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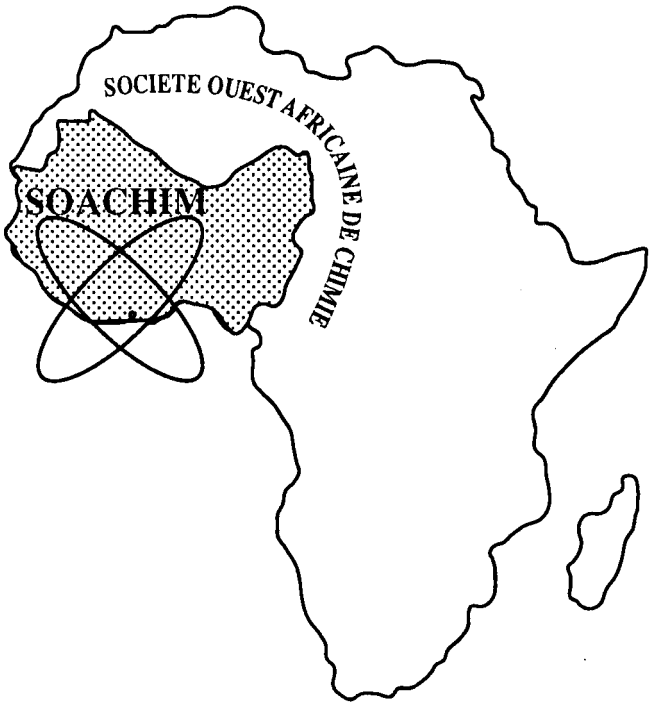

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Organic ginger (*Zingiberofficinale*) inhibits COX-1 and -2 enzymes and lipid peroxidation

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Abstract: The antioxidant, anti-inflammatory and tumor cell proliferation inhibitory activities of organically grown ginger (*Zingiberofficinale*) were evaluated. The water extract afforded a polysaccharide fraction with strong antioxidant activity as indicated by MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay. The methanolic extract of the resulting residue yielded compounds 10-gingerol (**1**), 6-gingerol (**2**), 8-gingerol (**3**) and hexahydrocurcumin (**4**). Compounds **1**, **2**, **3** and **4** inhibited lipid peroxidation (LPO) by 37, 78, 54 and 52%, respectively. Compound **1** showed the strongest inhibition (55 and 96%) against cyclooxygenase enzymes (COX-1 and -2). However, compound **2** showed selective COX-2 enzyme inhibitory activity (92%) at 25 µg/mL. Extracts and pure compounds showed weak human tumor cell proliferation inhibitory activity. Organic ginger contained at least three times more bioactive ingredients than the non organic ginger.

Keywords: Organic ginger, antioxidant, anti-inflammatory, anticancer.

Le gingembre organique (*Zingiber officinale*) inhibe les enzymes COX-1 et -2 et la peroxydation des lipides

Résumé: Les activitésantioxydante, anti-inflammatoire et anti prolifération des cellules cancéreuses du gingembre organique ont étéévaluées. L'extrait aqueux à forte teneur en polysaccharides a montré une forte activité anti-oxydante par la méthode de MTT[3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide]. L'extrait méthanoïque obtenu à partir des résidus issus de l'extraction aqueuse, a permis d'isoler le 10-gingérol (**1**), le 6-gingérol (**2**), le 8-gingérol (**3**) et l'hexahydrocurcumin (**4**). Les composés **1**, **2**, **3** et **4** ont inhibé la peroxydation des lipides (LPO) à 37, 78, 54 et 52%, respectivement. Le composé**1** a montré la plus forte inhibition (55 and 96%) contre les enzymes de cyclooxygénase de l'inflammation (COX-1 and -2). Cependant, le composé**2** a montré une inhibition sélective de l'enzyme COX-2 à92%à la dose de 25 µg/mL.Les extraits bruts du gingembre de même que les composés purs qui ont été isolés ont montré une faible activité anti-cancéreuse.Le gingembre organique contient au moins trois fois plus de matière active que le gingembre non-organique.

