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Simultaneous isolation of gallotannins and a related phenolic from *Mangifera indica* kernels and assessment of their anti-*Trichomonas vaginalis* activities

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ABSTRACT

Therapeutic alternatives are being searched for trichomoniasis as a result of the increased prevalence of metronidazole-resistant infections. *Mangifera indica* L. (Anacardiaceae) is an important tree with a long history in medicine. Traditionally, it has been used as an anti-diarrheal and anti-diabetic, and recently, its gallotannin-rich leaves and stem bark extracts have shown antiparasitic activities against various parasites. Aiming at exploring the anti-*Trichomonas vaginalis* activity of mango's gallotannins, an aqueous ethanol extract of fresh kernels of *M. indica* was phytochemically investigated. Based on a simple gel chromatographic procedure, ethyl gallate (**2**), a group of five isomeric tetragalloyl-glucoses (**3**–**7**), and a pentagalloyl-glucose (**8**) were simultaneously isolated from a single fraction by a preparative Reversed-phase-high performance liquid chromatography. The isolates were identified based on spectroscopic analyses and comparison with reported data. They showed structural-dependent inhibitory effects on the growth of *T. vaginalis* trophozoites in an *in vitro* investigation. Ethyl gallate and 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (**7**) exhibited elevated anti-*T. vaginalis* activity (IC_{50} = 1.3, 2.4 µg/ml, respectively). This is the first report exploring the potential of gallotannins as trichomonacidal agents.

INTRODUCTION

Trichomonas vaginalis (*T. vaginalis*) is the most common sexually transmitted parasite. It affects the external genitalia of men and women leading to a genitourinary disease trichomoniasis. The infections are sometimes associated with preterm birth of pregnant women (Silver *et al*., 2014) and prostatic tumor of men (Sutcliffe *et al*., 2012). The development of resistance, allergy, and other side effects due to repeated treatment with imidazole derivatives urged exploring safe alternative anti-trichomoniasis (Cudmore *et al*., 2004). The gallotannins-rich leaves and stem bark of the tree *Mangifera indica* L. (Anacardiaceae)(Núñez Sellés *et al*., 2002) have shown antiparasitic activities against *Entamoeba histolytica* (Tona *et al*., 1998); *Histomonas meleagridis*, *Tetratrichomonas gallinarum*, and *Blastocystis* sp (Grabensteiner *et al*., 2008); *Plasmodium falciparum* (Awe *et al*., 1998; Ruiz *et al*., 2011; Zirihi *et al*., 2005); and *Giardia lamblia* (Amaral *et al*., 2006). The mango kernels largely produce gallotannins, gallic acid, and benzoic acid derivatives (Masibo and He, 2008). The kernels are a very rich source of gallotannins (15.5 mg/g dry kernel; Berardini *et al*., 2004). It was traditionally employed to expel tapeworms and as an anti-diarrheal agent (Sairam *et al*., 2003). Extracts of the kernels have also demonstrated antimicrobial activities against a wide range of the Gram-positive organisms which were ascribed to its gallotannins content (Ahmed *et al*., 2007; Ka buki *et al*., 2000). In our previous report, gallotannins, benzophenones, and xanthone *C*-glucosides from the mango stem bark were shown

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together with their antiviral and cytotoxic activities (Abdel-Mageed *et al*., 2014). To explore the anti-*T. vaginalis* activity of the tannin constituents of the mango seed kernels, we conducted a phytochemical investigation focusing on the isolation of gallotannins. As a result, gallic acid (**1**), ethyl gallate (**2**), 2,3,4,6-tetra-*O*-galloyl-β-D-glucose (**3**), 1,2,3,4-tetra-*O*-galloyl-β-D-glucose (**4**), and a combination of almost equal proportions of 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**5**) and 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**6**), 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (**7**), and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**8**) were purified. This study reports an easy chromatographic procedure for the simultaneous isolation of ethyl gallate (**2**), and the six gallotannins (**3-8**, Fig. 1) from seed kernels of *M. indica* variety *Tymor*. The effect of these seven compounds on the viability of *T. vaginalis* trophozoites is also reported here for the first time. A growth inhibitory mechanism of the trophozoite is suggested in view of the known protein binding and iron-binding affinity of the tannins.

MATERIALS AND METHODS

General experimental procedures

The one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectra were recorded on a Varian INOVA AS 600 instrument (Agilent, Santa Clara, CA, USA; 0.151 GHz for ^{13}C and 0.6 GHz for ^{1}H). The chemical shift values were shown in δ (ppm) relative to the solvent signals [$(CD_3)_2CO$-d_6 (δ_H 2.04; δ_C 29.8) on the tetramethylsilane (TMS) scale. Reversed-phase-high performance liquid chromatography (RP-HPLC) was done on a YMC-Pack ODS-A A-303 (product of YMC, Japan) column (4.6 i.d. × 250 mm) using acetonitrile – water (2:8, v/v) with 0.1% acetic acid. The flow rate was set at 1 ml/min and the oven temperature at 40°C. The eluates were monitored by a Ultraviolet (UV) detector at λ_{max} = 280 nm. Preparative RP-HPLC was carried out on a YMC-Pack ODS-A, A-324 column (10 i.d. × 300 mm) using the mobile phase acetonitrile – water (2:8, v/v) with 0.1% acetic acid. The flow rate of 2 ml/min at column oven temperature 40°C, and UV detection (λ_{max} = 280 nm) were applied. The gels, Dia-ion HP-20 and MCI-gel CHP-20P (products of Mitsubishi Chemical, Japan), were used for the chromatographic experiments.

Plant material

Fresh seed kernels of *M. indica* variety *Tymor* were collected from mature ripe fruits which were purchased from a private farm in Assiut. The plant species was identified by Prof. Dr. Ayman Kotb, Horticulture Department, Faculty of Agriculture, Assiut University. An authentic sample (No. 2012MT) was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Extraction and isolation procedures

Mangifera indica fresh seed kernels (~2.5 kg) were extracted by maceration in EtOH/water [7:3, v/v, (3 L × 4)] at ambient temperature. The obtained extract was concentrated under vacuum at ~40°C. The obtained concentrate (~400 ml) was applied to a Diaion HP-20 column (5.5 i.d. × 63 cm), which was eluted with distilled water (6 L), methanol/water (2.5:7.5, v/v, 5 L), methanol/water (5:5, v/v, 5 L), methanol/water (7.5:2.5, v/v, 5 L), and methanol (5 L) successively. The respective eluates were dried under vacuum and yielded the respective water (54 g), methanol/water (2.5:7.5, v/v) (98.5 g), methanol/water (5:5, v/v) (55 g), methanol/water (7.5:2.5, v/v) (4.7 g), and methanol (4.4 g) fractions. A portion (3 g) of the methanol/water (2.5:7.5, v/v) fraction was subjected to chromatographic purification on MCI-gel CHP-20P (1.1 i.d. × 37 cm) with water, water/methanol (9:1 → 8.5:1.5 → 8:2 → 7.5:2.5 →7:3 → 6.5:3.5 → 6:4 → 3:7 and 0:10, v/v). All the 700 drops of

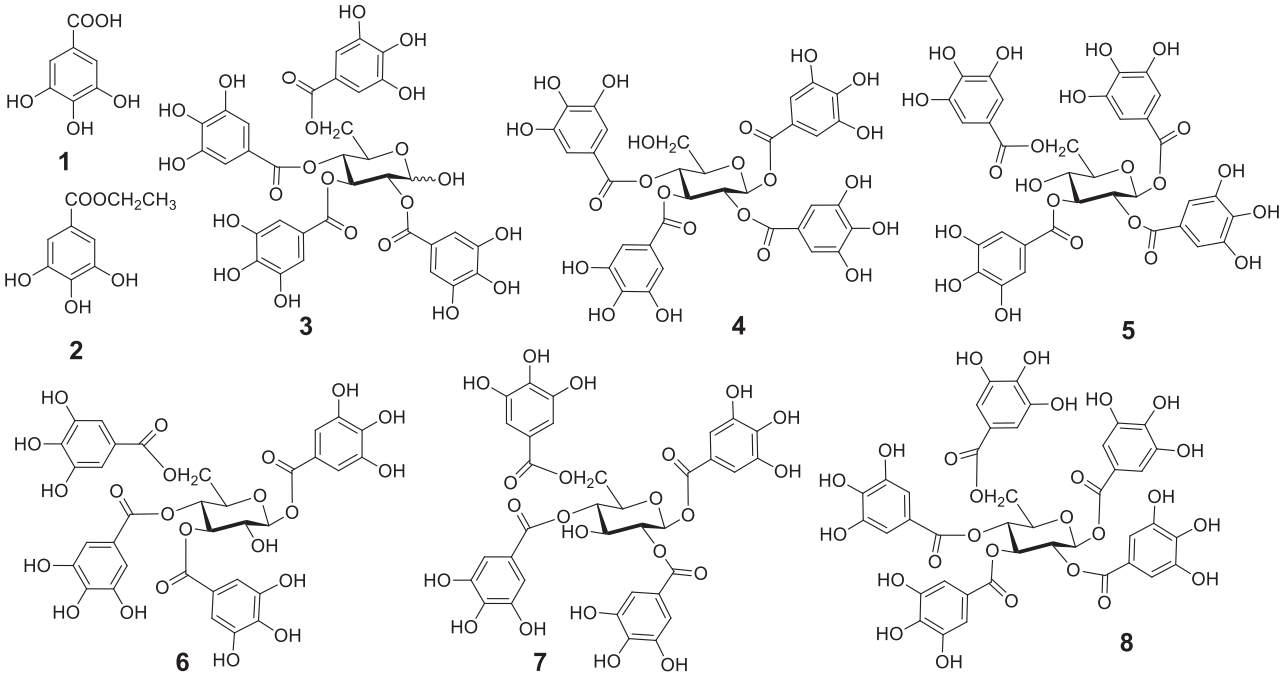

Figure 1. Structures of gallic acid (**1**) ethyl gallate (**2**), and the gallotannins **3–8**.

the column eluates were gathered separately in a test tube using a fraction collector. The fractions were visualized by monitoring the RP-HPLC. The water eluate furnished crude gallic acid [**1** (519 mg)]. The water/methanol (8.5:1.5, v/v) eluate provided pure ethyl gallate [**2**, (383 mg)]. The water/methanol (8:2, 133 mg), (7.5:2.5, 222 mg), and (7:3, v/v, 221.4 mg) eluates demonstrated identical RP-HPLC profiles (Fig. 2), indicating the presence of the same compounds in these elutes. A preparative HPLC purification of part (300 mg) of the water/methanol (7.5:2.5, v/v) eluted fraction led to purification of 2,3,4,6-tetra-*O*-galloyl-β-D-glucose [**3**, (4.2 mg)], 1,2,3,4-tetra-*O*-galloyl-β-D-glucose [**4**, (3 mg)], mixture (13.4 mg) of almost equal proportions of 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**5**) and 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**6**), 1,2,4,6-tetra-*O*-galloyl-β-D-glucose [**7**, (5 mg)], 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose [**8**, (29 mg)], and another pure sample of the ethyl gallate [**2**, (34.8 mg)]. In addition, the water/methanol (6:4, v/v) eluted fraction also afforded 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose [**8**, (188.5 mg)].

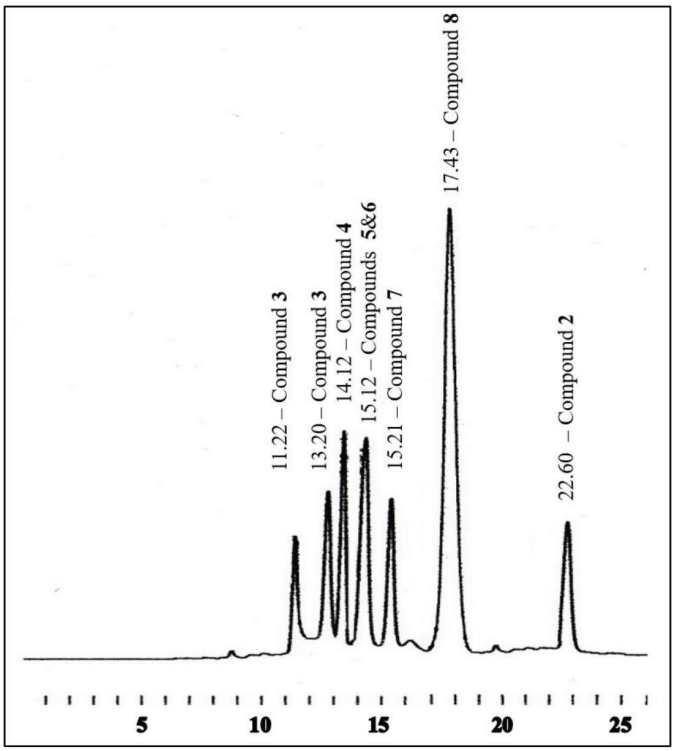

Figure 2. RP-HPLC chromatogram (at λ_{max} = 280 nm) of the main compounds in the water/methanol (8:2—6.5:3.5, v/v) eluates from an MCI-gel CHP-20P column, from the water/methanol (8:2, v/v) eluate of the dia-ion HP-20 chromatographic fractionation for the aq. EtOH extract of mango seed kernels.

Compounds physicochemical and spectroscopic data

Gallic acid (1): White, non-crystalline powder; RP-HPLC analysis and co-chromatography with authentic sample (t_R = 3.80 min); ^{1}H NMR [0.6 GHz; $(CD_3)_2CO$-d_6/D_2O, 9:1] δ_H: 7.08 (2H, s, H-2,6); Electrospray ionization mass spectrometry (ESIMS) *m/z* 171 $[M + H]^+$ ($C_7H_7O_5$).

