* statically in liquid medium and Chen et al. (2013 a) who produced EPS from *Penicillium griseofulvum* statically.

Effect of different media under static condition

Potato dextrose, malt, glucose peptone and Capek's media were used to investigate the EPSs production by *P.commune* and initial pH was 6 at constant incubation temperature of 25°C (Fig. 2). The maximum EPS production was achieved in GP medium after incubation of 5 days in static incubator which produced 0.3420.008± g/100 ml of mycelial dry weight and 1.660.155± mg/ml EPS. This result agreed with Peiqin et al., (2012), who reportd that glucose peptone media with different concentration increased exopolysaccharide production of *Berkleasmium* sp.

Effect of different temperatures

The data in Fig 3, it is evident that temperature 30°C was the optimum for EPSs production and mycelial growth from *P. commune.* The EPS concentration was 1.42 ± 0.707 mg/ml under static condition. Mycelial dry weight was 0.378± 0.04 g/100 ml. Elshamy & Nehad (2010) proved that 30°C was suitable for EPS production by *Alternaria alternata* and Chen et al. (2013b) reported that 28°C was suitable for EPS production by *Penicillium commune*.

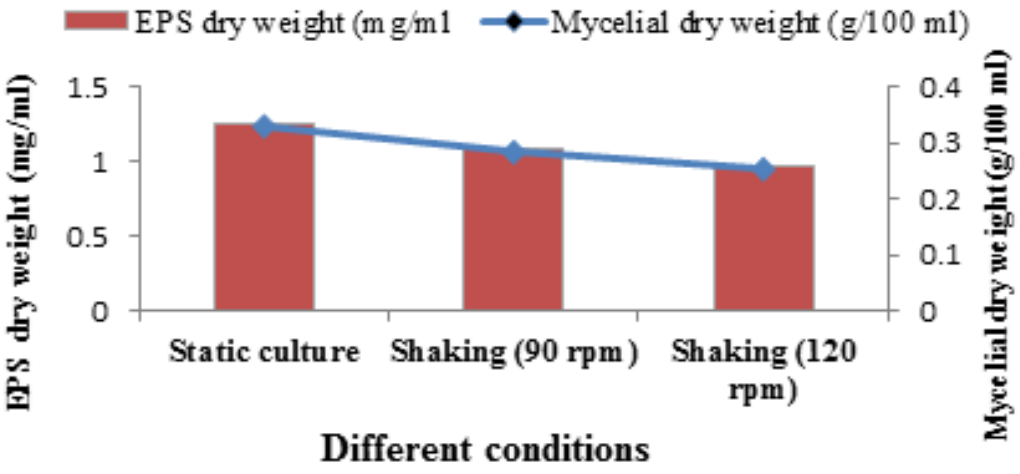

Fig. 1. Effect of static/shaking conditions on mycelial growth and EPS production by *P. commune*

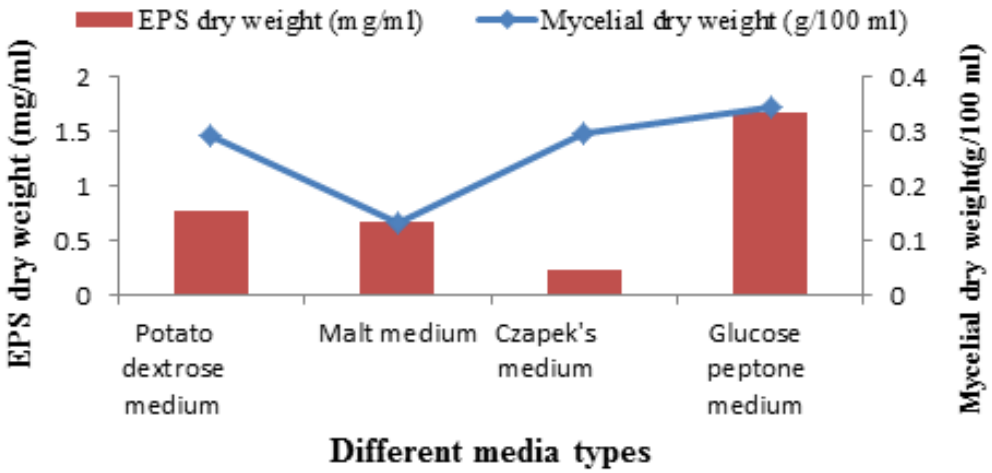

Fig. 2. Effect of different media on mycelial growth and EPS production of *P. commune* under static condition

