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Herein we report the first mychomical and cytotoxic activity investigation of *Sarcosphaera crassa* (Santi) Pouzar, an edible mushroom when baked. The dried mushroom material was divided into two parts, and one was baked. The baked and unbaked extracts were subjected column chromatography. As results, one known phthalate and four sugar moieties were obtained. Their structures were elucidated by GC/MS, FT-IR, 1D and 2D NMR spectroscopy and X-ray crystallography.

Sugars and phthalate from mushroom *Sarcosphaera crassa* (Santi) Pouzar

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ABSTRACT

Herein we report the first mychomical and cytotoxic activity investigation of *Sarcosphaera crassa* (Santi) Pouzar, an edible mushroom when baked. The dried mushroom material was divided into two parts, and one was baked. The baked and unbaked extracts were subjected column chromatography. As results, one known phthalate and four sugar moieties were obtained. Their structures were elucidated by GC/MS, FT-IR, 1D and 2D NMR spectroscopy and X-ray crystallography.

Keywords: *Sarcosphaera crassa* (Santi) Pouzar, Extraction, Isolation, Elucidation

INTRODUCTION

Sarcosphaera is a monotypic genus of family Pezizaceae, containing only one species *Sarcosphaera crassa* (Santi) Pouzar (*Sarcosphaera eximia* (Dur. et Lév.) R. Maire or *Sarcosphaera coronaria* (Jacq.: Cke.) Boud [2]. *Sarcoshpaera crassa* is known as pink crown or violet star cup, having large apothecia, 3-10 cm broad, first globose, hypogenous, deeply cup-shaped, margin splitting into irregular, with thick flesh [5]. This specie is consumed particularly in south-west Anatolia. *S. crassa* is a poisonous mushroom [1], but, edible when baked [3, 8]. The local people collect the mushroom during spring to early summer [2]. In Muğla and surroundings, it is called as "Kulak, Göbek kulağı, Çanak" and sold in markets. *S. crassa* is the most edible

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mushroom in Turkey due to its special taste. This mushroom is never studied for its chemical constituents. Herein we report the first isolation study on *S. crassa*.

RESULT & DISCUSSION

The extracts were afforded one disaccharide, one erythriol and one D-mannitol from unbaked methanol and acetone extracts, one known phthalate (GCMS), and one D-sorbitol from baked methanol and acetone extracts were isolated. Various experimental techniques and extensive spectroscopic techniques were applied for the structure determination of these compounds. The results of these experimental studies are discussed in this paper.

Structure Elucidation: The molecule formula of the compound **1** was determined to be $C_{12}H_{22}O_{11}$ based on the FAB^+ and ESI-MS, 343 $[M + 1]^+$, and 365 [342 + Na], respectively. IR spectrum of the compound **1** showed the absorption at $\tilde{V}$ 3362.10 cm^{-1}, 2908.9 cm^{-1}. The ^{1}H-NMR spectra recorded in D_2O (Table 2) showed the presences of anomeric proton resonated at δ 5.07 (1H, *d*, *J* = 3.6 Hz, H-1), 3.73 (1Ha, *d*, *J* = 3.6 Hz, H-5) and 3.70 (1Hb, *t*, *J* = 5.4 Hz), 3.50 (1H, *dd*, *J* = 9.9, 3.6 Hz, H-2), 3.30 (1H, t, *J* = 9.6 Hz, H-3), 3.69 (1H, *m*, H-4), 3.63 (1H, *dd*, *J* = 12.0, 5.4 Hz, H-6).

^{13}C-NMR and Dept135 spectrum of **1** (Table 1) exhibited six signals in the broad band spectrum due to symmetry in the molecule, each peak was considered double to arrive at the molecular mass. The broad band spectrum confirmed the presence of anomeric carbon (δ 95.9), four sp^3 hydroxy methines (δ 75.2, 74.8, 73.7, 72.4), and one hydroxy methylene (δ 63.2). All the sugar ^{13}C- and ^{1}H-NMR assignment for sugar was established by comparison with the reported data of the compound α, α-trehalose 1→1-Linked glucopyranosyl glucopyranoside [6, 7]. XRD also showed the same sugar as elucidated (Figure 2). The GC-MS analysis also confirmed the mentioned structure (Figure 3).