Ethyl gallate (2): Off-white, non-crystalline powder; ^{1}H NMR [0.6 GHz; $(CD_3)_2CO$-d_6/D_2O, 9:1] δ_H: 7.07 (2H, s, galloyl H-2,6), 4.22 (2H, q, J = 7.2 Hz, CH_2), 1.27 (3H, t, J = 7.2 Hz, CH_3); ^{13}C NMR [0.151 GHz; $(CD_3)_2CO$-d_6/D_2O, 9:1]: δ_C 167 (C-7), 145.9 (2C, C-3,5), 138.7 (C-4), 121.6 (C-1), 109.6 (2C, C-2,6), 61 (C-8) and 14.5 (C-9); ESIMS *m/z* 199 $[M + H]^+$ ($C_9H_{11}O_5$).

2,3,4,6-Tetra-O-galloyl-β-D-glucose (3): Off-white, non-crystalline powder; ^{1}H NMR [0.6 GHz; $(CD_3)_2CO$-d_6/D_2O, 9:1] δ_H: 7.13, 7.02, 7.00, 6.92 [each s, 2H, galloyl H-2,6 × 4], galloyls of the β-anomer of the D-glucose, 7.15, 7.03, 7.028, 6.98 [each s, 2H, galloyl H-2,6 × 4], galloyls of the α-anomer of the D-glucose, glucose protons (Table 1); ESIMS *m/z* 789 $[M + H]^+$ ($C_{34}H_{29}O_{22}$).

1,2,3,4-Tetra-O-galloyl-β-D-glucose (4): Off-white, non-crystalline powder; ^{1}H NMR [0.6 GHz; $(CD_3)_2CO$-d_6/D_2O, 9:1] δ_H: 7.07, 7.025, 6.98, 6.95 (each s, 2H, galloyl H-2,6 × 4), glucose protons (Table 1); ESIMS *m/z* 789 $[M + H]^+$ ($C_{34}H_{29}O_{22}$).

1,2,3,6-Tetra-O-galloyl-β-D-glucose (5): Off-white, non-crystalline powder; ^{1}H NMR [0.6 GHz; $(CD_3)_2CO$-d_6/D_2O, 9:1] δ_H: 7.13, 7.06, 7.04, 6.98 (each s, 2H, galloyl H-2,6 × 4], glucose protons (Table 1); ESIMS *m/z* 789 $[M + H]^+$ ($C_{34}H_{29}O_{22}$).

1,3,4,6-Tetra-O-galloyl-β-D-glucose (6): Off-white, non-crystalline powder; ^{1}H NMR [0.6 GHz; $(CD_3)_2CO$-d_6/D_2O, 9:1] δ_H: 7.16, 7.10, 7.05, 7.02 (each s, 2H, galloyl H-2,6 × 4], glucose protons (Table 1); ESIMS *m/z* 789 $[M + H]^+$ ($C_{34}H_{29}O_{22}$).

1,2,4,6-Tetra-O-galloyl-β-D-glucose (7): Off-white, non-crystalline powder; ^{1}H NMR [0.6 GHz; $(CD_3)_2CO$-d_6/D_2O, 9:1] δ_H: 7.14, 7.11, 7.08, 7.05 (each s, 2H, galloyl H-2,6 × 4), glucose protons (Table 1); ESIMS *m/z* 789 $[M + H]^+$ ($C_{34}H_{29}O_{22}$).

1,2,3,4,6-Penta-O-galloyl-β-D-glucose (8): Off-white, non-crystalline powder; ^{1}H NMR [0.6 GHz; $(CD_3)_2CO$-d_6/D_2O,

Table 1. ^{1}H NMR data (δ in ppm, J in Hz) of glucose moiety of the galloylglucoses **3**–**8** (0.6 GHz, $(CD_3)_2CO$-d_6/D_2O, 9:1, 27°C).

glc. H	3		4	5	6	7	8
	3α	3β					
1	5.55 (d, 3.6)	5.16 (d, 7.8)	6.19 (d, 8.4)	6.10 (d, 8.4)	5.96 (d, 8.4)	6.03 (d, 8.4)	6.27 (d, 8.4)
2	5.11 (dd, 3.6, 10.2)	5.26 (dd, 7.8, 10.2)	5.55 (dd, 8.4, 9.6)	5.45 (dd, 8.4, 9.6)	4.023 (dd, 8.4, 9.6)	5.37 (dd, 8.4, 9.6)	5.62 (d, 8.4, 9.6)
3	6.04 (t, 10.2)	5.75, t	5.92, t	5.64, t	5.65, t	4.35, t	6.00, t
4	5.55 (t, 10.2)	5.47 (t, 10.2)	5.46 (t, 9.6)	4.04 (t, 9.6)	4.04 (t, 9.6)	5.38 (t, 9.6)	5.65 (t, 9.6)
5	4.58 (ddd, 1.8, 6.6, 10.2)	4.24 (ddd, 1.8, 6.6, 10.2)	4.16 (ddd, 1.8, 4.8, 10.2)	4.14 (ddd, 2.4, 5.4, 9.6)	4.35 (ddd, 2.4, 5.4, 9.6)	4.29 (ddd, 1.8, 4.8, 9.6)	4.54 (ddd, 1.8, 4.8, 9.6)
6	4.24 (dd, 4.8, 12)	4.24 (dd, 4.8, 12)	3.73 (dd, 1.8, 12)	4.63 (dd, 1.8, 12)	4.51 (dd, 2.4, 12)	4.52 (dd, 1.8, 12.6)	4.57 (dd, 1.8, 12.6)
	4.50 (dd, 1.8, 13.2)	4.50 (dd, 1.8, 13.2)	3.65 (dd, 4.8, 13.2)	4.46 (dd, 4.8, 12)	4.21 (dd, 4.8, 12)	4.19 (dd, 4.8, 12.6)	4.3 (dd, 4.8, 12.6)

9:1] δ_H: 7.13, 7.07, 7.04, 6.99, 6.97 (each s, 2H, galloyl H-2,6 × 5), glucose protons (Table 1); ESIMS *m/z* 941 $[M + H]^+$ ($C_{41}H_{33}O_{26}$).

Anti-*T. vaginalis* activity of the isolated compounds

Culture of T. vaginalis

Fresh *T. vaginalis* was isolated from a female patient visiting the Patient Treatment Center, Gynecology and Obstetrics Hospital, Faculty of Medicine, Assiut University. After explaining the aim of the study, informative consent was signed by the patient. This study was endorsed by the Research Ethics Committee, Faculty of Medicine, Assiut University, Egypt. The live trophozoites were grown in trypticase–yeast extract–maltose (TYM) medium (Innocente *et al*., 2014) in 15-ml screw-stoppered glass tubes at 37°C. The medium was complemented with 1-ml heat-inactivated fetal calf serum, crystalline penicillin (1,000,000 IU/ml), and streptomycin sulfate (100,000 µg/ml), and the pH was adjusted at 6.0. The isolate was regularly transferred to fresh TYM medium every 48 hours, and maintained in the Parasitology Department, Faculty of Medicine, Assiut University. The growth of *T. vaginalis* was monitored until it reached the logarithmic growth phase. The trophozoites exhibiting normal viability and morphology were harvested, isolated from the medium by centrifugation, and regrown in fresh TYM medium for the investigations; 10^5/ml cells were used for the evaluation of the anti-trichomonal effect of the isolated compounds. The organism count number was monitored using hemocytometer slides.

The trichomonacidal assay

The anti-*T. vaginalis* activity of the pure compounds (**2**–**8**) was determined *in vitro* based on the reported method (Ibrahim, 2013). The test compounds were diluted by DMSO as solubilizing vehicle at a concentration not more than 0.01% in the experiment medium. A series of two-fold dilutions for all compounds tested were obtained and used at final concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56 µg/ml. A 50 µl of 1 × 10^5 trophozoite/ml was inoculated in 150 µl of the test compound in a sterile 96-well culture plate at 37°C (Innocente *et al*., 2014). Three controls were considered: negative control with parasites only; vehicle control (DMSO); and the positive control [metronidazole (MTZ) (Sanofi-Aventis, Egypt). MTZ was dissolved in distilled H_2O, diluted by the incubation medium giving four concentrations, 3.12, 1.56, 0.75, and 0.0037 µg/ml, and used as the reference standard. The 96-well plate was incubated for 72 hours at 37°C, 5% CO_2. The number of living organisms was determined by counting the parasites by a hemocytometer using the trypan blue dye (0.2%) exclusion method. Control cultures were treated by the same procedure as that of the test cultures. All assays were carried out in triplicate and repeated three times. For evaluation of the activity of the compound at different contact times, samples from the cultures were taken after 24-, 48-, and 72-hours incubations. The mortality percentage of the parasites was determined from the equation {100 × [100–a/b]} where "a" is the living organisms count in test wells and "b" is the living organisms count in the control wells (Ali, 2007).

Statistical analysis

The IC_{50} values were computed from a non-linear regression curve using the GraphPad Prism 5.0 software.

RESULTS AND DISCUSSION

Simultaneous isolation of gallotannins

The development of analytical technique Liquid chromatography mass spectrometry/mass spectrometry (LCMS/MS) made profiling and identification of multi-components of plant extracts more rapid. Identification of a series of gallotannins in extracts of morphological parts of mango (peel, pulp, and kernel) by such technique has recently been reported (Berardini *et al*., 2004). However, estimation of the activity of the individual gallotannins, as well as its pharmacokinetic and pharmacodynamic parameters, are largely dependent on the tannin structure (Berardini *et al*., 2004, Gan *et al*., 2018), which necessitate obtaining the tannin in pure form. We herein succeeded to simultaneously purify a group of five isomeric tetragalloyl glucoses (**3**–**7**), a pentagalloyl glucose (**8**), and a tannin related compound, ethyl gallate (**2**), based on a preparative RP-HPLC purification of a crude tannin mixture obtained from gel chromatography. Briefly, a concentrated aqueous EtOH extract of fresh mango kernels was fractionated on a Diaion HP-20 column with methanol/water gradients (0:10 → 2.5:7.5 → 5:5 → 10:0, v/v). The methanol/water (2.5:7.5, v/v) eluted fraction was further fractionated on an MCI-gel CHP-20P with water, water/methanol gradients (9:1 → 8.5:1.5 → 8:2 →7.5:2.5 →7:3 → 6.5:3.5 → 6:4 → 3:7), and methanol. Then, simultaneous isolation of the compounds **2**–**8** (Fig. 1) was attained by RP-HPLC purification of the water/methanol (7.5:2.5, v/v) eluate on A-324 (10 i.d. × 300 mm) ODS-A column (YMC-Pack) using CH_3CN–water (2:8, v/v) acidulated with 1 ml/L acetic acid. A 2 ml/min flow rate at column oven 40°C was used, and the eluates were monitored by a UV detector set at λ_{max} 280 nm.

The easy and fast isolation procedures described here for gallotannins could be useful for the rapid purification of such compounds from other gallotannins-rich plants including other morphological parts of *M. indica*.

Identification of the isolated compounds

Analysis of the data obtained from ESIMS and NMR spectroscopic experiments, ^1H, ^{13}C ^1H–^1H correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC), and comparison of the found data with literature ones, allowed identification of compounds **1** and **2** as gallic acid (Lee *et al*., 2005) and ethyl gallate (Cui *et al*., 2002), and galloylglucoses (**3**–**8**) as 2,3,4,6-tetra-*O*-galloyl-β-D-glucose (**3**) (Haddock *et al*., 1982), 1,2,3,4-tetra-*O*-galloyl-β-D-glucose (**4**) (Berardini *et al*., 2004), mixture of almost equal proportions of 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**5**) (Hagenah and Gross, 1993) and 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**6**) (Haddock *et al*., 1982), 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (**7**) (Haddock *et al*. 1982), and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**8**) (Haddock *et al*., 1982). Worthy, gallic acid (**1**), ethyl gallate (**2**), and the pentagalloylglucose (**8**) are the most abundant isolates.

Anti-T. vaginalis *activities of the isolated compounds*

Secondary metabolites from natural sources have played a unique role in the discovery of anti-infectious compounds. Studies on the inhibitory effects of plant extracts on the growth of *T. vaginalis* trophozoites evidenced that extracts from the volatile oils producing plants *Mentha piperita*, *Salvia officinalis*,

and *Tanacetum parthenium* demonstrate anti-*T. vaginalis* activity identical to MTZ (Ezz Eldin and Badawy, 2015; Sharafi *et al*., 2013). A commercial garlic-based product (Tomex®) has been also shown to significantly reduce the multiplication and motility of the *T. vaginalis* trophozoites (Ali, 2007). In another study, the hydrolyzable tannins containing ethyl acetate fraction of a *Eucalyptus* extract exhibited MTZ-like activity on the growth of the *Trichomonas* trophozoites (Hassani *et al*., 2013). Meanwhile, limited research on the effects of pure compounds from natural sources, such as berberine alkaloid (de Brum Vieira *et al*., 2015; Sharafi *et al*., 2013) and piperazinyl derivatives of betulinic acid (Innocente *et al*., 2014), have been reported.