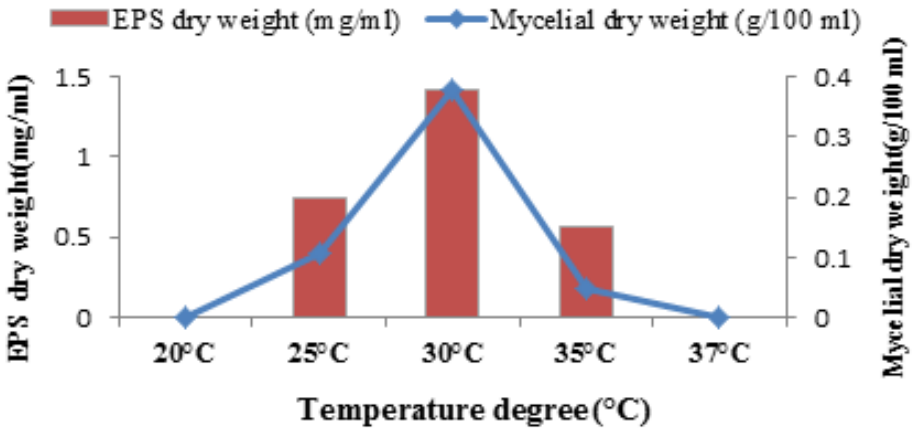

Fig 3. Effect of different temperature on mycelial growth and EPS production of*P. commune* under static condition

Effect of different pH values

It is evident that pH 5 was the most promising for EPSs production and mycelial growth of *P. commune*. It was clear that there was a gradual decrease in the fungal growth until reaching pH 8 (Fig.4). Therefore, pH 5 was applied for the other experiments. EPS concentration was 1.580.56± mg/ml under static condition at 30ºC. Mycelial dry weight was 0.3710.028± g/100 ml.NourELdein et al. (2004) reported that pH 5 was optimum for EPS production from *Pleurotus pulmonarius*. Also, Rong et al. (2010) found that optimum pH for EPS by *Hirsultella pat*was 5.5

Effect of different incubation period

The incubation for 9 days was the most promising for EPSs production and mycelial growth from *P. commune*. It was clear that there was a gradual increase in the fungal growth from 3 to 9 days (Fig. 5). So, incubation for 9 days was applied for the other experiments.

The EPS concentration was 1.720.028± mg/ml under static condition at 30ºC. Mycelial dry weight was 0.412 ±0.0007 g/100ml and this was consistent with Elshamy & Nehad (2010) for *Alterneria alternata*. Chen et al. (2013b) reported that 10 days was suitable for EPS production by *Penicillium commune*.

Effect of different carbon sources

The Data in Fig.6 showed that the most suitable carbon source was sucrose. The EPS concentration in sucrose was 1.740.03± mg/ml and mycelial dry weight was 0.4020.003± g/100 ml. It was reported that sucrose the most suitable carbon source for mycelium growth production in *Cordyceps militaris* **(**Park et al., 2001). Yadav et al. (2014**)** reported that sucrose was the best for exopolysaccharides production by *Aureobasidium sp*.

Effect of different nitrogen sources

Different nitrogen sources have performed effect on EPS and biomass production. From Fig.7, it is evident that, EPS concentration using peptone as nitrogen source was 1.810± mg/ml and fungal growth was 0.3950± g/100 ml. The same conclusions were detected by Fraga et al. (2014) has stated that a higher supply of peptone (4.80 gL^{-1}) is needed for maximum EPS production. The highest level of EPS was obtained when peptone was used as a sole nitrogen source by *Hirsutella sp*. (Rong et al., 2010).

Effect of different inoculum size

It was obvious that three disc sized 1cm in diameter from *P. commune* gave high EPS dry weight and high mycelial dry weight. Cultivation of three fungal discs with diameter 1cm from tested organism on GP media at static condition for 9 days gave high quantity of EPS(1.89 ± 0 mg/ml) and dry mycelial (0.420 ± 0 g/100 ml) (Fig. 8). Bae et al. (2000) and Yun et al. (2003) found that inoculum size (5mm) of *Cordyceps militaris* was the most favourable for exopolysaccharide production. Osman et al. (2014) observed that increasing the inoculum size led to a significant increase in growth and polysaccharides production.

Purification of EPSs

It is used only for purification of exopolysaccharide from impurities to complete characterization and application.

Characterization of EPS obtained from marine-derived P. commune

Three main spectroscopic analyses (FTIR, ^{1}HNMR and HPLC) were employed to characterize the EPS obtained from marine-derived *P. commune*.