Keywords: Gingembre organique, activités antioxydant, anti-inflammatoire, anti-cancer.

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1. Introduction

The rhizome of *Zingiberofficinale* (Zingiberaceae) is one of the most widely used spices around the world for over 2000 years. It is a common condiment for a variety of compounded foods, beverages and traditional medicine [1]. The rhizome is rich source of pungent bioactive compounds of great importance, which has been recognized long before. These phenolic compounds include the gingerols as well as the shogaols[2]. Recently, ginger has received extensive attention as a botanical dietary supplement in the U.S.A. and Europe because of its anecdotal biological activities. Several studies reported that gingerol-related compounds from *Z. officinale* roots showed antioxidant, anti-inflammatory, antidiabetic, anti-tumor, antifungal activities [3-7]. However, the biological activity of water soluble compounds in ginger is not known. In addition, the effect of its extract on prostate, pancreatic and lung tumor cell proliferation inhibition is not yet reported. Therefore, the aim of thisstudywas to investigate the water and methanolic extracts of organic *Z.officinale* grown in USA, for their antioxidant, anti-inflammatory and human tumor cell proliferation inhibitory activities by using MTT, LPO, COX-1 and -2, and human tumor cell proliferation inhibitory assays.

2. Results and Discussion

2.1. Isolated compounds

The major compounds isolated from the methanolic extract by silica gel CombiFlash column, PTLC and identified by ^{1}H and ^{13}C NMR were (**1**)10-gingerol (0.015% of rhizome), (**2**) 6-gingerol (0.035%), (**3**) 8-gingerol (0.010%) and (**4**) hexahydrocurcumin (0.017%) (Fig.1). Mass balance calculation showed that water extract contained mainly polysaccharides (0.21%). The TLC profile indicated that polysaccharides fraction possessed four major compounds. The NMR spectrum showed that the polysaccharides contained a number of carboxylic acid moieties. The ^{13}C NMR spectrum and the TLC profiles of hydrolyzed polysaccharides suggested that the monosaccharide components were α– and β-pyranogalactoses. Further investigations are needed to elucidate the complete structure of the polysaccharides fraction.

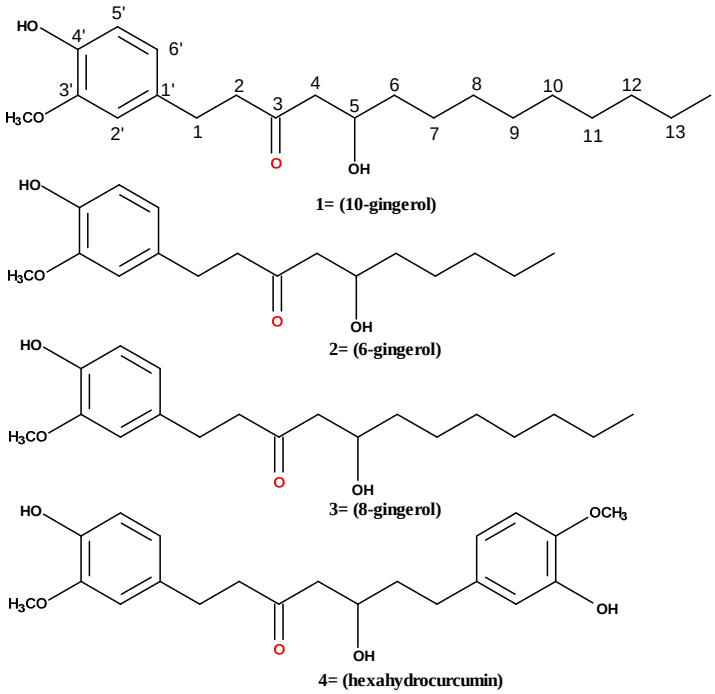

Figure 1: Structures of compounds **1-4** isolated from *Z. officinale* methanol extract

2.2. Antioxidant activity

The antioxidant activity was evaluated by using MTT and LPO assays. In the MTT assay, the water extract showed the strongest UV absorbance value of 0.6 at 250 µg/mL.At 25µg/mL, polysaccharides fraction showed an absorbance value of 0.27 where compounds **1-4** showed very weak absorbance indicating that the isolates were not efficient in reducing MTT (**Figure 2**).