Herein, the effect of a group of gallotannins and ethyl gallate from kernels of *M. indica* on the viability of *T. vaginalis* clinical isolates was estimated (see experimental section). Although the common chemical nature of the investigated compounds, galloyl esters of a glucose core (Fig. 1), their antiprotozoal activities were varied based on the molecular structure (Fig. 3). All the examined compounds (**2**–**8**) exhibited a remarkable dose-dependent and time-dependent decrease in the percentage of living trophozoites (Fig. 3). After 24 hours of incubation, ethyl gallate, a tannin-related compound, showed the highest inhibitory effect on the *T. vaginalis* trophozoites viability (IC_{50} 1.3 µg/ml, Fig. 4). The gallotannin with an unacylated O-3 position of the glucose core, 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (**7**), exerted a potent effect (IC_{50} 2.4 µg/ml), while the inhibitory effect of ~1:1 mixture of **5** and **6** on the *T. vaginalis* trophozoites was relatively low (IC_{50}36.1 µg/ml). The other gallotannins (**3**, **4** and **8**) exhibited

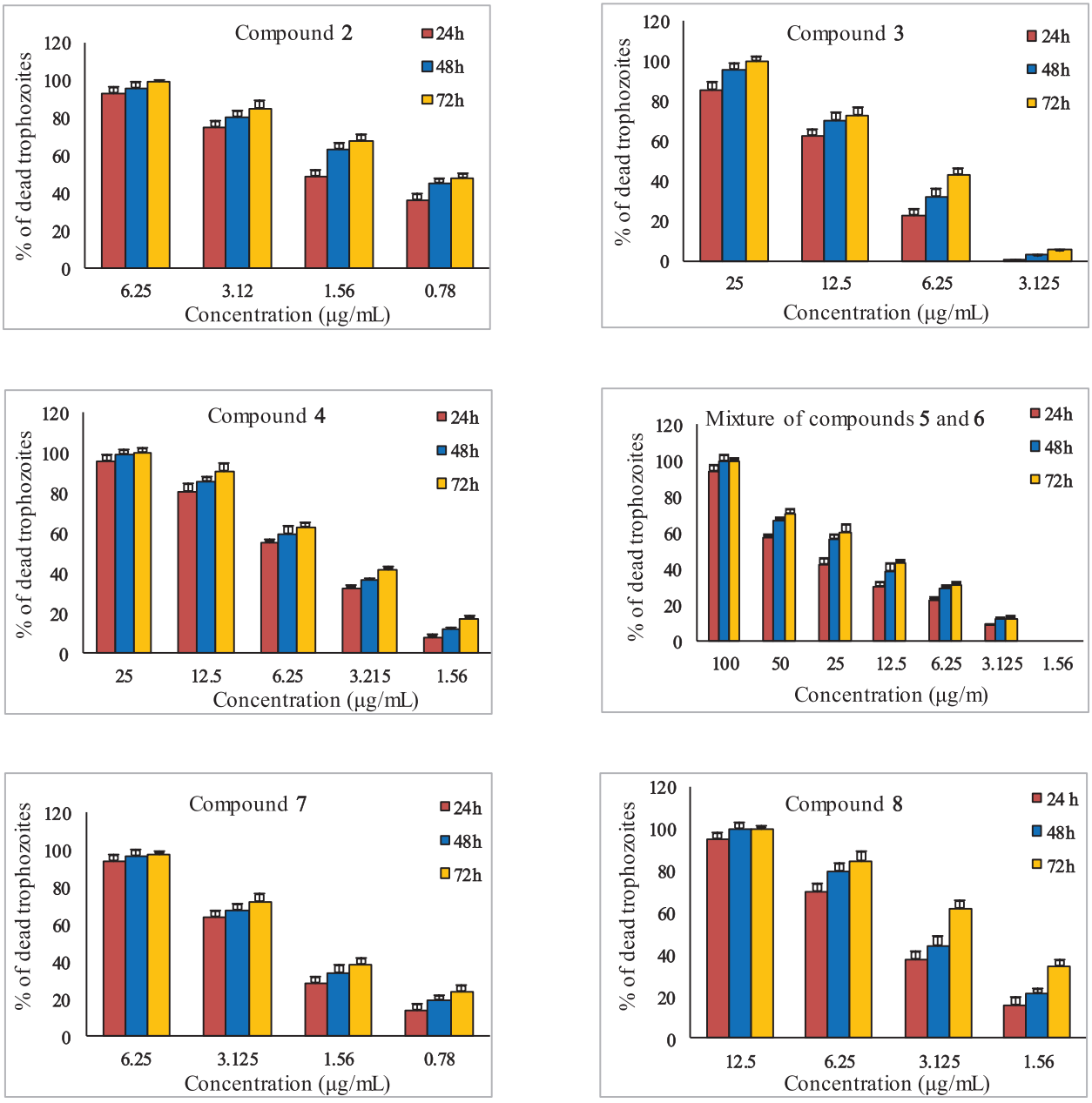

Figure 3. *In vitro* inhibitory effects of the compounds **2**–**8** on viability of *T. vaginalis* trophozoites. The bars represent standard division (SD) of the means.

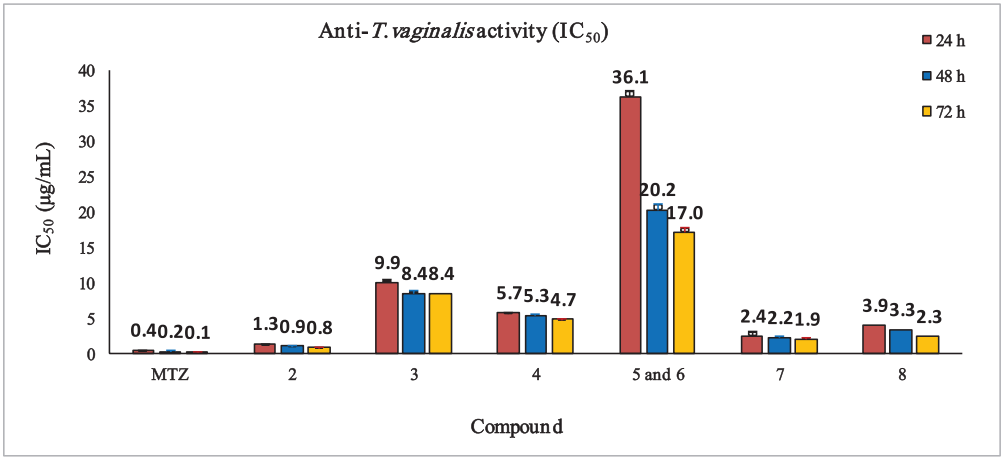

Figure 4. IC_{50} of the compounds **2–8** on *T. vaginalis* clinical isolate compared to a standard control (MTZ). The bars represent SD of the means.

noticeable anti-*T. vaginalis* activities with comparable potencies (IC_{50} 3.9 – 9.9 µg/ml). Due to the presence of some differences in the activity of the examined gallotannins, a structural activity relationship can't be generated because of the limited number of the investigated compounds.

The broad-spectrum antiprotozoal activities of the mango leaf and stem bark extracts (Núñez Sellés *et al*., 2002) against *Entamoeba histolytica* (Tona *et al*., 1998), *Histomonas meleagridis*, *Tetratrichomonas gallinarum* and *Blastocystis* sp (Grabensteiner *et al*., 2008), *Plasmodium falciparum* (Awe *et al*., 1998; Ruiz *et al*., 2011; Zirihi *et al*., 2005), and *Giardia lamblia* (Amaral *et al*., 2006) agree with our herein obtained results. In addition, the reported leishmanicidal activity of several tannins against amastigotes of *Leishmania donovani* (Kolodziej *et al*., 2001) also supports our results. Likewise, the antibacterial activity of gallotannins and/or extracts of mango kernels on different bacterial species have been also shown in several reports (El-Gied *et al*., 2012; Engels *et al*., 2010; Ka buki *et al*., 2000; Rajan *et al*., 2011; Rakholiya *et al*., 2015; Shabani and Sayadi, 2014; Subbiya *et al*., 2013). The antimicrobial properties of the kernel gallotannins were ascribed to its ability to intermingle with proteins, hinder the enzyme activity (Rajan *et al*., 2011), and/or its ability to make a complex with metal ions such as iron (Engels *et al*., 2009). Altogether, the anti-*T. vaginalis* activity of the isolated gallotannins may be attributed to either or all of the aforementioned mechanisms. *T. vaginalis* uses the iron-containing proteins lactoferrin and hemoglobin (Sehgal *et al*., 2012 delivered by the menstruation blood (Figueroa-Angulo *et al*., 2012). A study on the effect of iron deficiency in the host on *T. vaginalis* demonstrated changes in the parasite propagation, cytotoxicity, and immune evasion (Alvarez-Sánchez *et al*., 2007). Therefore, iron deficiency by complexation with gallotannins may be the cause of the trophozoite cellular damage and the parasite survival inhabitation at the experimental conditions.

CONCLUSION

We are reporting here on the accumulation of gallotannins (galloylglucoses), gallic acid, and gallate derivatives in mango kernels. The procedure as described here is an easy and fast isolation for gallotannins that could be useful for the preparation of these compounds either as a crude fraction or single pure sample from the kernels and from gallotannins-rich plant extracts including other morphological parts of *M. indica*. The present study demonstrated, to the first time, that the mango kernels along with its isolated gallotannins and ethyl gallate could be used for further studies on the development of novel preventive or therapeutic agents for the treatment of trichomoniasis. However, it is still required to achieve animal lab-work and more mechanistic studies to approve our *in vitro* finding.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdel-Mageed WM, Bayoumi SA, Chen C, Vavricka CJ, Li L, Malik A, Dai H, Song F, Wang L, Zhang J, Gao GF, Lv Y, Liu L, Liu X, Sayed HM, Zhang L. Benzophenone *C*-glucosides and gallotannins from Mango tree stem bark with broad-spectrum anti-viral activity. Bioorg Med Chem, 2014; 22 (7):2236–43.

Ahmed A, Saeid D, Eman A, Reham E. Egyptian mango by-product 1. Compositional quality of mango seed kernel. Food Chem, 2007; 103(4):1141–52.

Ali NM. *In vitro* activity of commercially available Egyptian propolis on *Trichomonas vaginalis*. New Egypt J Med, 2007; 36(1):7–15.

Alvarez-Sánchez ME, Solano-González E, Yañez-Gómez C, Arroyo R. Negative iron regulation of the CP65 cysteine proteinase cytotoxicity in *Trichomonas vaginalis*. Microbes Infect, 2007; 9(14–15):1597–605.

Amaral FM, Ribeiro MNS, Barbosa-Filho JM, Reis AS, Nascimento FR, Macedo RO. Plants and chemical constituents with giardicidal activity. Revista Brasileira de Farmacognosia, 2006; 16: 696–720.

Awe SO, Olajide OA, Oladiran OO, Makinde JM. Antiplasmodial and antipyretic screening of *Mangifera indica* extract. Phytother Res, 1998; 12(6):437–8.

Berardini N, Carle R, Schieber A. Characterization of gallotannins and benzophenone derivatives from Mango (*Mangifera indica* L. cv.'Tommy Atkins') peels, pulp and kernels by high-performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun Mass Sp, 2004; 18(19):2208–16.

Cudmore SL, Delgaty KL, Hayward-McClelland SF, Petrin DP, Garber GE. Treatment of infections caused by metronidazole-resistant *Trichomonas vaginalis*. Clin Microbiol Rev, 2004; 17(4):783–93.

Cui CB, Zhao QC, Cai B, Yao XS Osadsa H. Two new and four known polyphenolics obtained as new cell-cycle inhibitors from *Rubus aleaefolius* Poir. J Asian Nat Prod Res, 2002; 4(4):243–52.

de Brum Vieira P, Giordani RB, Macedo AJ, Tasca T. Natural and synthetic compound anti-*Trichomonas vaginalis*: an update review. Parasitol Res, 2015; 114(4):1249–61.

El-Gied AAA, Joseph MR, Mahmoud IM, Abdelkareem AM, Al Hakami AM, Hamid ME. Antimicrobial activities of seed extracts of Mango (*Mangifera indica* L.). Adv Microbiol, 2012; 2(4):571–6.

Engels C, Gänzle MG, Schieber A. Fractionation of gallotannins from mango (*Mangifera indica* L.) kernels by high-speed counter-current chromatography and determination of their antibacterial activity. J Agric Food Chem, 2010; 58(2):775–80.

Engels C, Knödler M, Zhao YY, Carle R, Gänzle MG, Schieber A. Antimicrobial activity of gallotannins isolated from Mango (*Mangifera indica* L.) kernels. J Agric Food Chem, 2009; 57(17):7712–18.

Ezz Eldin HM, Badawy AF. *In vitro* anti-*Trichomonas vaginalis* activity of *Pistacia lentiscus* mastic and *Ocimum basilicum* essential oil. J Parasit Dis, 2015; 39(3):465–73.

Figueroa-Angulo EE, Rendón-Gandarilla FJ, Puente-Rivera J, Calla-Choque JS, Cárdenas-Guerra RE, Ortega-López J, Arroyo R. The effects of environmental factors on the virulence of *Trichomonas vaginalis*. Microbes Infect, 2012; 14(15):1411–27.

Gan RY, Kong KW, Li HB, Wu K, Ge YY, Chan CL, Shi XM, Corke H. Separation, identification, and bioactivities of the main gallotannins of red sword bean (*Canavalia gladiata*) coats. Front Chem, 2018; 6:39.

Grabensteiner E, Liebhart D, Arshad N, Hess M. Antiprotozoal activities determined in vitro and in vivo of certain plant extracts against *Histomonas meleagridis*, *Tetratrichomonas gallinarum* and *Blastocystis* sp. Parasitol Res, 2008; 103(6):1257–64.

Haddock EA, Gupta RK, Al-Shafi SM, Haslam E, Magnolato D. The metabolism of gallic acid and hexahydroxydiphenic acid in plants. Part 1. Introduction. Naturally occurring galloyl esters. J Chem Soc Perkin Transactions, 1982; 1:2515–24.

Hagenah S, Gross GG. Biosynthesis of 1, 2, 3, 6-tetra-*O*-galloyl-β-D-glucose. Phytochemistry, 1993; 32:637–41.

Hassani S, Asghari G, Yousefi H, Kazemian A, Rafieiean M, Darani HY. Effects of different extracts of *Eucalyptus camaldulensis* on *Trichomonas vaginalis* parasite in culture medium. Adv Biomed Res, 2013; 2:47.

Ibrahim AN. Comparison of *in vitro* activity of metronidazole and garlic-based product (Tomex®) on *Trichomonas vaginalis*. Parasitol Res, 2013; 112(5):2063–7.

Innocente AM, de Brum Vieira P, Frasson AP, Casanova BB, Gosmann G, Gnoatto SCB, Tasca T. Anti-*Trichomonas* vaginalis activity from triterpenoid derivatives. Parasitol Res, 2014; 113(8):2933–40.

Ka buki T, Nakajima H, Arai M, Ueda S, Kuwabara Y, Dosako S. Characterization of novel antimicrobial compounds from Mango (*Mangifera indica* L.) kernel seeds. Food Chem, 2000; 71(1):61–6.