The molecule formula of the compound **2** was determined to be $C_4H_{10}O_4$, based on EI-MS showed molecular ion peak at 122.1 $[M + 1]^+$. IR spectrum of the compound **2** showed the absorption at $\tilde{V}$ 3330 cm^{-1}, 2925 cm^{-1}. The ^{1}H-NMR spectrum recorded in D_2O (Table 2) showed a doublet at δ 3.69 (1H, *d*, *J* = 10.8 Hz) and a triplet at δ 3.53 (1H, *t*, *J* = 4.5 Hz, H-2, 2′) and a singlet at δ 3.55 (1H, *s*, H-1, 1′).

^{13}C-NMR and Dept135 spectrum of **2** (Table 1) exhibited one sp^3 hydroxy methine (δ 74.6) and one hydroxy methylene (δ 65.3). As the structural is symmetrical so each peak was considered double to reach the mass. All the ^{13}C- and ^{1}H-NMR assignment for sugar was established by comparison with the reported data of the compound erythriol [12]. The GC-MS analysis also confirmed the compound as erythriol (Figure 4).

The molecule formula of the compound **3**, colorless shiny needlelike crystal, was determined to be $C_6H_{14}O_6$ based on the FAB$^+$-MS and ESI-MS showed molecular peaks at 183 $[M + 1]^+$, and 205 $[M + Na]^+$, respectively. FTIR spectrum of the compound **3** showed the absorption at $\tilde{V}$ 3330 cm^{-1}, 2925 cm^{-1}. The ^{1}H-NMR spectrum recorded in D_2O (Table 2.) of **3** showed signals at δ 3.70 (1Ha, *dd*, *J* = 12.0, 2.5 Hz) and 3.50 (1Hb, *dd*, *J* = 11.5, 6.5 Hz, H-1, 5), 3.59 (1H, *dd*, *J* = 6.0, 2.5 Hz, H-2, 4), 3.61 (1H, *s*, H-3, 4).

^{13}C-NMR and Dept135 spectrum of **3** (Table 1) exhibited four sp^3 methine signals (δ 70.7, 70.6, 69.1, 69.0), and two hydroxy sp^2 methylene signals (δ 63.1, 63.0). Observed chemical shifts are like the previously reported values of the D-sorbitol with small differences caused by the solvent. All the sugar ^{13}C- and ^{1}H-NMR assignment for sugar was established by comparison with the reported data of the compound D-sorbitol [11].

The molecule formula of the compound **4**, colorless shiny solid needlelike crystal, was determined to be $C_6H_{14}O_6$, based on ESI-MS, which showed molecular peak at 205 $[M + 23]^+$. The FTIR shows peaks at $\tilde{V}$ 3385.56 cm^{-1}, 2876.50 cm^{-1}. The ^{1}H-NMR recorded in D_2O (Table 2) of the compound **4** showed signals at δ 3.74 (1Ha, *dd*, *J* = 9.12, 2.5 Hz,) and 3.54 (1Hb, *q*, *J* = 11.82, 6.18 Hz, H-1, 1′), 3.66 (1H, *dt*, *J* = 6.0, 2.5 Hz H-2, 2′), 3.69 (1H, *d*, *J* = 6.0 Hz, H-3, 3′).

^{13}C-NMR and Dept135 spectrum of the compound **4** (Table 1) showed two hydroxy methines (δ 70.8, 69.2) and one hydroxy sp^3 methylene (δ 63.1). Hence the compound is in symmetric form so only three peaks can be observed in ^{13}C-NMR. Each peak was considered double to reach the mass. All the ^{13}C and ^{1}H-NMR assignment for sugar was established by comparison with the reported data of the D-mannitol with small differences caused by the solvent [4].

GCMS analysis confirmed the compound **5** to be Di-octyl phthalate (Figure 5)