LPO assay is an in vitro model for in vivo cellular lipid peroxidation. The reaction of free radicals with lipids, proteins, nucleic acids and other cellular molecules result in oxidative damage which is implicated in the etiology of a number of diseases including cancer, cardiovascular disease and arthritis [8]. The potential LPO inhibition by *Z. officinale* extracts and the isolates were tested at 50 and 25 µg/mL respectively. The water and methanolic extracts inhibited the LPO by 3 and 33%, compounds 1–4 by 37, 78, 54 and 52% and polysaccharides by 5%, respectively (**Figure 3**). Compound 2 showed the highest LPO inhibition by 78% comparable to BHA. Compounds 3 and 4 showed similar activity. The polysaccharide fraction was not active.

MTT and LPO assays showed that 6-gingerol had the best antioxidant activity followed by 8-gingerol, hexahydrocurcumin and 10-gingerol. The polysaccharide fraction and water extract showed high MTT activity but little or no LPO inhibition. It has been reported that polysaccharides had strong DPPH radical scavenging and hydroxyl radical-scavenging activities [9-11]. The apparent antioxidant activity of polysaccharides shown by MTT assay may be explained by the chelation of metal ions with polysaccharide chain. Therefore, the bonded metal ions could undergo redox reaction with MTT to form formazan blue. It is common that compounds with structure containing functional groups such as -OH, -SH, -COOH, $-PO_3H_2$, -CO, $-NR_2$, -S- and –O- are excellent chelators of transition metal ions [9,12].

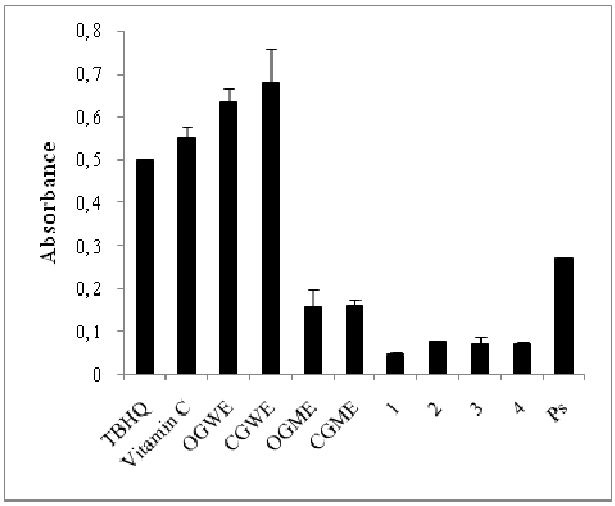

Figure 2: MTT activities of extracts and isolated compounds from *Z. officinale*. Commercial antioxidants, TBHQ and vitamin C, were tested at 25µg/mL, Compounds**1–4**, polysaccharides (Ps), water and methanolic extracts (OGWE, OGME, CGWE and CGME) were tested at 25 and 250µg/mL respectively.