Kolodziej H, Kayser O, Kiderlen AF, Ito *H*, Hatano T, Yoshida T, Foo LY. Antileishmanial activity of hydrolyzable tannins and their modulatory effects on nitric oxide and tumour necrosis factor-alpha release in macrophages in vitro, Planta Medica, 2001; 67(09):825–32.

Lee TH, Chiou JL, Lee CK, Kuo YH. Separation and determination of chemical constituents in the roots of *Rhus javanica* L. var. *roxburghiana*. J Chin Chem Soc, 2005; 52(4):833–41.

Masibo M, He Q. Major mango polyphenols and their potential significance to human health. Compr Rev Food Sci Food Saf, 2008; 7(4):309–19.

Núñez Sellés AJ, Vélez Castro HT, Agüero-Agüero J, González-González J, Naddeo F, De Simone F, Rastrelli L. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. J Agri Food Chem, 2002; 50(4):762–6.

Rajan S, Thirunalasundari T, Jeeva S. Anti—enteric bacterial activity and phytochemical analysis of the seed kernel extract of *Mangifera indica* Linnaeus against *Shigella dysenteriae* (Shiga, corrig.) Castellani and Chalmers. Asian Pac J of Trop Med, 2011; 4(4):294–300.

Rakholiya KD, Kaneria MJ, Chanda SV. *In vitro* assessment of novel antimicrobial from methanol extracts of matured seed kernel and leaf of *Mangifera indica* L.(Kesar Mango) for inhibition of *Pseudomonas* spp. and their synergistic. Am J Drug Discovery Dev, 2015; 5:13–23.

Ruiz L, Ruiz L, Maco M, Cobos M, Gutierrez-Choquevilca AL, Roumy V. Plants used by native Amazonian groups from the Nanay River (Peru) for the treatment of malaria. J Ethnopharmacol, 2011; 133(2): 917–21.

Sairam K, Hemalatha S, Kumar A, Srinivasan T, Ganesh J, Shankar M, Venkataraman S. Evaluation of anti-diarrhoeal activity in seed extracts of *Mangifera indica*. J Ethnopharmacol, 2003; 84(1):11–15.

Sehgal R, Goyal K, Sehgal A. Trichomoniasis and lactoferrin: future prospects. Infect Dis Obstet Gynecol, 2012; 2012:1–8.

Shabani Z, Sayadi A. The antimicrobial in vitro effects of different Mango kernel. J App Pharma Sci, 2014; 4(5):075–9.

Sharafi SM, Yousefi M, Yousefi HA, Asghari G, Darani HY. *In vitro* effects of various plants extracts on the growth of *Trichomonas vaginalis*. Infect Disord Drug Targets, 2013; 13(5):322–7.

Silver BJ, Guy RJ, Kaldor JM, Jamil MS, Rumbold AR. *Trichomonas vaginalis* as a cause of perinatal morbidity: a systematic review and meta-analysis. Sex Trans Dis, 2014; 41(6):369–76.

Subbiya A, Mahalakshmi K, Pushpangadan S, Padmavathy K, Vivekanandan P, Sukumaran VG. Antibacterial efficacy of *Mangifera indica* L. kernel and *Ocimum sanctum* L. leaves against *Enterococcus faecalis* dentinal biofilm. J Conserv Dent, 2013; 16(5):454–7.

Sutcliffe S, Neace C, Magnuson NS, Reeves R, Alderete JF. Trichomonosis, a common curable STI, and prostate carcinogenesis—a proposed molecular mechanism. PLoS Pathogens, 2012; 8(8):e1002801.

Tona, L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J Ethnopharmacol, 1998; 61(1):57–65.

Zirihi GN, Mambu L, Guédé-Guina F, Bodo B, Grellier P. *In vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. J Ethnopharmacol, 2005; 98(3):281–5.

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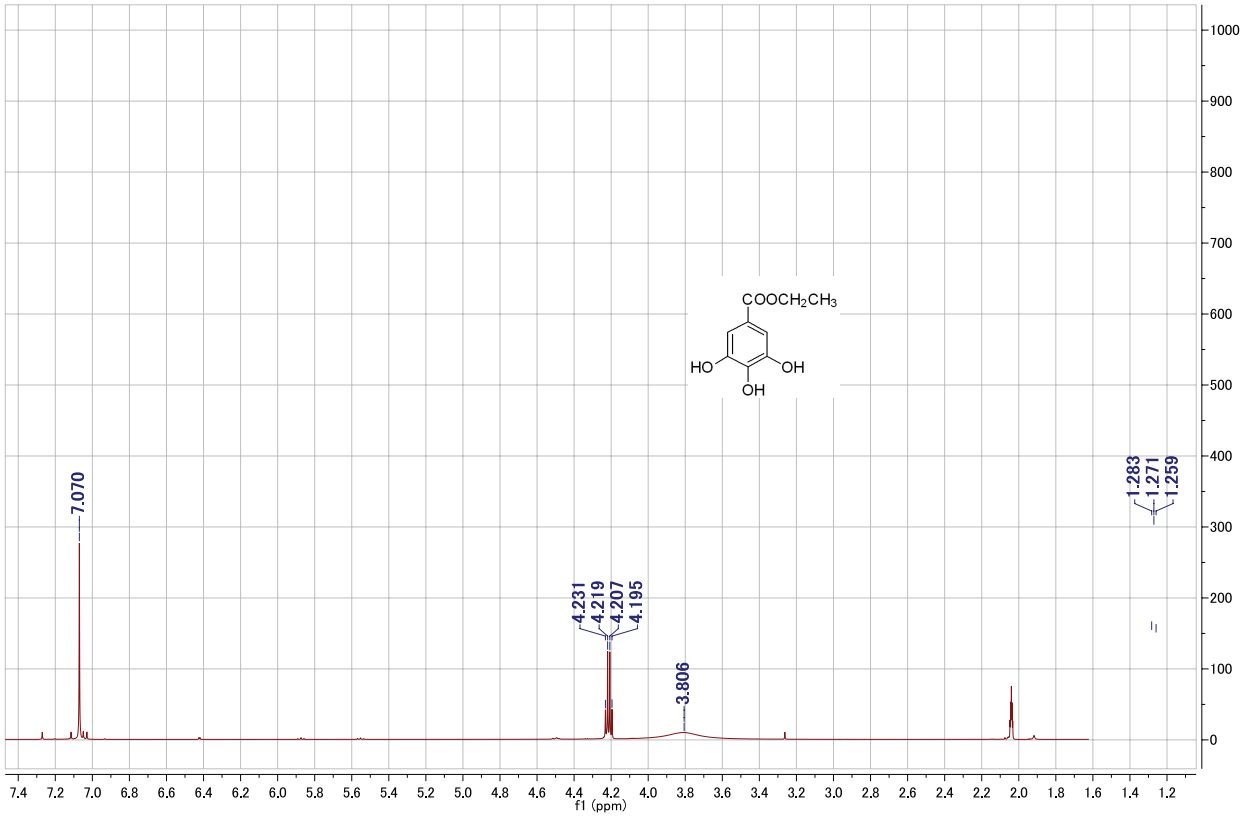
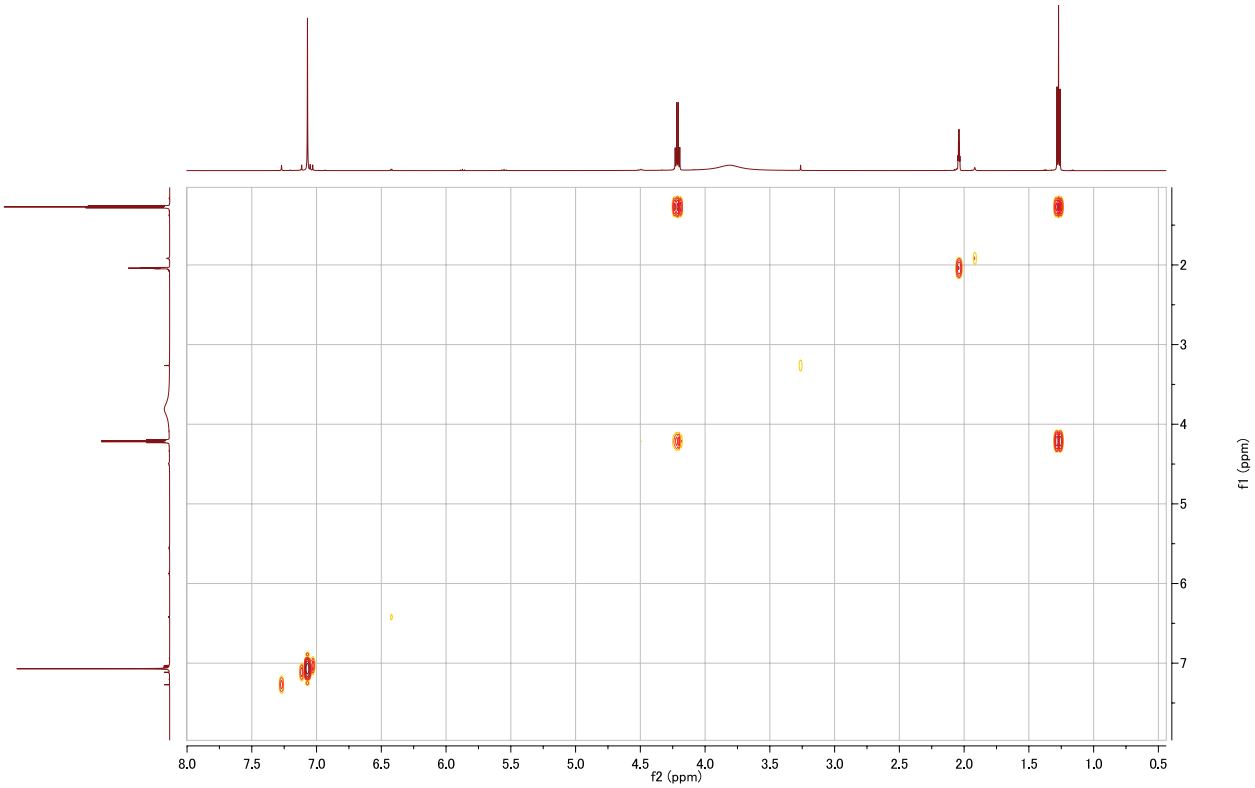
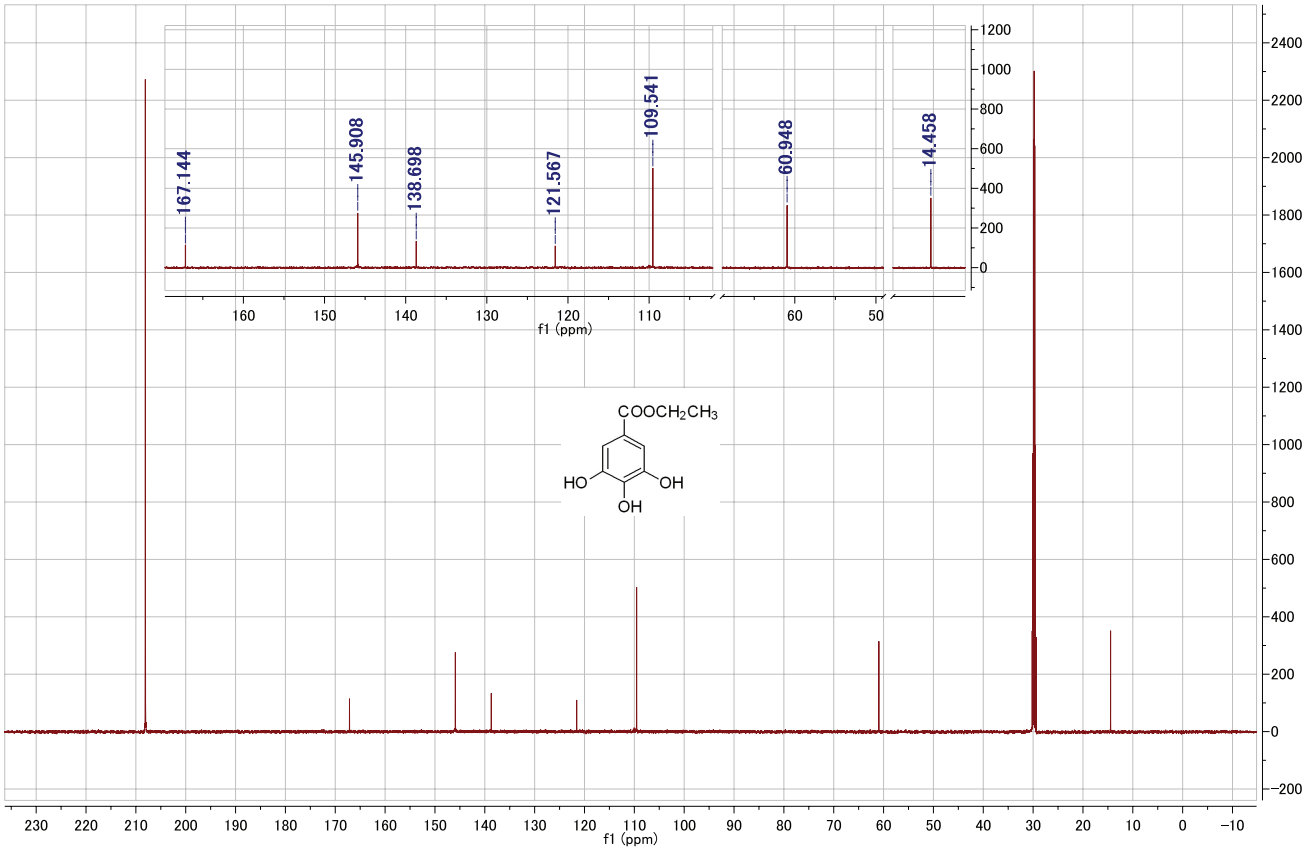
SUPPLEMENTARY MATERIAL