FT-IR analysis of EPS

The EPS obtained from *P. commune* was subjected to IR spectroscopy and the FTIR spectra of the EPS exhibited bands at various levels (Fig. 9). Data obtained revealed that adominant absorption that is often attributed to O-H stretching vibration at 3361.74 of O-H in carboxylic acid which is accompanied with the bands at 2923.88 cm^{-1} corresponds to H stretching in carboxylic group. The band at 1740.04 cm^{-1} approves the stretching vibration of C=O carbonyl group of an aldehyde or ketone. The peak at 2933.2 cm^{-1} identifies the vibration stretching of alkyl hydrogen (CH_2-CH_3) in aliphatic alkyl group (R-CH_2-CH_3). The obvious absorption peaks at 825 cm^{-1} revealed the existence of β-galatopyranosyl linked IR spectrum of polysaccharide units as shown in Figure. This result agreed with Sharmila et al. (2014) that the absorption peaks at 825 cm^{-1} revealed the existence of β-galatopyranosyl of *Syncephalastrum* sp.

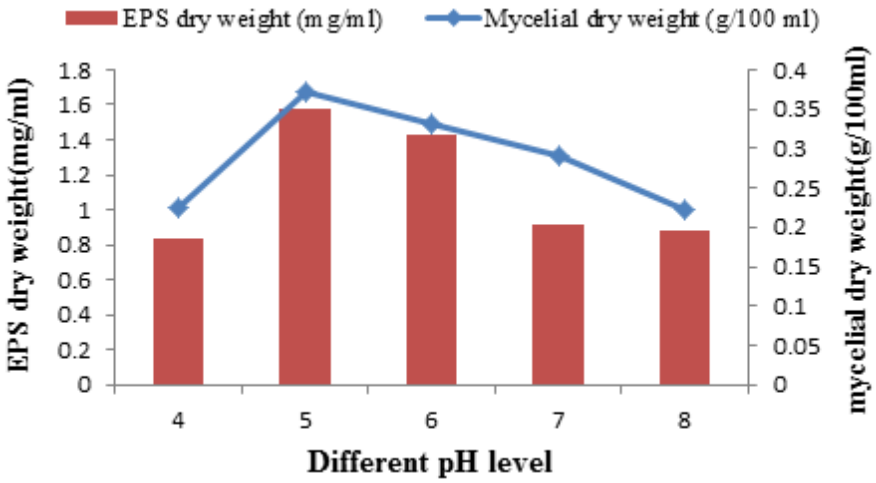

Fig. 4. Effect of different pH level on mycelial growth and EPS production of *P. commune* under static condition

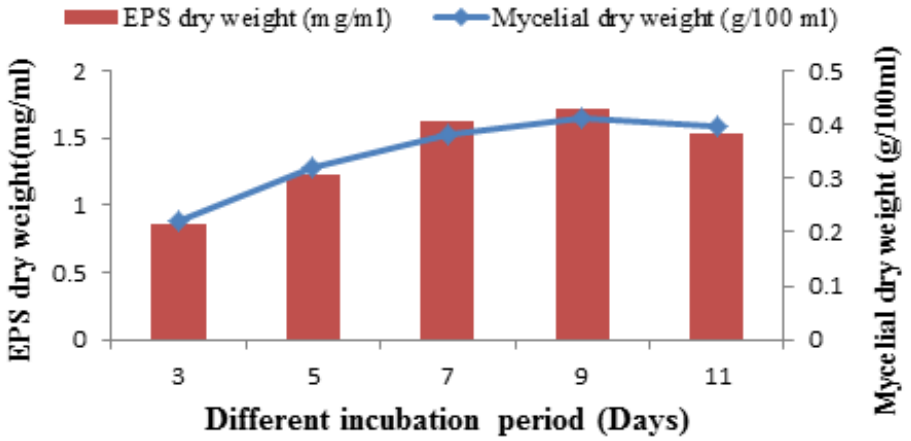

Fig. 5. Effect of different incubation periods onmycelial growth and EPS production of*P. commune* under static condition.

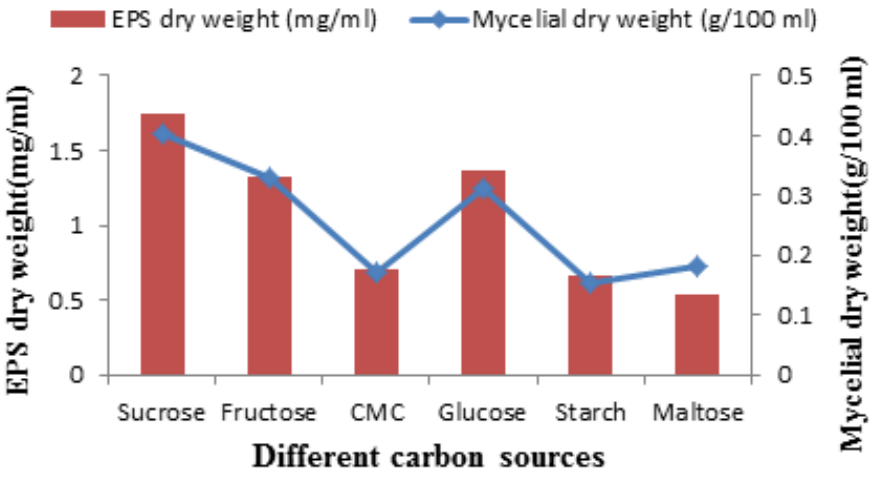

Fig. 6. Effect of different carbon source on mycelial growth and EPS produced by *P. commune* under static condition