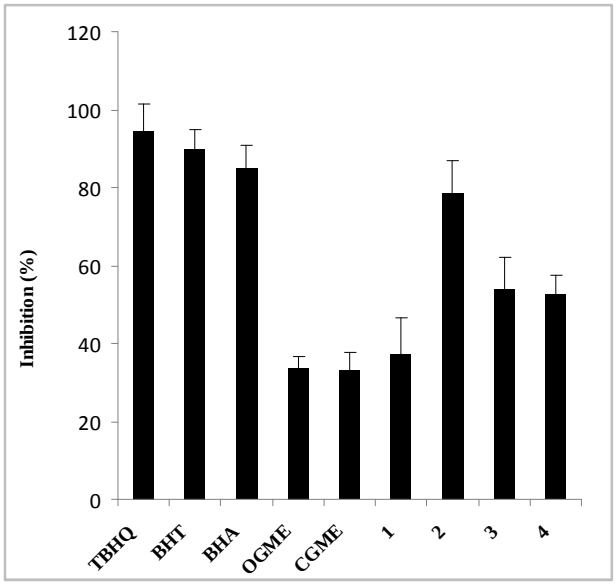

Figure 3: Lipid peroxidation (LPO) inhibitory activities of the extracts and isolated compounds from *Z. officinale*. Commercial antioxidants, TBHQ, BHT and BHA, were tested at 1.67, 2.2 and 1.67 µg/mL respectively. Oxidation of lipid was initiated by the addition of Fe^{2+} ions. Compounds **1–4**, polysaccharides (Ps), water and methanol extracts (OGWE, OGME, CGWE and CGME) were tested at 25 and 50 µg/mL respectively. The vertical bars represent the standard deviation of each data point (n = 2). The water extract and polysaccharides fraction were not active

2.3. Cyclooxygenase inhibition activity

The cyclooxygenase enzyme catalyzes the conversion of arachidonic acid to prostanoids including prostaglandins. Inhibition of COX-1 and COX-2 isozymes can result in the alleviation of symptoms of inflammation and pain. The COX-2 isozyme is over expressed in several pathological conditions such as diabetes, Alzheimer's disease and cancer [8].

Studies have shown that its selective inhibition was correlated with delayed onset or reduced progression of disease [13]. Anti-inflammatory activity was determined by COX-1 and COX-2 enzyme. *Z. officinale* water and methanolic extracts at 100 μg/mL inhibited COX-1 enzyme by 0 and 32% and COX-2 enzyme by 14 and 64%, respectively (Figure 4). The isolated compounds (1–4) and polysaccharide fraction inhibited COX-1 and -2 enzymes by 55 and 96%, 0 and 92%, 27 and 26%, 10 and 43% and 0 and 36%, respectively, at 25 μg/mL (Figure 4). The water extract, compound 2 and polysaccharides showed selective COX-2 inhibition but methanolic extract, compounds 1, 3 and 4 inhibited both COX-1 and -2 enzymes. The similarities in activities were evident between compound 1 and Celebrex, compound 2 and Vioxx and compound 3 and ibuprofen.

2.4. Tumor cell proliferation inhibition activity

Human tumor cell proliferation inhibitory assay showed weak activity for extracts and pure compounds from ginger (below 8% of inhibition) on all tumor cell lines tested.

3. Experimental Section

3.1. General experimental

All solvents used for isolation and purification were of ACS reagent grade (Aldrich Chemical Co., Inc., Milwaukee, WI). ^{1}H NMR spectra were recorded on a 500 MHz VRX instruments. ^{13}C NMR spectra were recorded at 125 MHz on the same instruments. Positive controls used in LPO assay, t-butyl hydroquinone (TBHQ), butylatedhydroxyanisol (BHA) and butylatedhydroxytoluene (BHT), were purchased from Sigma–Aldrich Chemical Company. Ram seminal vesicles [Oxford Biomedical Research, Inc. (Oxford, MI)] were used to prepare the COX-1 enzyme and the COX-2enzyme was prepared from insect cells cloned with human PGHS-2 enzyme. Arachidonic acid was purchased from Oxford Biomedical Research, Inc. (Oxford, MI). Positive controls (Aspirin, Naproxen and Ibuprofen) used in COX inhibitory assay were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Similarly, the non-steroidal anti-inflammatory drugs Celeberex™,and Vioxx were physician's professional samples and provided by Dr. Subash Gupta, Sparrow Pain Center, Michigan. HCT-116 (tumor origin, colon; histologic type, colon carcinoma) and AGS (tumor origin, stomach; histologic type, stomach carcinoma) were purchased from American Type Culture Collection (ATCC, Rockville, MD). MCF-7 (tumor origin, breast; histologic type, mammary adenocarcinoma; host strain, athymic nude),