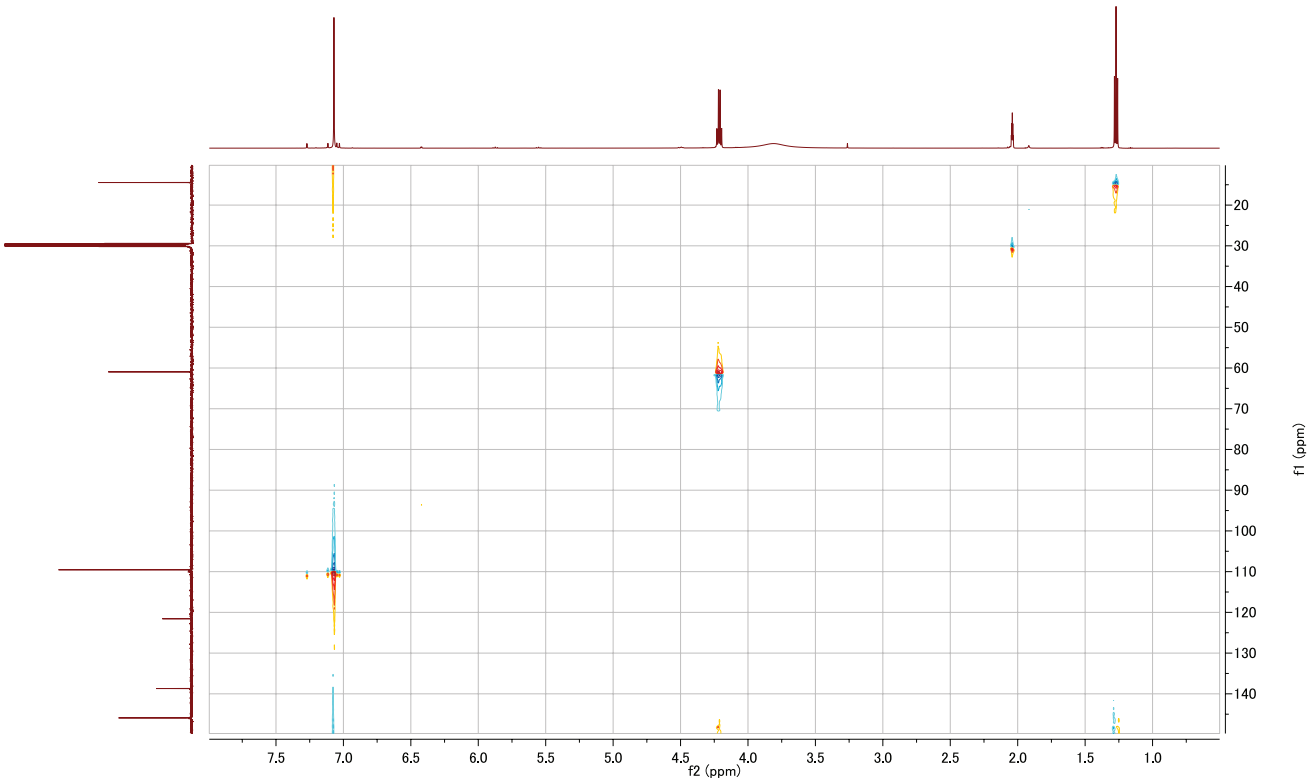
S1. ^{1}H NMR spectrum of **2** [600 MHz, (acetone-d_6 – D_2O, 9:1), 27 °C].



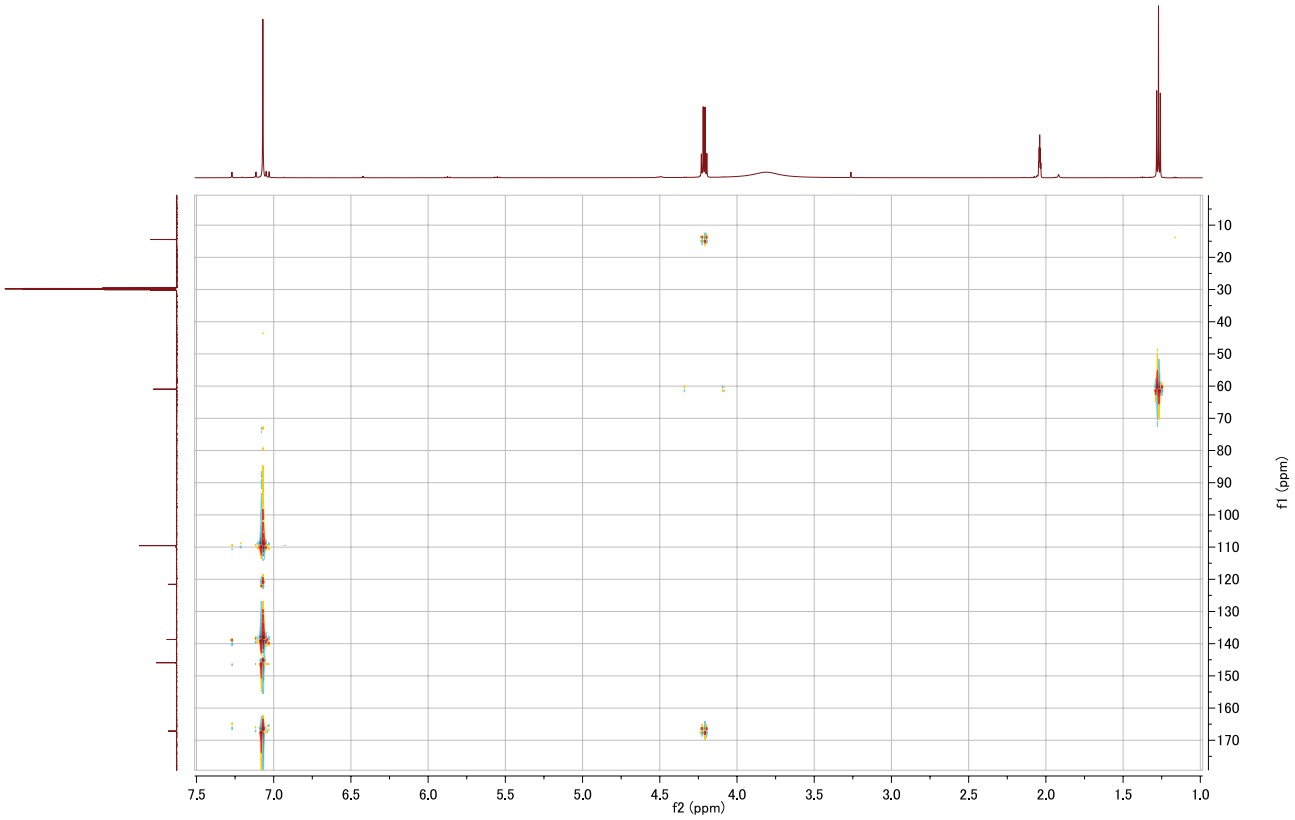
S2. ^{1}H–^{1}H COSY spectrum of **2** [600 MHz, (acetone-d_6 – D_2O, 9:1), 27 °C].



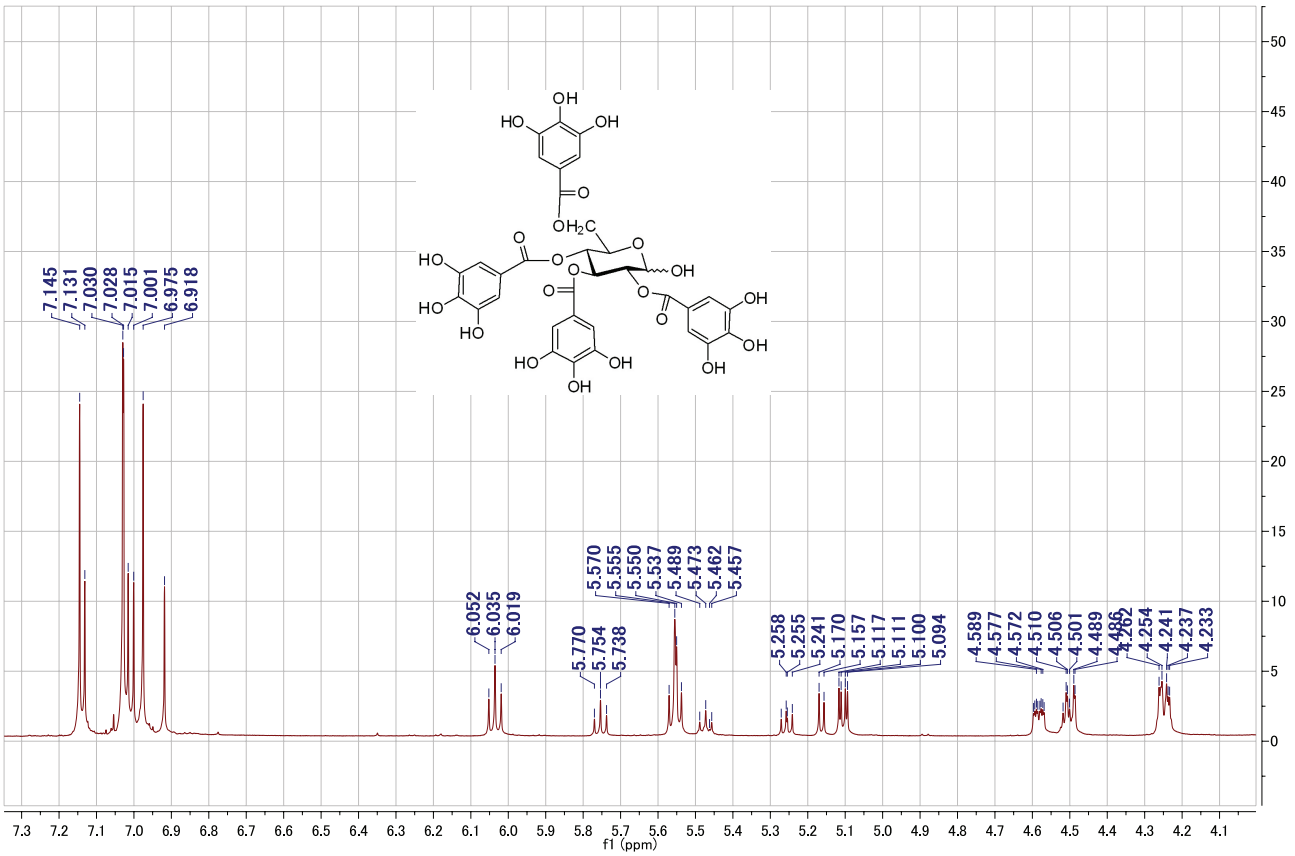
S3. ^{13}C NMR spectrum of **2** [151 MHz, (acetone-d_6 – D_2O, 9:1), 27 °C].



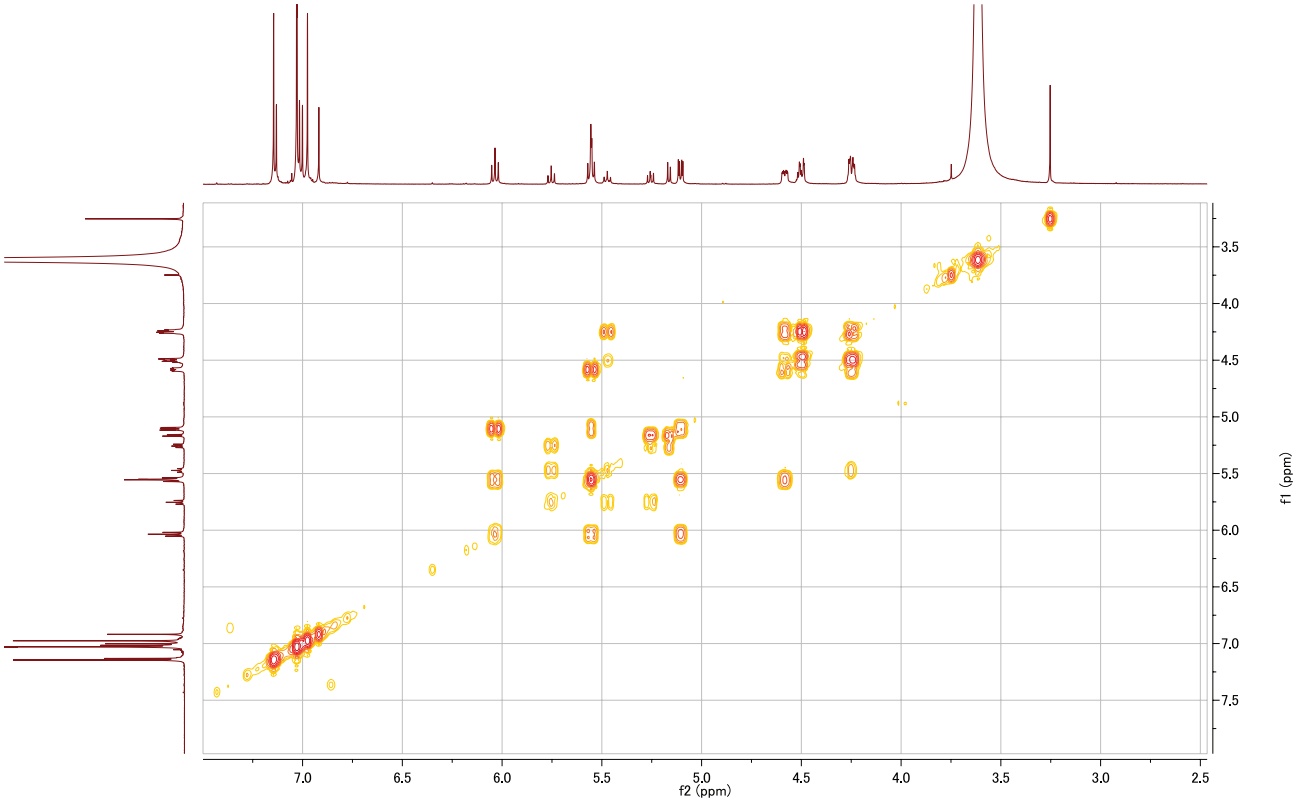
S4. HSQC spectrum of **2** [600 MHz, (acetone-d_6 – D_2O, 9:1), 27 °C].