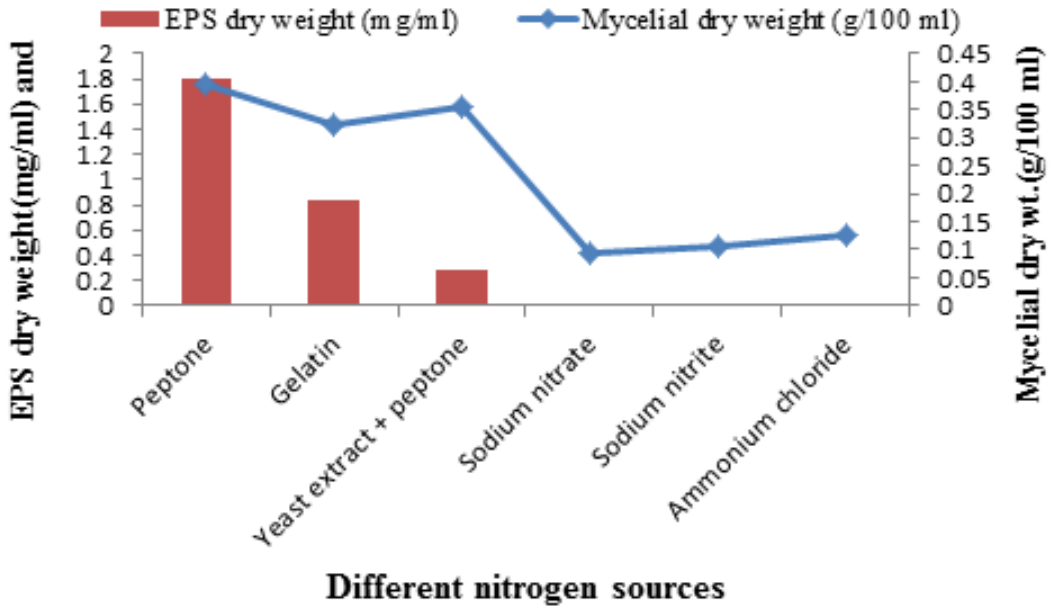

Fig. 7. Effect of different nitrogen source on mycelial growth and EPS production by *P. commune* TE2 under static condition

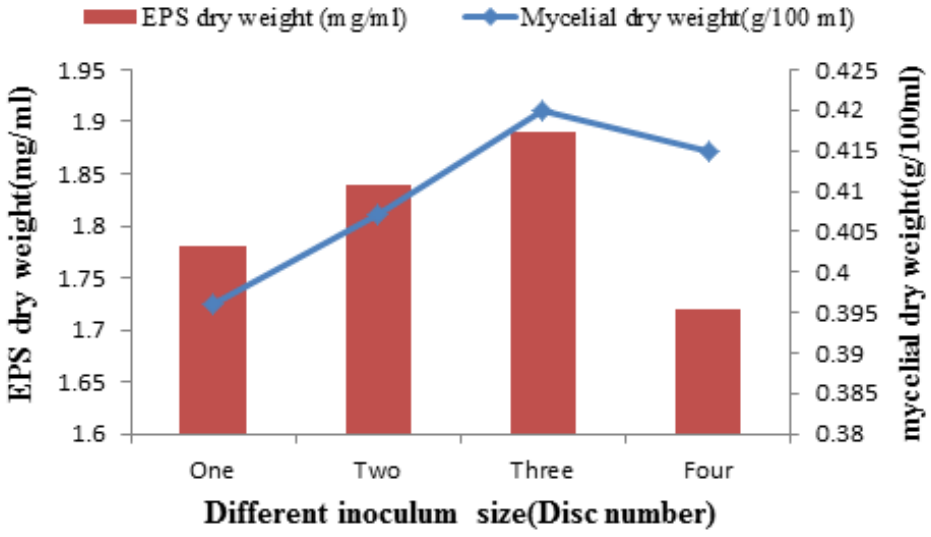

Fig. 8. Effect of different inoculum size (discs number) on mycelial growth and EPS produced by *P. commune* using static culture