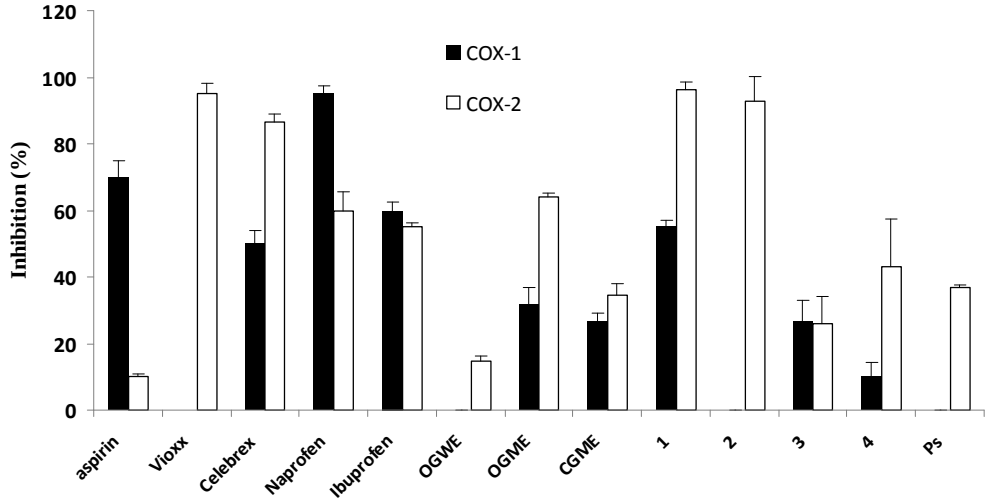

Figure 4: COX-1 and COX-2 enzyme inhibitory activity of extracts, isolated compounds **1–4** and polysaccharides (Ps) from *Z. officinale*. The positive controls, commercial NSAIDs, Aspirin, Celebrex, Vioxx, Naproxen and Ibuprofen, were tested at 108, 1, 1, 15 and 12 μg/mL. Water and methanolic extracts (OGWE, OGME, CGWE and CGME), compounds **1–4** and polysaccharides (Ps) were tested at 100 μg/mL and 25 respectively. Vertical bars represent the standard deviation of each data point (n = 2). Water extract from conventional ginger (CGWE) was not active.

SF-268 (tumor origin, central nervous system (CNS); histologic type, glioblastoma; host strain, athymic nude) and NCI-H460 (tumor origin, lung; histologic type, large cell carcinoma; host strain, athymic nude) were obtained from the National Cancer Institute (NCI, Bethseda, MD). MCF-7, SF-268, and NCI-H460 are the cell lines used by the Developmental Therapeutics Program (DTP) of the NCI as a prescreen tool for the discovery of anticancer agents. All cell lines were maintained in the Bioactive Natural Products and Phytoceuticals Laboratory at Michigan State University.

3.2. Plant material

The rhizomes of organic and conventional ginger (*Z. officinale*) were purchased fromorganic food store and supermarket, respectively, in Okemos, MI(USA) and stored at -20°C for two days till extraction.

3.3. Extraction isolation and identification

3.3.1 General

Organic ginger (960 g) was blended with water (1.5L) and centrifuged. The supernatant was lyophilized to yield dried water extract (39.5 g). The residue from centrifugation was soaked in methanol (1.5 L) for 2 h, centrifuged (10 min) and the supernatant was evaporated under vacuum to obtain methanolic extract (4.9 g). All the extractions were performed in triplicate. The conventional ginger was extracted in the similar manner.