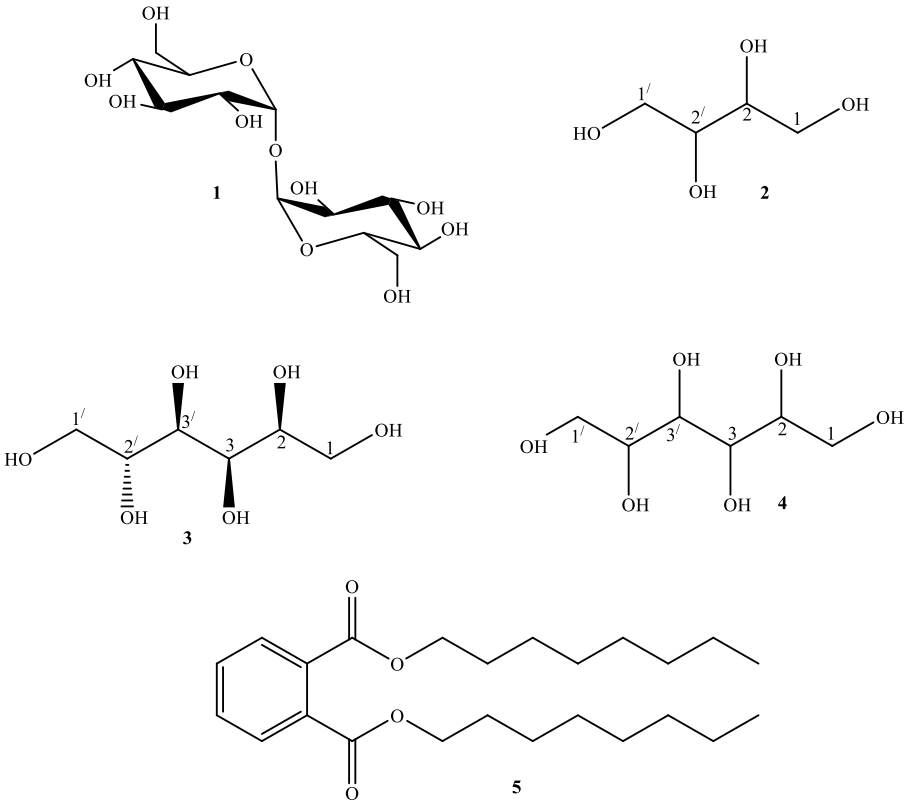

Figure 1. Structures of the compounds 1-5

Table 1. ^{1}H-NMR of compounds 1-4

	1	2	3	4
1	5.07 (1H, *d*, *J* = 3.6 Hz)	3.55 (1H, *s*)	3.70 (1H, *dd*, *J* = 12.0, 2.5 Hz), 3.50 (1H, *q*, *J* = 11.5, 6.5 Hz)	3.74 (1H, *dd*, *J* = 9.12, 2.5 Hz), 3.54 (1H, *q*, *J* = 11.82, 6.18 Hz)
2	3.50 (1H, *dd*, *J* = 9.9, 3.6 Hz)	3.69 (1H, *d*, *J* = 10.8 Hz), 3.53 (1H, *t*, *J* = 4.5 Hz)	3.61 (1H, *s*)	3.66 (1H, *dt*, *J* = 6.0, 2.5 Hz)
3	3.30 (1H, *t*, *J* = 9.6 Hz)		3.59 (1H, *dd*, *J* = 6.0, 2.5 Hz)	3.69 (1H, *d*, *J* = 6.0 Hz)
4	3.69 (1H, *m*)		3.59 (1H, *dd*, *J* = 6.0, 2.5 Hz)	
5	3.73 (1H, *d*, *J* = 3.6 Hz)		3.61 (1H, *s*)	
6	3.70 (1H, *t*, *J* = 5.4 Hz), 3.63 (1H, *dd*, *J* = 12.0, 5.4 Hz)		3.70 (1H, *dd*, *J* = 12.0, 2.5 Hz), 3.50 (1H, *q*, *J* = 11.5, 6.5 Hz)	

Table 2. ^{13}C-NMR of compounds 1-4

	1	2	3	4
1	95.9	74.6	63.1	63.1
2	73.7	65.3	69.1	70.8
3	72.4		70.7	69.2
4	74.8		70.6	
5	75.2		69.0	
6	63.2		63.0	