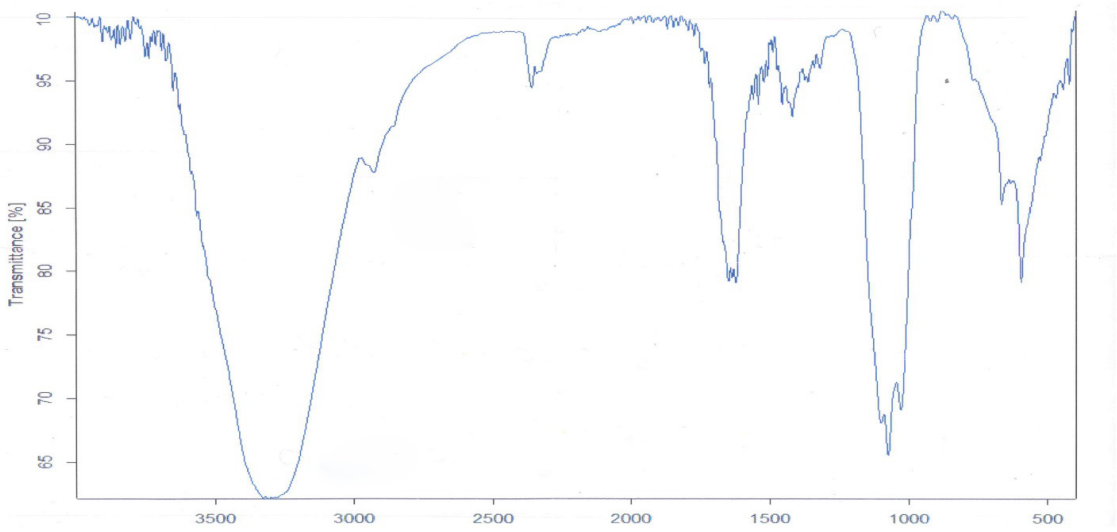

Fig. 9. Chromatogram of FT-IR of EPS produced from *P. commune.*

[1]HNMR pattern of EPSs

In the [1]HNMR spectrum of polysaccharide unit, methoxy carbon was observed in the region, 3.45 to 3.60 ppm integrals. The signals around 3.38 to 3.42 ppm were assigned to methylene proton of β-D-galactopyronosyl units. Anomericporton H-1 was observed at 5.11 ppm, and it was attached with carbon atom C-1. The remaining methane protons were observed at 4.53, 3.14 and 3.12 ppm, respectively. [1]HNMR spectrum of polysaccharide is shown in Fig.10. This result similar to Sharmila et al. (2014) which indicate the presence of methylene proton of β-D-galactopyronosyl units at signals around 3.38 to 3.42 ppm of *Syncephalastrum* sp. Ahrazem et al. (1999) found that *Penicilliumv ermoesenii* have similar residues, mainly 2,6-di-O-substituted galactofuranose (2,6)- Gal*f*-(1), and terminal glucopyranose (Glc*p*-(1), and almost identical "H-NMR spectra .