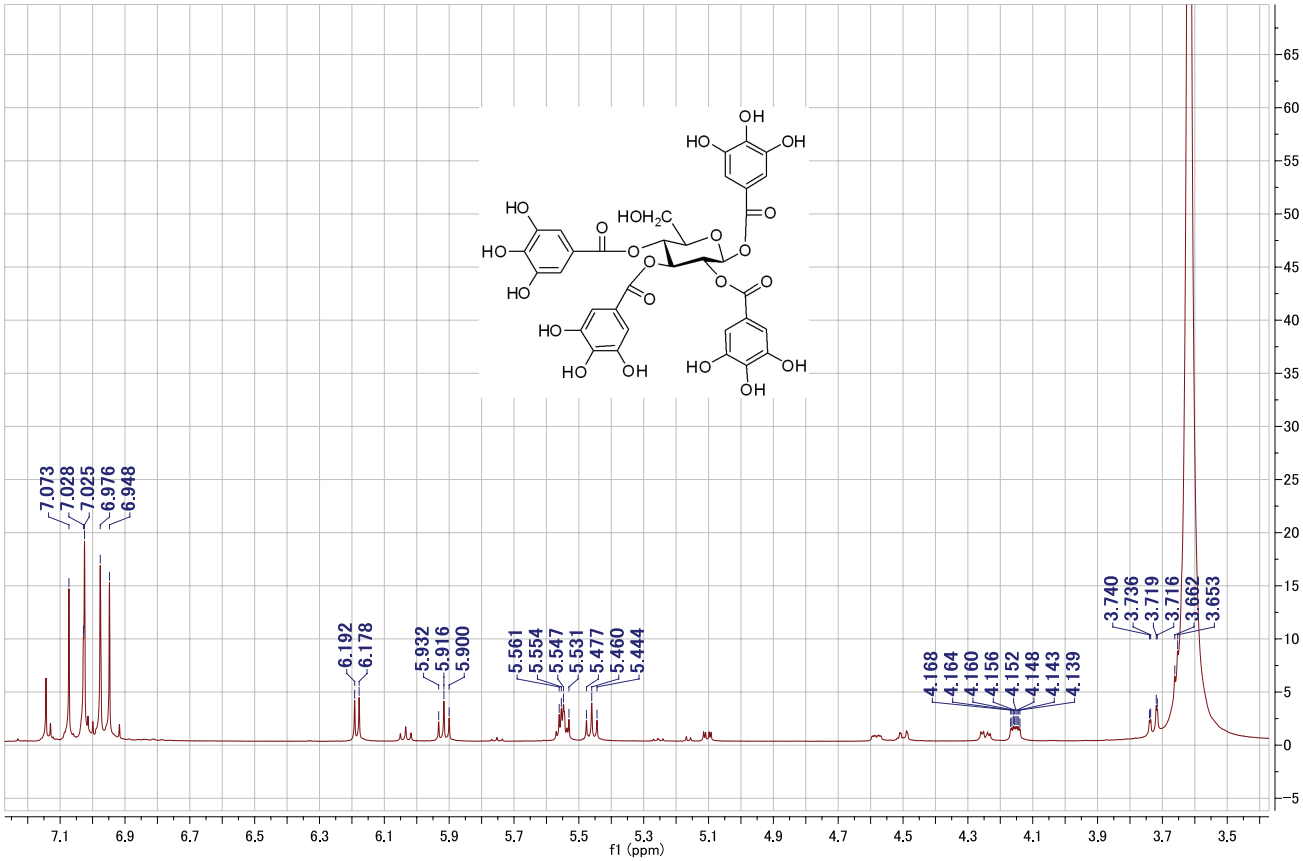
S5. HMBC spectrum of **2** [600 MHz, (acetone-d_6 – D_2O, 9:1), 27 °C].



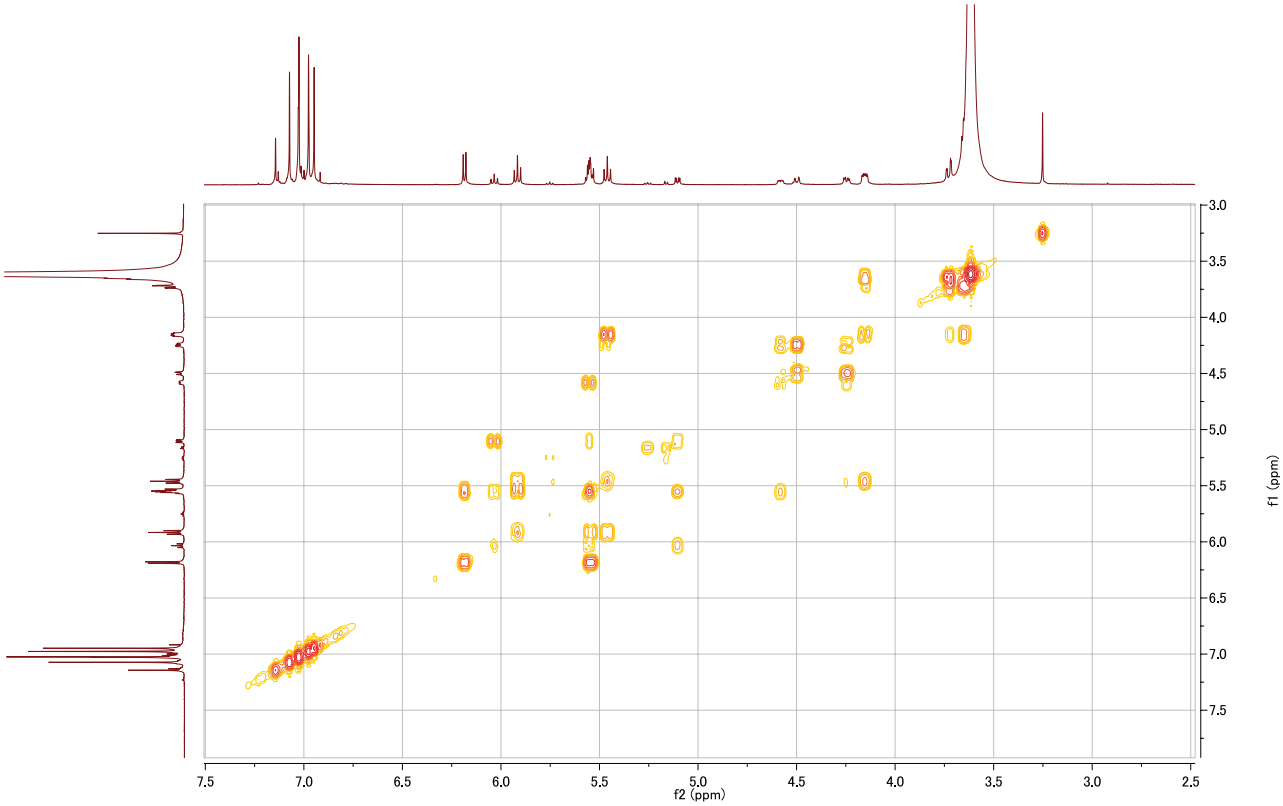
S6. ^{1}H NMR spectrum of **3** [600 MHz, (acetone-d_6 – D_2O, 9:1), 27 °C].



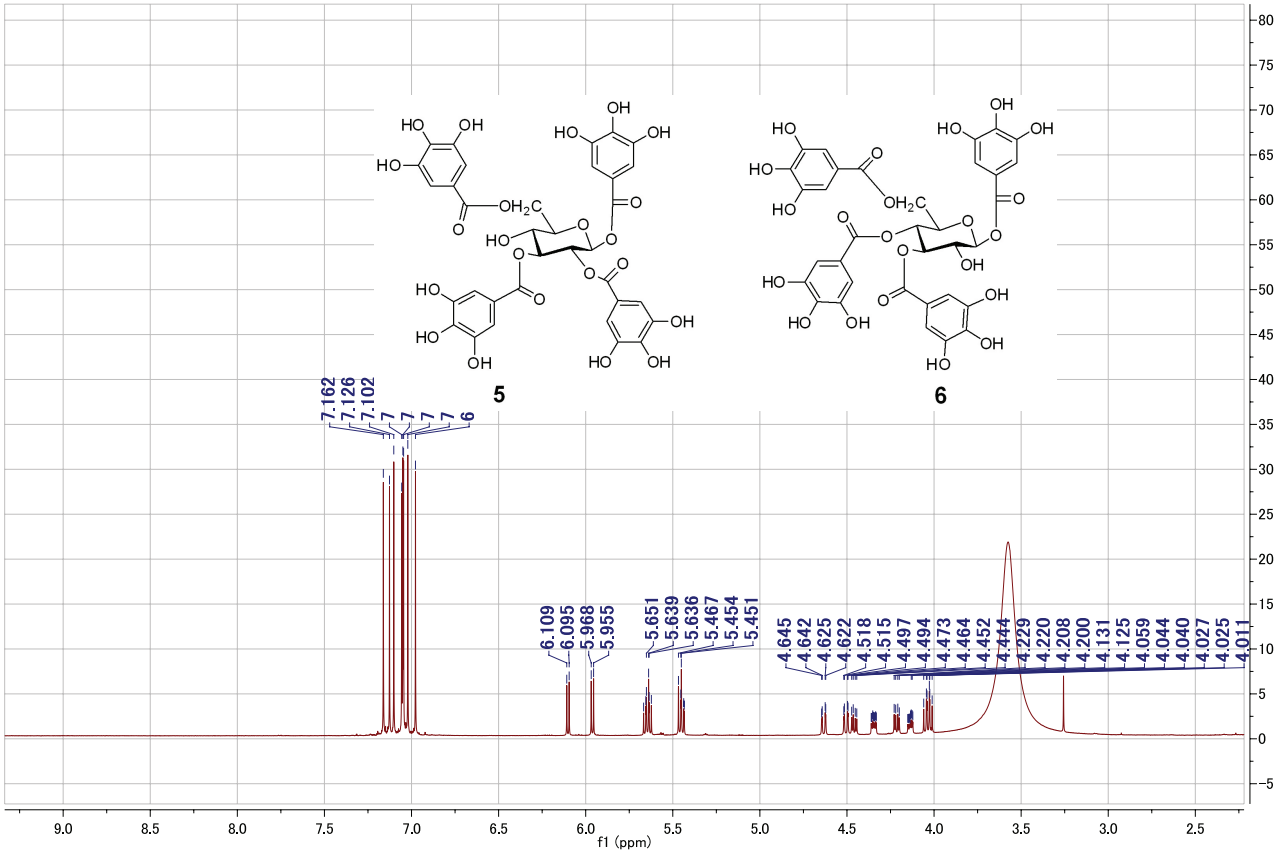
S7. ^{1}H–^{1}H COSY spectrum of **4** [600 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



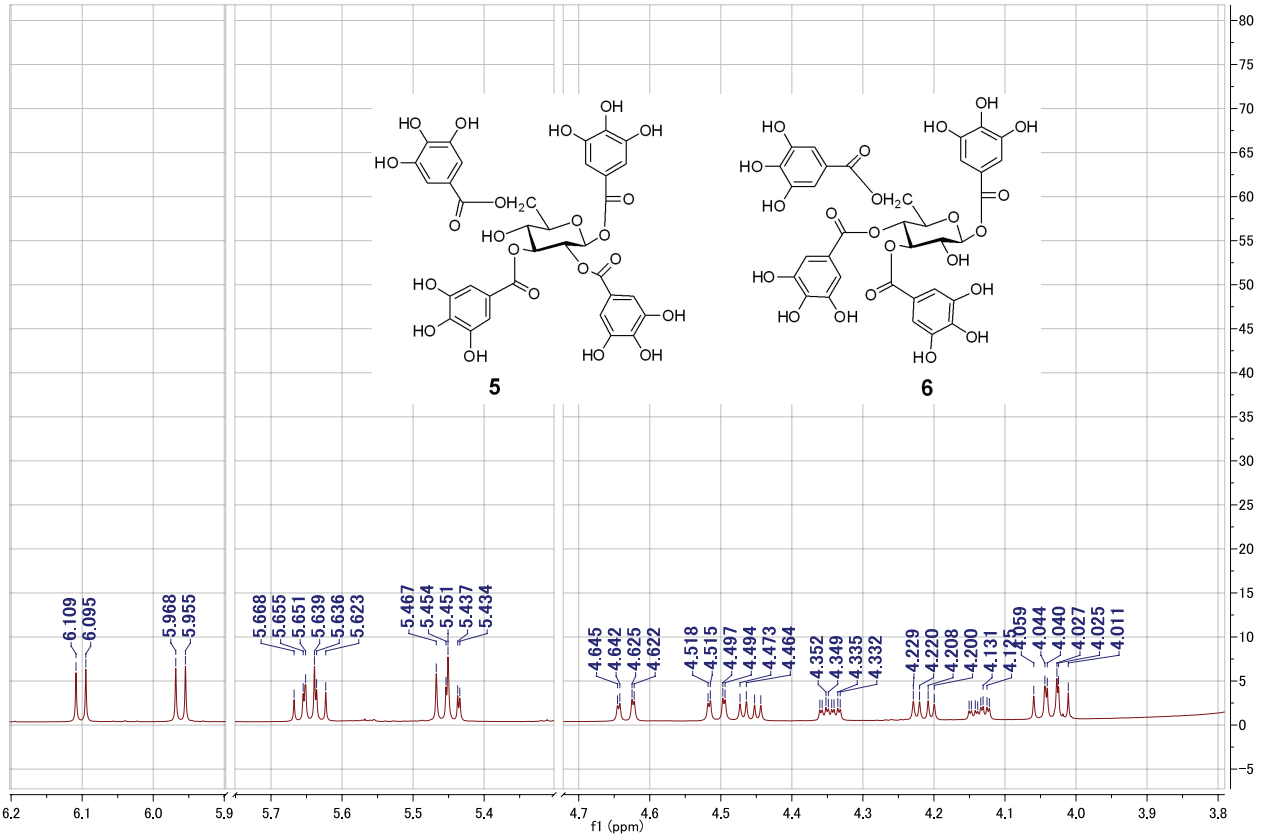
S8. ^{1}H NMR spectrum of **4** [600 MHz, (acetone-d_6 – D_2O, 9:1), 27 °C].