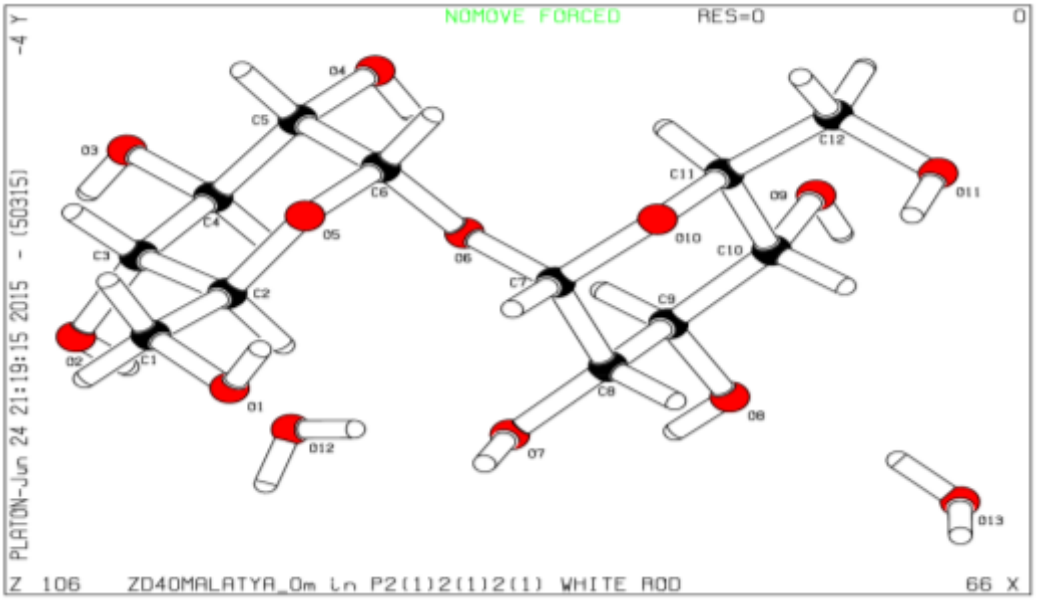

Figure 2. XRD crystallography of compound 1

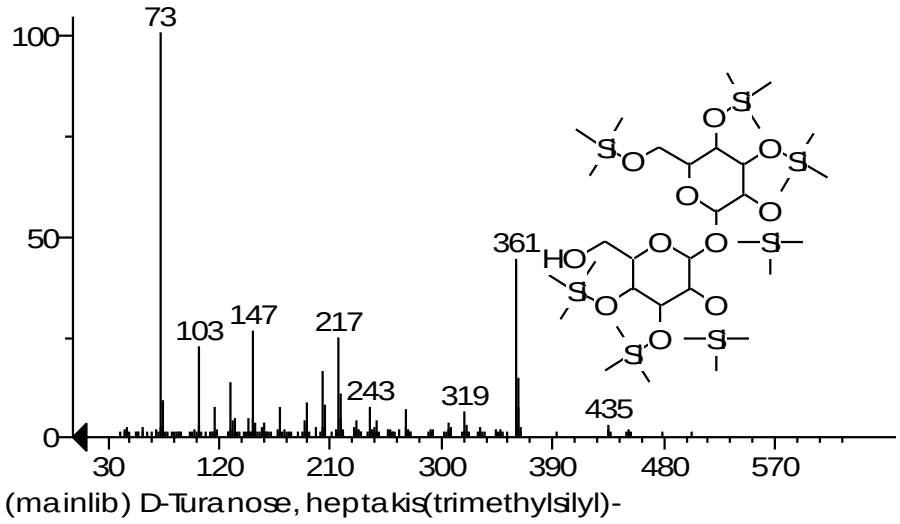

Figure 3. GCMS chromatogram of compound 1

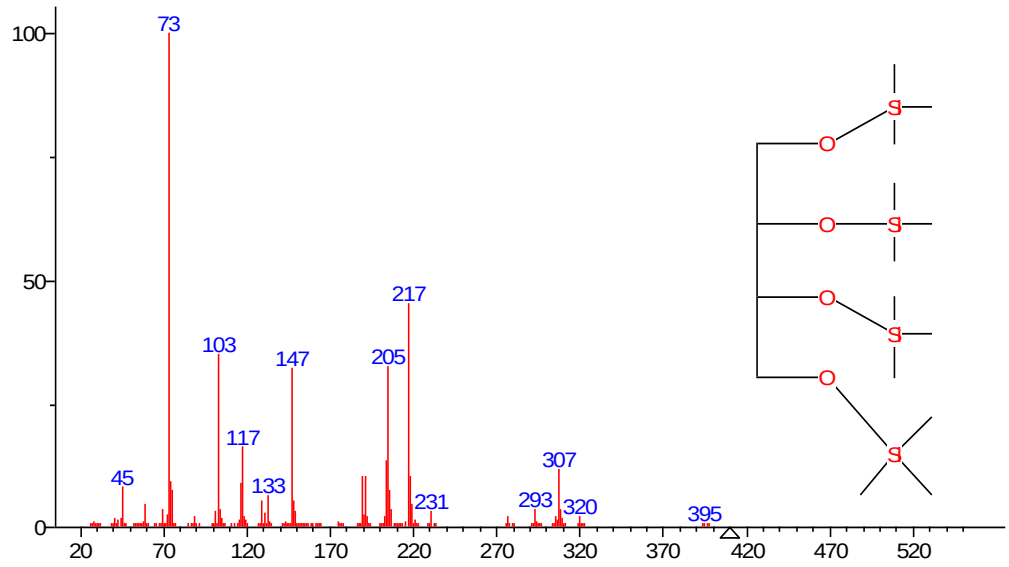

Figure 4. GCMS chromatogram of compound 2

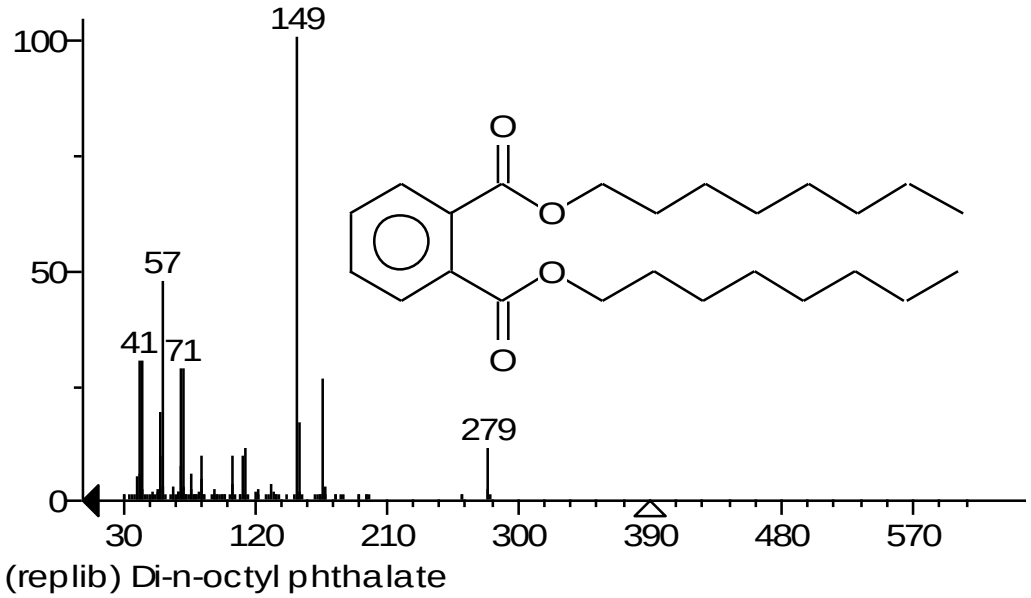

Figure 5. GCMS chromatogram of 5

EXPERIMENTAL

Sarcosphaera crassa (Santi) Pouzar. was purchased from local bazar which had been collected from Yılanlı mountain, in Muğla, Turkey, in January 2015. The mushroom was identified by Ms.

Cansu Korkmaz under Voucher specimen number (CK-3231), and was deposited in the Herbarium of Biology Department, Mugla Sitki Kocman University.

Extraction of crude extracts: The freshly obtained mushroom (20 kg) separated into two equal parts. One part was baked under 200 °C for 40 minutes. Then both baked and unbaked parts were air-dried under shadow. Both unbaked and baked samples were grinded and extracted with petroleum ether, acetone and methanol, successively.

Each extract was dissolved in minimum amount of methanol and adsorbed on silica (60-120 mesh) for the preparation of slurry. It was dried and chromatographed over silica gel column packed in petroleum ether (b.p. 60-80 °C). The columns were eluted with petroleum ether (100%), and then with mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, and 1:3 v/v), and then chloroform (100%), and then chloroform-methanol (99:1, 49:1, 19:1, 9:1 v/v), and ended with methanol (100%), successively, in order of increasing polarity to isolate the following compounds. From the methanol extract of unbaked sample **1** and **2**, and from the methanol extract of baked sample **3**, and from the acetone extract of unbaked sample **4**, was isolated. Additionally, the obtained **5** from baked methanol extract through column chromatography were further analyzed and elucidated using GC-MS.