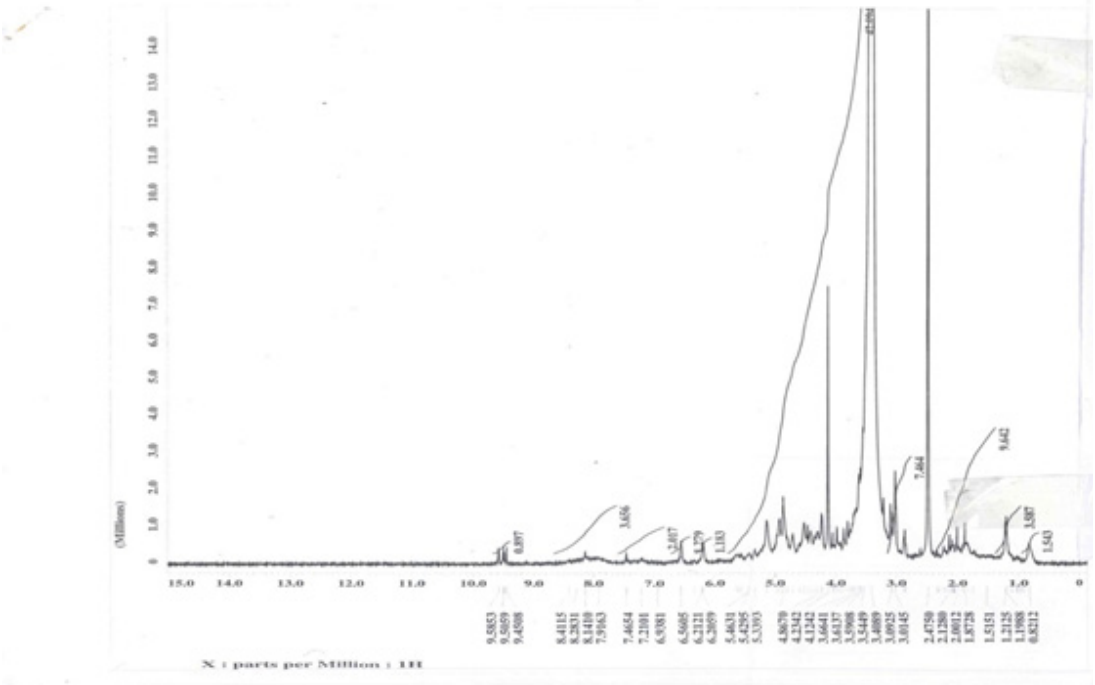

Fig. 10. The [1]HNMR spectrum of EPSs obtained from *P. commune.*

HPLC analysis of EPSs

The HPLC chromatography of EPS (Fig. 11) showed that the appearance of two peaks the first peak was at retention time 3.5 min and represented 53.49% and the second was at retention time 6.1 min with area 46.51%, which indicated that the EPS is a heteroploysaccharide consisting of raffinose and rhamnose with concentration of 3.542 and 6.104mg/g, respectively. This result agreed with Sun et al. (2015) who extracted the crude EPS (0.55 g/l) from the broth of the coral symbiotic fungus *Penicillium* sp. Two EPSs, GX11- and GX21- were 93.5% and 89.7%, respectively the monosaccharide composition differed significantly between the two samples. GX11- contained only glucose, while GX21- consisted of mannose, glucose and galactose. Chen et al. (2012) reported polysaccharide were mannopyranose and mannoglucan from *Aspergillus versicolor***.**

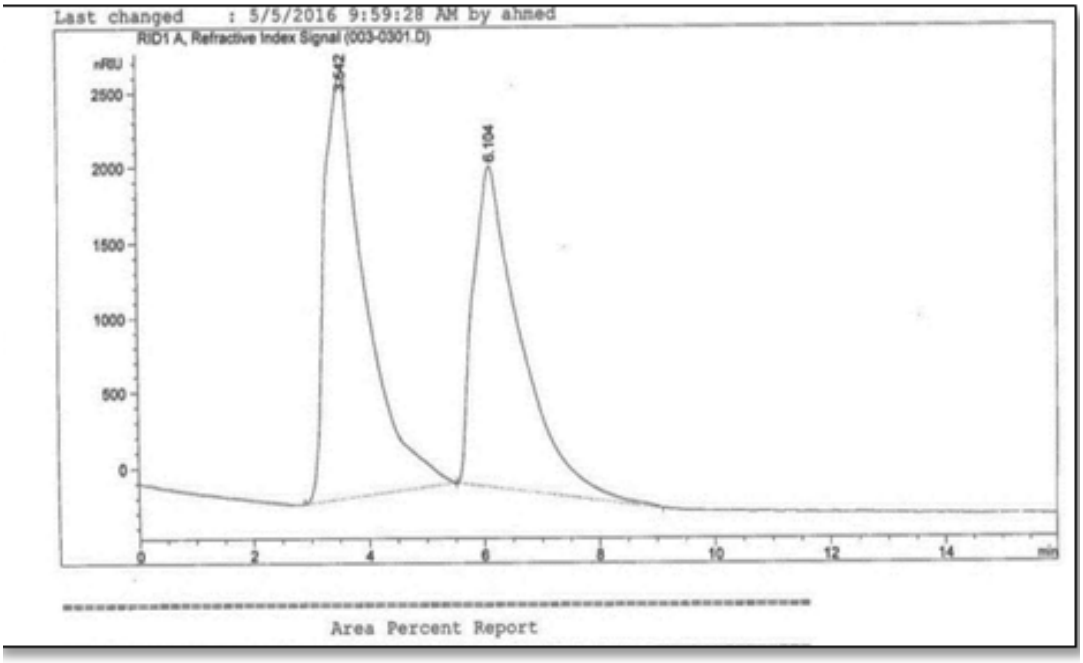

Fig. 11. The HPLC chromatogram of EPS produced from marine-derived *P. commune.*

Anti-tumor activity of EPSs from marine-derived P. commune

In the present study, the growth inhibitory effects of the EPS obtained from marine-derived *P. commune* against human colon cancer cell (Caco-2) and Mcf - 7 breast cancer cells (Mcf-7) were examined. The inhibitory effect was estimated using different concentrations (10, 5, 6.25, 12.5, and 25.50 µg) of EPS on both cell lines included. Results showed that the produced EPS exhibited various degree of antitumor effect toward the tested cell lines and increasing concentrations of EPS resulted in increased rates of tumor inhibition. The EPS presented antiproliferative activity at dose 10 mg/ml where inhibit 85% of tumor viability cell of colon. In Mcf-7 (breast cell), EPS inhibit 87% of tumor cell at dose 10 mg/ml. The IC_{50} for colon was 3.21 mg/ml, while it was 5.5 mg/ml for Mcf-7. The effects of EPS on cell line are exhibited in Fig.12. Liu et al. (2013) also evaluated that cytotoxicities of *Penicillium commune* EPSs were against five human carcinoma cell lines (Hela, A549, MCF7, HCT116, T24).Latha & Baskar (2014) approved that polysaccharides of *Pleurotus florida*-EPS and *Hypsizygus ulmarius*-EPS had effect against breast cancer cell llines, where it exhibited percentage of cell viability at 66.48% and 47.63%, respectively.