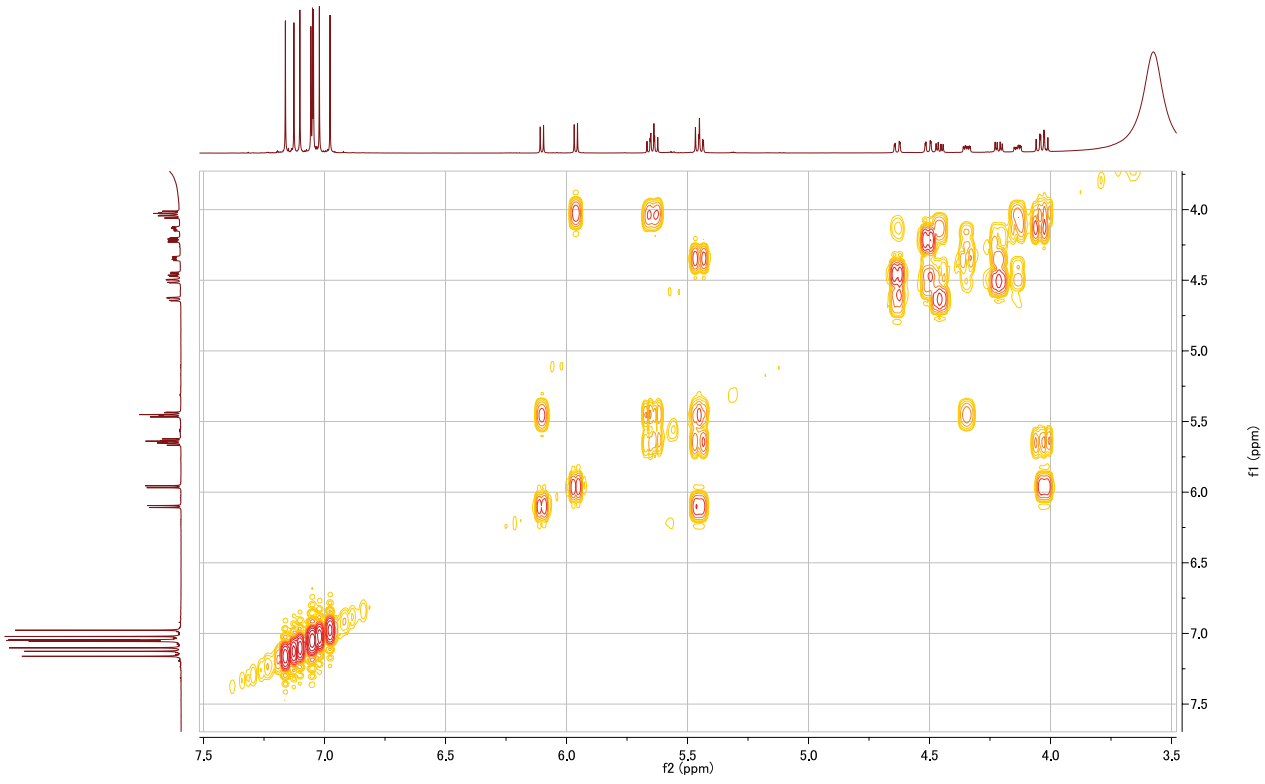
S9. ^{1}H–^{1}H COSY spectrum of **4** [600 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



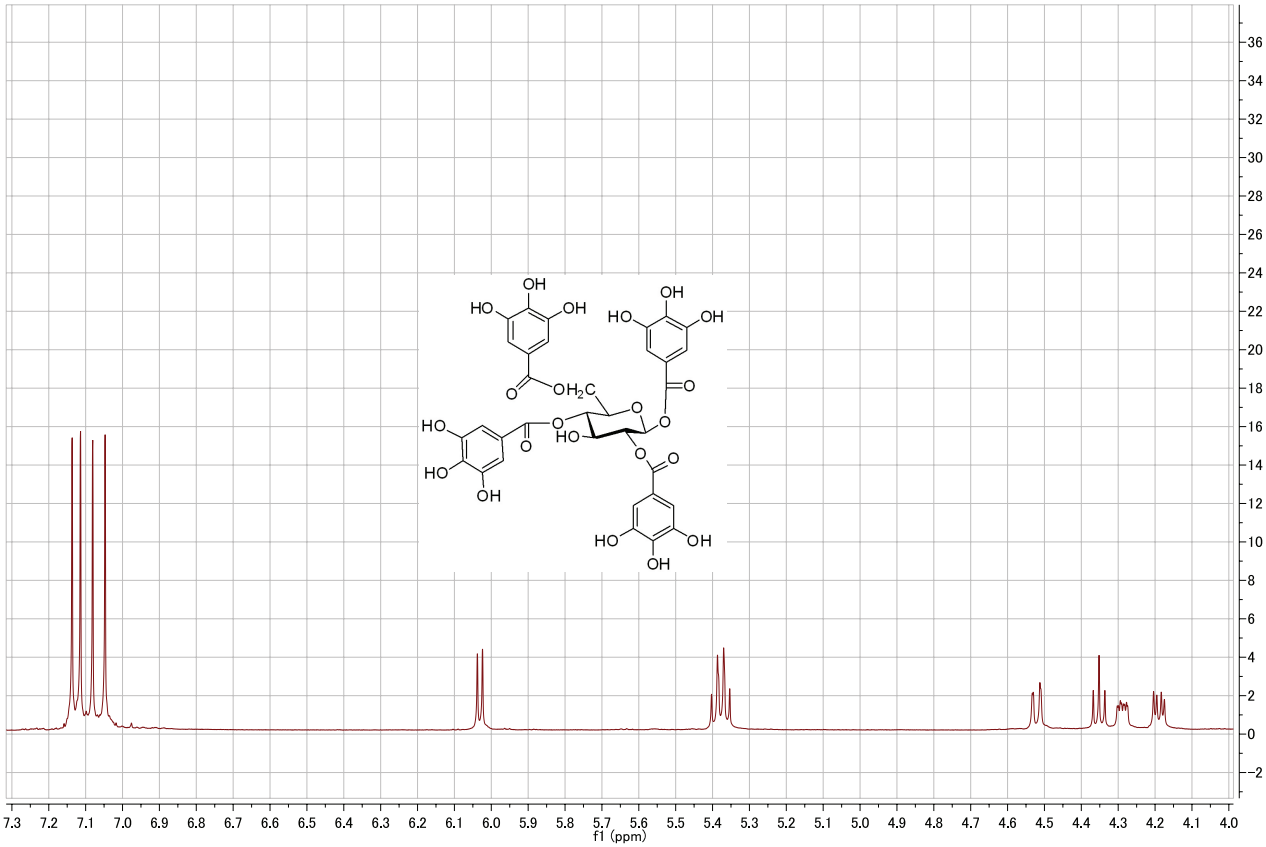
S10. ^{1}H NMR spectrum of mixture of **5** and **6** [600 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



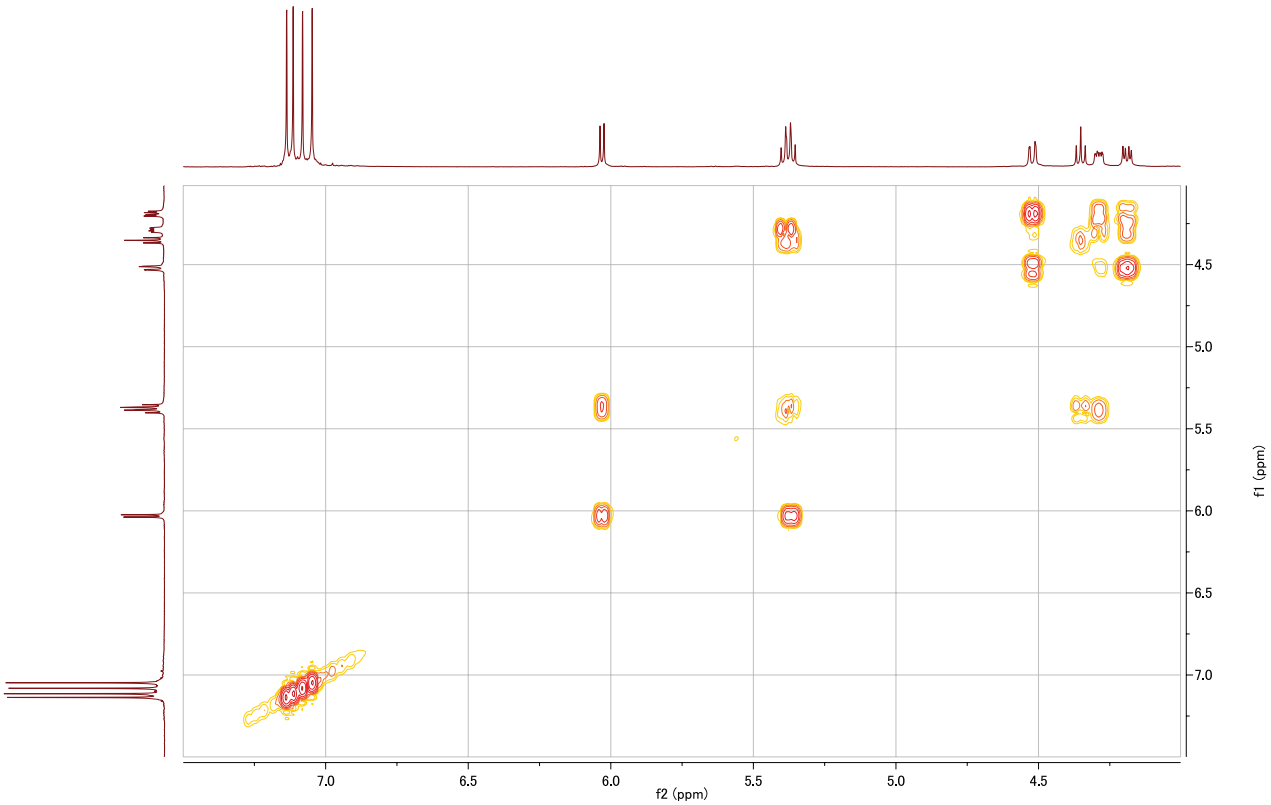
S11. Expanded sugar protons region of ^{1}H NMR spectrum of mixture of **5** and **6** [600 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



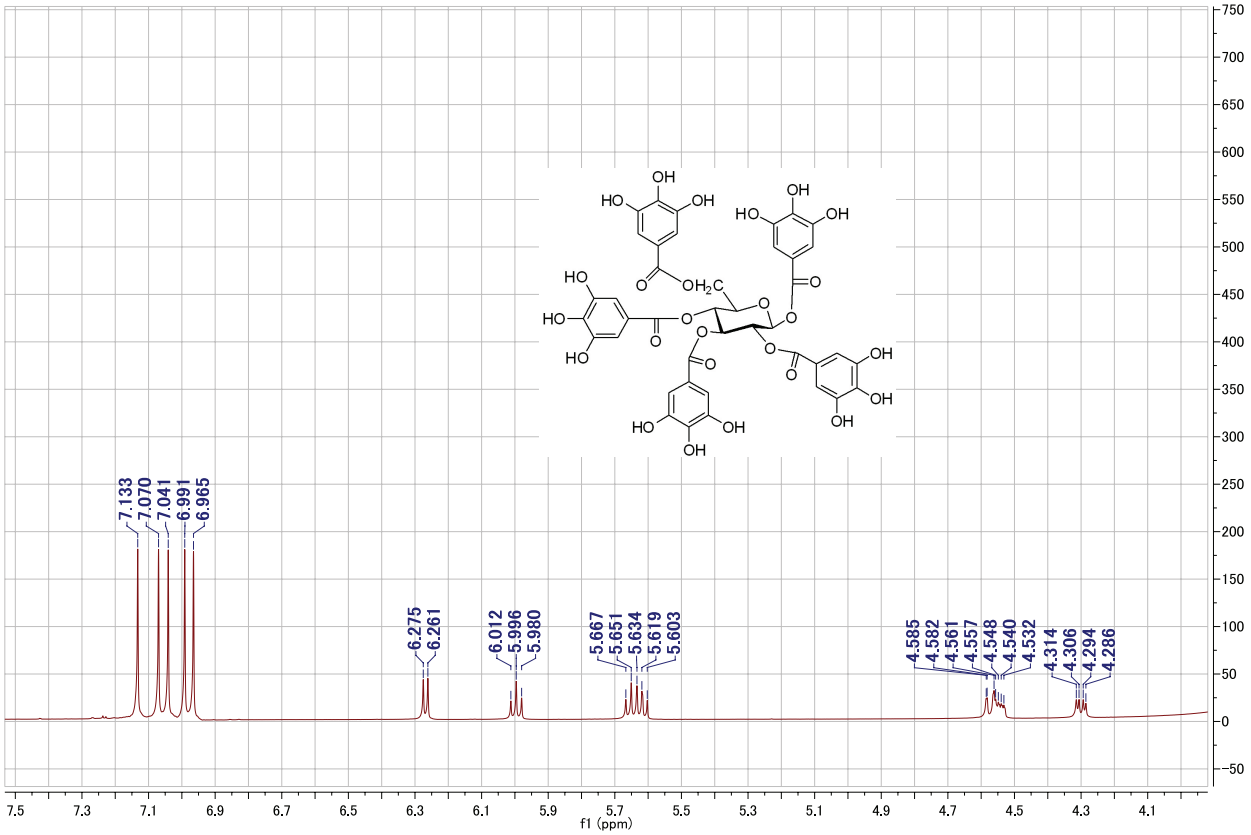
S12. ^{1}H – ^{1}H COSY spectrum of mixture of **5** and **6** [600 MHz, (acetone-d_6 D_2O, 9:1), 27 °C].