3.3.2 Methanol extract

An aliquot of the methanolic extract (1.3 g) was fractionated on a silica gel CombiFlash column by using hexane-ethyl acetate mixtures of increasing polarity (4:1 Hexane:EtOAC to 100% EtOAC) as the mobile phase. A total of three fractions was then obtained (Fr. 1, 0.33 g; Fr. 2, 0.66 g; Fr. 3, 0.18 g). Fr. 2 was purified by silica PTLC (1000 μm) using hexane-EtOAC (2:1) as the mobile phase to yield compound **1** (39.4 mg) and **2** (86 mg). Fr. 3 was subjected to silica PTLC using $CHCl_3$-MeOH (20:1) as the mobile phase to afford compounds **3** (27 mg) and **4** (26 mg). Based on NMR spectral data, compounds **1-4** were identified as 10-gingerol, 6-gingerol, 8-gingerol and hexahydrocurcumin[14-16].

*Compound **1***(figure 5 and 6)

Colourless powder.^{1}H NMR (500 MHz, CD_3OD): δ 0.84 (3H, t, J = 7 Hz, H-14), 1.26–1.47 (16H, m, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 2.51 (1H, dd, J = 17.5, 8.9 Hz, H-4), 2.56 (1H, dd, J = 17.5, 2.9 Hz, H-4), 2.72 (2H, m, H-2), 2.84 (2H, m, H-1), 3.87 (3H, s, CH_3O-), 4.05 (1H, m, H-5), 6.65 (1H, dd, J = 7.9, 1.9 Hz, H-6′), 6.67 (1H, d, J = 2 Hz, H-2′), 6.82 (1H, d, J = 8.1 Hz, H-5′). ^{13}C NMR (125 MHz, CD_3OD): δ211.6(C-3), 146.5 (C-3′), 144.1 (C-4′), 132.7 (C-1′), 120.8 (C-6′), 114.5 (C-5′), 111.1 (C-2′), 67.8 (C-5), 56.0 (CH_3O-), 49.4 (C-4), 45.5 (C-2), 36.6 (C-6), 32.0 (C-1), 29.7 (C-7), 29.6 (C-8), 29.4 (C-9), 25.5 (C-10), 22.8 (C-11, C-12 and C-13), 14.2 (C-14).

*Compound **2***

Yellow oil.^{1}H NMR (500 MHz, CD_3OD): δ 0.80 (3H, t, J = 4.0 Hz, H-10), 1.17–1.42 (8H, m, H-6, H-7, H-8, H-9), 2.42 (1H, dd, J = 20.9 Hz, H-4), 2.49 (1H, dd, J = 20.3 Hz, H-4); 2.66 (2H, m, H-2), 2.76 (2H, m, H-1), 3.79 (3H, s, CH_3O-), 3.96 (1H, m, H-5), 5.6 (1H, s, HO-Ar), 6.58 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.60 (1H, dd, J = 8.0, 2.0 Hz, H-2′), 6.75 (1H, d, J = 8.0 Hz, H-5′). ^{13}C NMR (125 MHz, CD_3OD): δ 212.4 (C-3), 147.4 (C-3′), 144.9 (C-4′), 133.5 (C-1′), 121.6 (C-6′), 115.3 (C-5′), 111.9 (C-2′), 68.6 (C-5), 56.8 (CH_3O-), 50.2 (C-4), 46.3 (C-2), 37.5 (C-6), 32.6 (C-1), 30.2 (C-7), 26.0 (C-8), 23.5 (C-9), 14.9 (C-10).