Anti-viral activity of EPSs from marine-derived P. commune

In the present study, the growth inhibitory effects of the EPS obtained from *P. commune* against HAV (Hepatitis A Virus) was examined. The HAV was injected to Vero cellcausing 60% toxicity of Vero cell which represents 100% of its actual virulent power. By application of EPS crude extract, the toxicity of virus to Vero cell became 46.7% which represent 77.1% of viral activity. So, the EPS of *P. commune* exhibited antiviral activity in percentage

of 22.8% (Fig.13). Arena et al. (2009) reported that EPS from *Bacillus licheniformis* and *Geobacillus thermodenitrificans* are known to interfere with the adsorption and penetration of viruses into host cells, as well as inhibit various retroviral reverse transcriptases.

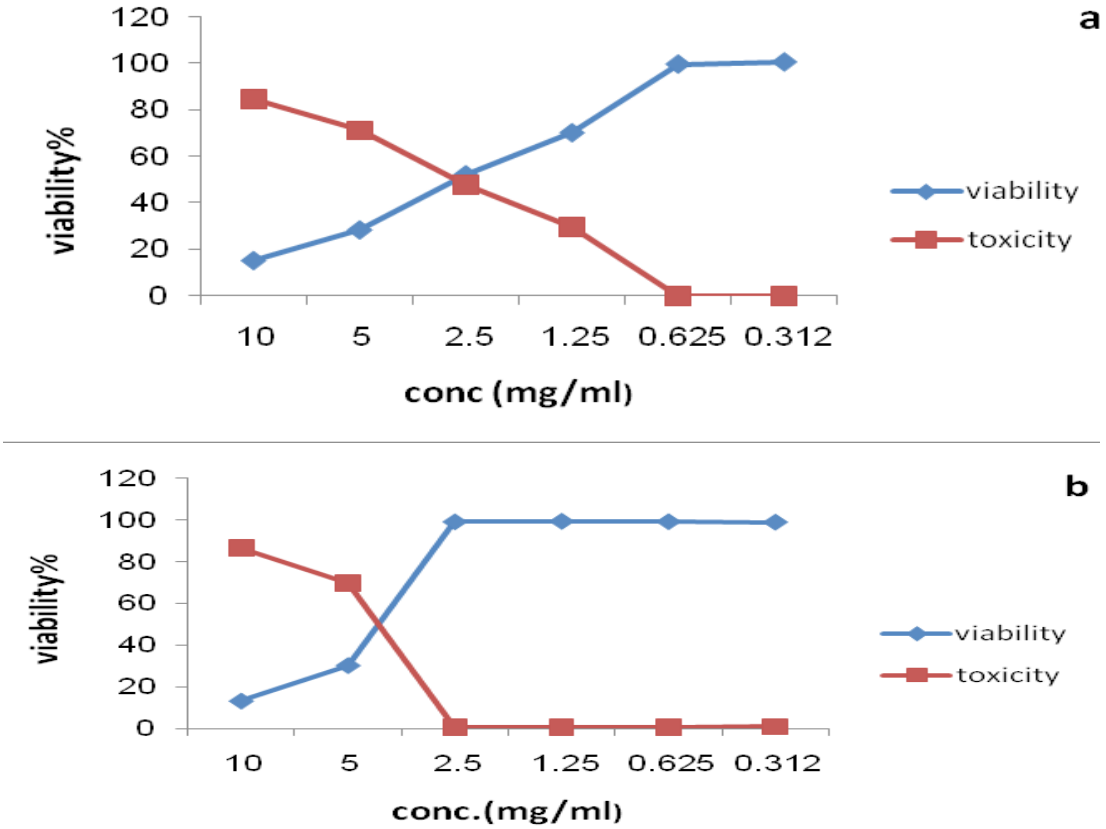

Fig. 12. (a) Effect of different concentrations of EPS on Caco-2 cells, (b) Effect of different concentrations of EPS on Mcf-7 cells