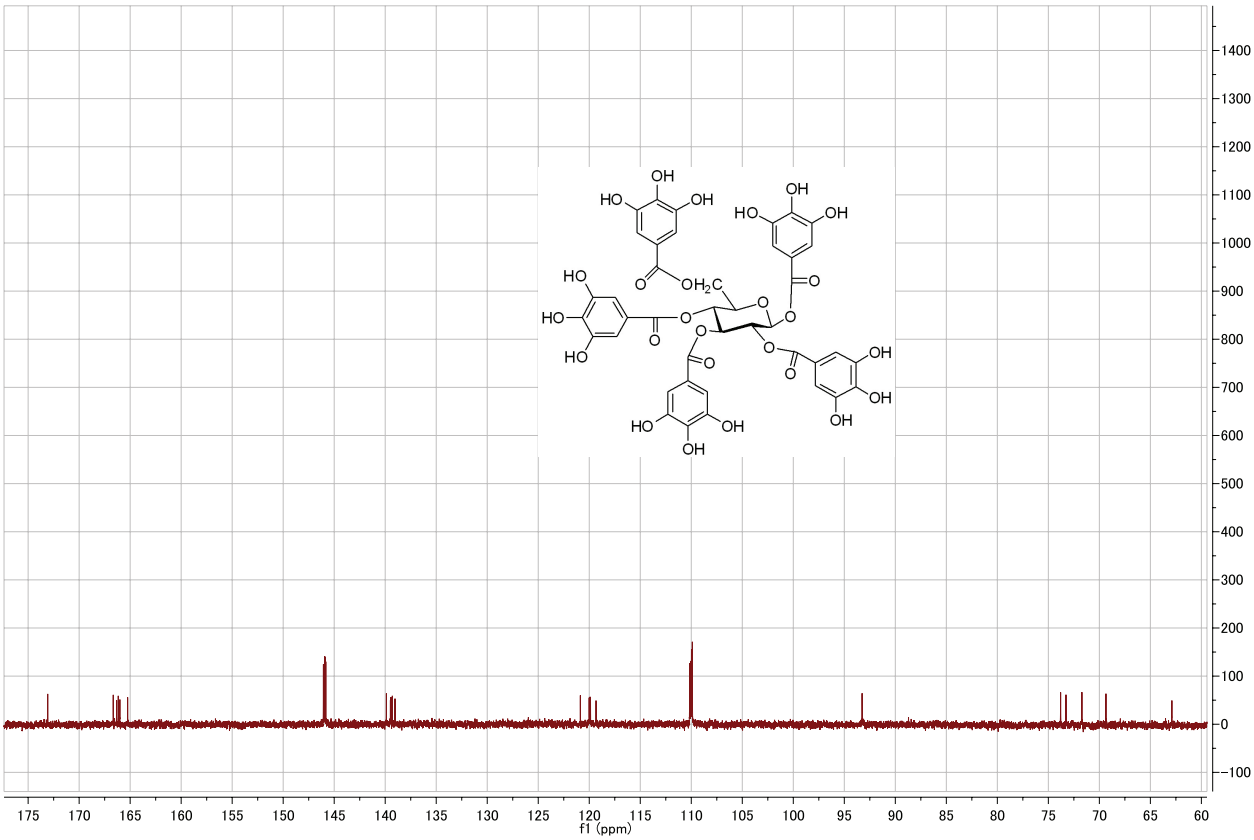
S13. ^{1}H NMR spectrum of **7** [600 MHz, (acetone-d_6 – D_2O, 9:1), 27 °C].



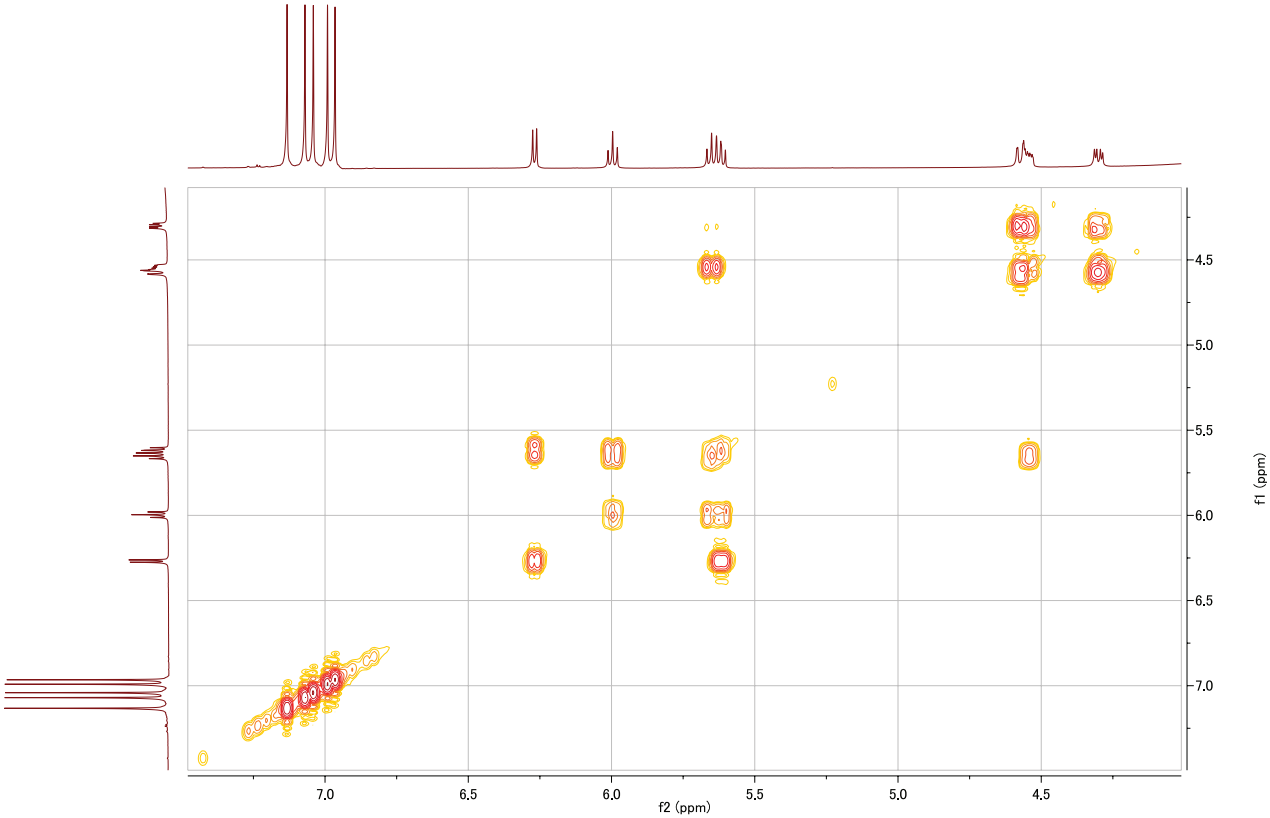
S14. ^{1}H – ^{1}H COSY spectrum of **7** [600 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



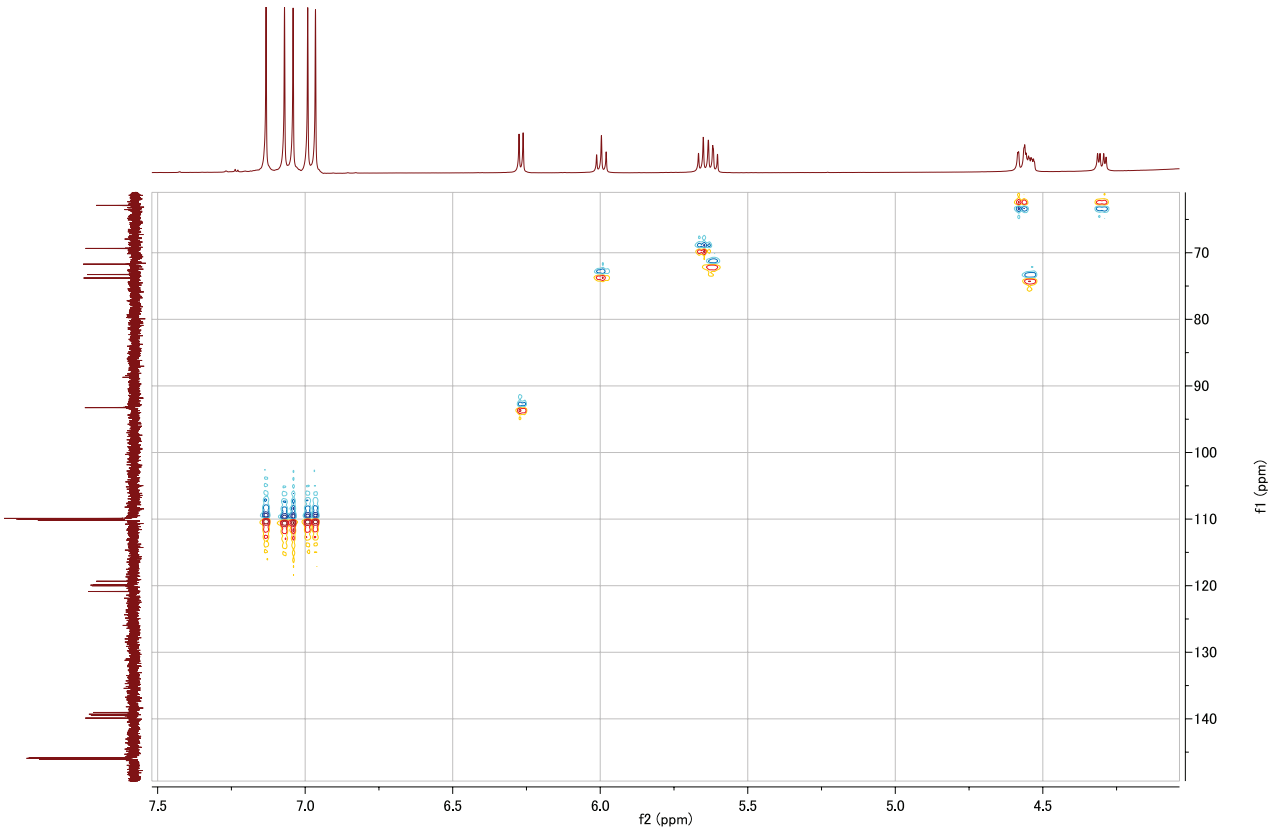
S15. ^{1}H NMR spectrum of **8** [600 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



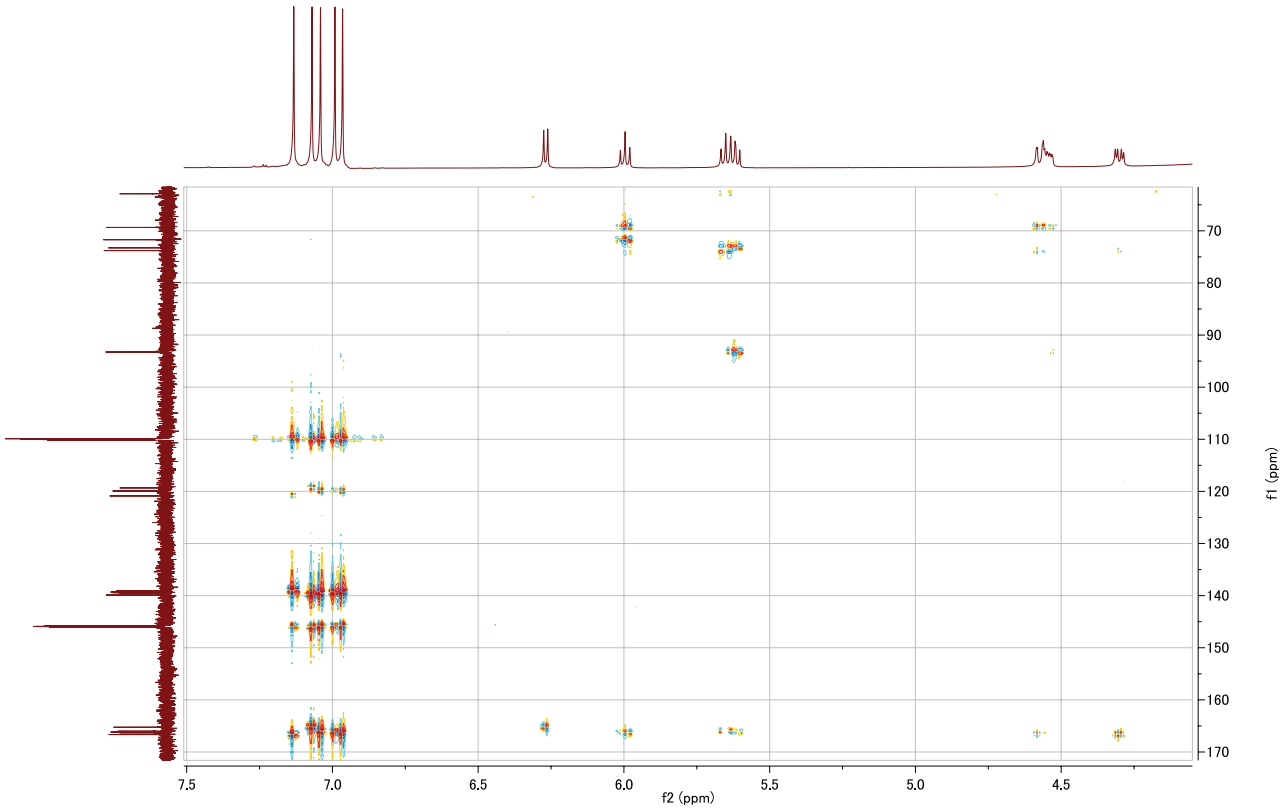
S16. ^{13}C NMR spectrum of **8** [151 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



S17. ^{1}H–^{1}H COSY spectrum of **8** [600 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



S18. HSQC spectrum of **8** [600 MHz, (acetone-d_6 –D_2O, 9:1), 27 °C].



S19. HMBC spectrum of **8** [600 MHz, (acetone-d_6–D_2O, 9:1), 27 °C].