Compound (1); White solid crystals (D_2O), eluted through *n*-hexane: dichloromethane. FTIR (neat) max ($\tilde{V}$); 3362.10 cm^{-1}, 2908.09 cm^{-1}. The ^1H- and ^{13}C-NMR spectroscopic data (D_2O) see Table 1 and 2, for XRD and GC/MS see Figure 2 and 3. ^1H-NMR (D_2O, 600 MHz) δ 5.07 (1H, *d*, *J* = 3.6 Hz, H-1), 3.73 (1Ha, *d*, *J* = 3.6 Hz, H-5) and 3.70 (1Hb, *t*, *J* = 5.4 Hz, H-5), 3.69 (1H, *m*, H-4), 3.63 (1H, *dd*, *J* = 12.0, 5.4 Hz, H-6), 3.50 (1H, *dd*, *J* = 9.9, 3.6 Hz, H-2), 3.30 (1H, *t*, *J* = 9.6 Hz, H-3), [6, 7]. ESI-MS; 365 $[M + Na]^+$.

Compound (2); White amorphous (D_2O), FTIR (neat) max ($\tilde{V}$); 3425 cm^{-1}, 2910 cm^{-1}. ^1H- and ^{13}C-NMR spectroscopic data (D_2O) see Table 1 and 2, GC/MS see Figure 4. ^1H-NMR (D_2O, 500 MHz) δ 3.69 (IHa, *d*, *J* = 10.8 Hz) and 3.53 (1Hb, *t*, *J* = 4.5 Hz, H-2, $2^{/}$), 3.55 (1H, *s*, H-1, $1^{/}$), [12]. EIMS; *m/z* 122.1 $[M + 1]^+$.

Compound (3); White amorphous (D_2O), FTIR (neat) max ($\tilde{V}$); 3330 cm^{-1}, 2925 cm^{-1}, ^1H- and ^{13}C-NMR spectroscopic data (D_2O) see Table 1 and 2. ^1H-NMR (D_2O, 600 MHz) δ 3.70 (1Ha, *dd*,

J = 12.0, 2.5 Hz,) and 3.50 (1Hb, *dd*, *J* = 11.5, 6.5 Hz, H-1, 1/), 3.61 (1H, *s*, H-3, 3/), 3.59 (1H, *dd*, *J* = 6.0, 2.5 Hz, H-2, 2/). FAB^+; *m/z* 183 $[M + 1]^+$, ESI-MS: *m/z*; 205 $[M + Na]^+$.

Compound (4); White shiny amorphous (D_2O), FTIR (neat) max ($\tilde{V}$); 3385.56 cm^{-1}, 2876.50 cm^{-1}, ^1H- and ^{13}C-NMR spectroscopic data (D_2O) see Table 1 and 2. ^1H-NMR (D_2O, 600 MHz) δ 3.74 (1H, *dd*, *J* = 9.12, 2.5 Hz) and 3.54 (1H, *q*, *J* = 11.82, 6.18 Hz, H-1, 1/), 3.69 (1H, *d*, *J* = 6.0 Hz, H-3, 3/), 3.66 (1H, *dt*, *J* = 6.0, 2.5 Hz, H-2, 2/). ESI-MS; *m/z* 205 $[M + 23]^+$.

GC-MS analysis of 5: The GC-MS analysis was performed using Varian Saturn 2100T, coupled with ion trap analyzer, and equipped with DB-1 fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium (15 psi) at a flow rate of 1.3 mL/min. Injector and MS transfer line temperatures were set at 250 and 290 °C respectively. The oven temperature was held at 100 °C for 5 min, then increased up to 238 °C with 3 °C/min increments and held at this temperature for 9 min. Diluted samples (1/25, w/v, in chloroform) of 0.2 µL were injected manually in the split mode. Split ratio was 1:20. EI-MS were taken at 70 eV ionization energy. Mass range was adjusted from *m/z* 40 to 650 amu. Scan time 0.5 s with 0.1 inters scan delays. The library search was carried out using NIST and Wiley 2005 (gas chromatography–mass spectrometry) GC-MS libraries.