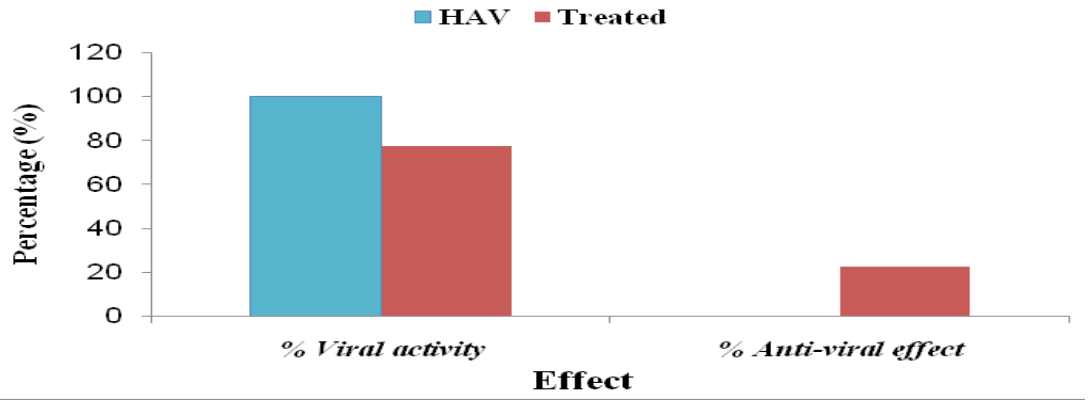

Fig. 13. Effect of different concentrations of EPS obtained from marine-derived *P. commune* on survival HAV (Dilution used was0.625mg/ml).

Anti-viral activity of EPSs from marine-derived P. commune

In the present study, the growth inhibitory effects of the EPS obtained from *P. commune* against HAV (Hepatitis A Virus) was examined. The HAV was injected to Vero cell causing 60% toxicity of Vero cell which represents 100% of its actual virulent power. By application of EPS crude extract, the toxicity of virus to Vero cell became 46.7% which represent 77.1% of viral activity. So, the EPS of *P. commune* exhibited antiviral activity in percentage of 22.8% (Fig.13). Arena et al. (2009) reported that EPS from *Bacillus licheniformis* and *Geobacillus thermodenitrificans* are known to interfere with the adsorption and penetration of viruses into host cells, as well as inhibit various retroviral reverse transcriptases.

Conclusion

The present study spotlighted on the importance of the EPS from marine-derived *P. commune* KP942881.1. Maximum production of the EPS from *P. commune* was achieved under static condition on sucrose peptone medium at 30°C and initial pH 5 for 9 days.The EPS was purified by successive extraction and precipitation by 95% isopropanol and followed by partial purification with dialysis membrane and FT-IR spectrum analysis of EPS indicated the presence of C=O, C-O-C, CH_2 and OH groups and also confirmed the presence of β-galatopyranosyl, ^{1}HNMR analysis of EPS confirmed presence of β-galatopyranosyl. HPLC chromatography showed that the EPS consisted of two peaks; raffinose and rhamnose.The EPS produced by *P. communes* howed valuable activities for medical purposes such as; antibacterial, antitumor, antiviral, antioxidant and anti-inflammatory.

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االنتاج عديد التسكر ووصفه من الفطر البحرى البينيسيليوم كومينى KP942881.1 مع بعض تطبيقاته الطبيه

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تم عزل سبعة فطريات بحريه من ميناء الإسكندرية الشرقى والغربى في مصر. وقد وجد أن أكثر الفطريات البحرية الواعدة التي تنتج عديد التسكر كانت بينيسيليوم كوميون (KP942881.1) التي تم تحديدها وفقا لخصائص المورفولوجية المجهرية وأكدت وراثيا من قبل جين الريبوسمي 18S . وقد وجد ان افضل ظروف لانتاج عديد التسكر من البينيسيليوم كومينى عند 40 ملليجرام/ملى من السكروز و 20ملليجرام/ملى من البيبتون و عند الاس الهيروجينى 5 و باستخدام ثلاثه افراص (1 سم) و درجه 30 درجه سيليلوزيه لمده 9 ايام حيث ان الظروف الثابته هى افضل ظروف. تم توظيف ثلاثه تحاليل طيفيه (FTIR, 1HNMR and HPLC) لتوصف عديد التسكر من البينيسيليوم كومينى . تم تحليل الرنين المغناطيسى و اكد على وجود بيتا جالكتوبيرنوزول. باستخدام جهاز HPLC اتضح ان عديد التسكر من البينيسيليوم كومينى يتكون من سكر رافينوز و رامنوز. وجد ان عديد التسكر له نشاط مضاد للاورام عند تركيز 10 ملجم / مل كانت نسبة تثبيط خلايا القولون المصابه بالورم 85% و في خلايا الثدي 87% من الخلايا السرطانية و ايضا وجد ان عديد التسكر من البينيسيليوم كومينى لها نشاط مضاد للفيروسات حيث انها ثبطت 22.8% .