Identification of sugars by GC-MS: The pure sugar compounds (**1 - 2**) obtained from unbaked methanol were silylated and analyzed by GC-MS [10]. For this each compound were solved in 50 µL of anhydrous pyridine, and then 75 µL of bis (trimethylsilyl) trifluroacetamite (BSTFA) were added. Finally, all were kept in the oven for 20 minutes at 80 °C. After cooled in desiccator, each sample was diluted to 375 µL using *n*-hexane. 0.2 µL were injected to GC-MS.

The silylated sugars were analyzed using the same GC–MS (Varian Saturn 2100T) and conditions mentioned above, except column program. Briefly, the oven temperature was held at 100 °C for 5 min, then increased up to 270 °C with 5 °C/min increments and held at this temperature for 15 min. The sugars were detected based on co-injection of silylated standard sugars (D-glucose, D-galactose, L-glucose, L-galactose, D-arabinose, L-arabinose), and the Wiley-Nist-2005 Mass Library, and the literature comparison.

CONCLUSION

Sarcosphaera crassa was studied for its sugar and phthalate content for the first time. From the baked and unbaked samples four sugar (**1-4**) and one phthalate (**5**) were isolated. The structures were elucidated according to 1D, and 2D NMR, and MS techniques.

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REFERENCES

[1] Afife, M. Türkiyede Mantar Zehirlenmeleri ve Zehirli Mantarlar, *Nobel Tıp Kitabevi Press*, 2. (**2000)**, Edition, Istanbul Turkey.

[2] Bas, H. Mugla Makrofunguslari Üzerinde Taksonomik Çalismalar, Mugla Üniversity, Institute of science and technology, **(2005)**, Mugla, M.Sc. Thesis.

[3] Bresisnsky, A. and Besl, H. "A colour of Poisonous fungi", *Wolf Publishing*, **(1990)**, London, 295p.

[4] Gaidamauskas, E., Norkus, E., Vaičiunien, J., Crans, D.C., Vuorinen, T., Jačiauskien, J. and Baltrūnas, G. Evidence of two-step deprotonation of D-mannitol in aqueous solution, *Carbohydr Res*, **(2005)**, 340 (8): 1553–1556.

[5] Kasom, G. and llinka, C. *Sarcosphaera coronaria* (Jack) j. Schrot. 1983-the distribution and conservation status in montenegro, *Natura Montenegrina, Podgorica*, **(2011)**, 10 (4): 377–383.

[6] Koto, S., Inada, S. and Zen S. Upfield Shift of CMH of Anoivieric Carbons of 1,1'-Linked Glycopyranosyl Glycopyranosides, *Chem Lett*, **(1980)**, 403–406.

[7] Larsen, P.I., Sydnes, L.K., Landfald, B. and Strata, A.R. Osmoregulation in *Escherichia eoli* by accumulation of organic osmolytes: betaines, glutamic acid, and trehalose, *Arc Microbiol*, **(1987)**, 147 (1): 1–7.

[8] Pacioni, G. "Mushrooms and Toadstools", *Mac Donald and Ltd*., **(1985)**, London, 249p.

[9] Saleem, M., Nazir, M., Akhtar, N., Onocha, P.A., Riaz, N., Jabbar, A., Ali, M.S. and Sultana, S. New phthalates from *Phyllanthus muellerianus* (Euphorbiaceae), *J Asian Nat Prod Res*, **(2009)**, 11 (11): 974–977.

[10] Torri, C., Soragnib, E., Pratia, S. and Fabbria, D. Py-SPME-GC-MS with on-fiber derivatization as a new solvent-less technique for the study of polar macromolecules: Application to natural gums, *Microchem J*, **(2013)**, 110: 719–725.

[11] Venditti, A., Frezza, C., Sciubba, F., Serafini, F. and Bianco, A. Primary and secondary metabolites of an European edible mushroom and its nutraceutical value: *Suillus bellinii* (Inzenga) Kuntze, *Nat Prod Res*, **(2016)**, 1-10.

[12] Xu, X-M., Lib, D-Y., Huaa, H-M., Li, Z-L and Liuc, Q. Two new threitol orsellinates from a sea mud-derived fungus, *Ascotricha* sp. ZJ-M-5, *J Asian Nat Prod Res*, **(2016)**.