*Compound **3***

Pale yellow powder.^{1}H NMR (500 MHz, CD_3OD): δ 0.87 (3H, t, J = 6.9 Hz, H-12), 1.26–1.49 (12H, m, H-6, H-7, H-8, H-9, H-10, H-11), 2.48 (1H, dd, J = 17.5, 9.2 Hz, H-4), 2.56 (1H, dd, J = 17.5, 2.9 Hz, H-5), 2.72 (2H, m, H-2), 2.82 (2H, m, H-1), 3.86 (3H, s, CH_3O-), 6.65 (1H, dd, J = 8.0, 1.9 Hz, H-6′), 6.67 (1H, d, J = 1.7 Hz, H-2), 6.83 (1H, d, J = 8.0 Hz, H-5′). ^{13}C NMR (125 MHz, CD_3OD): δ 211.6 (C-3), 146.5 (C-3′), 144.1 (C-4′), 132.7 (C-1′), 120.8 (C-6′), 114.5 (C-5′), 111.1 (C-2′), 67.8 (C-5), 56.0 (CH_3O-), 49.4 (C-4), 45.5 (C-

2), 36.6 (C-6), 31.9 (C-1), 29.6 (C-7), 29.4 (C-8), 29.3 (C-9), 25.5 (C-10), 22.7 (C-11), 14.2 (C-12).

*Compound **4***

Yellow pale powder; ^{1}H NMR (500 MHz, in CD_3OD): δ 1.63 (1H, dddd, *J*=14.0, 10.1, 6.7, 4.3 Hz,H-6), 1.77 (1H, dddd, *J*=14.0, 9.2, 9.2, 5.5 Hz, H-6), 2.51 (1H, dd, *J*=17.4, 8 Hz, H-4), 2.57 (1H, dd, *J* =17.4, 3.1 Hz, H-4), 2.59 (1H, ddd, *J*=12.9, 9.2, 6.7 Hz, H-7), 2.71 (2H, t, *J*=7.2 Hz, H-2), 2.72 (1H, ddd, *J*=12.9, 10.1, 5.5 Hz, H-7), 2.82 (2H, t, *J*=7.2 Hz, H-1), 3.85 (3H, s, CH_3O-), 3.86 (3H,s, CH_3O-), 4.03 (1H, dddd, *J*=9.2, 8.0, 4.3, 3.1 Hz, H-5), 6.64 (1H, dd, *J*=8, 2 Hz, H-6′ or H-6″), 6.66 (1H, d, *J*=2 Hz, H-2′ or H-2″), 6.67 (1H, dd, *J*=8, 2 Hz, H-6′ or H-6″), 6.70 (1H, d, *J*=2 Hz, H-2′ or H-2″), 6.81 (2H, d, *J*=8 Hz, H-5′and H-5″). ^{13}C NMR (125 MHz, in CD_3OD): δ210.7 (C-3),147.6 (C-3′ and C-3″), 144.3 (C-4′ and C-4″), 133.6 (C-1″), 132.8 (C-1′),120.6 (C-6″),120.5 (C-6′), 114.9 (C-5′ and C-5″), 111.9 (C-2′ and C-2″), 67.0 (C-5), 55.1 (CH_3O-), 50.1 (C-4), 45.1 (C-2), 39.2 (C-6), 31.2 (C-1), 29.0 (C-7).

3.3.2 Water extract

Water extract (3.4 g)was fractionated by SEPHADEX LH-20 M column, eluted with waterMeOH mixtures of increasing polarity (90%MeOH to 100% MeOH, flow rate of 1 mL/min). The column yielded a total of three 3 fractions (Fr. 1: 0.2 g, Fr. 2: 2.8 g, Fr. 3: 0.4 g). Fr.2 was MTT antioxidant active and it was further purified. This fraction is considered in this paper as polysaccharides fraction (Ps). The crude polysaccharides were precipitated two times from water solution with methanol and kept overnight at 4°C in the refrigerator. The colorless precipitate was washed three times with cold methanol, dissolved in water and lyophilized (240 mg). The NMR spectra (^{1}H and ^{13}C) confirmed the fraction as polysaccharides.

3.4. Biological assays

The assays, MTT [17], LPO [18], anti-inflammatory [19] and human tumor cell proliferation inhibition, described in the manuscript were performed according to previously published procedures form our laboratory [13].

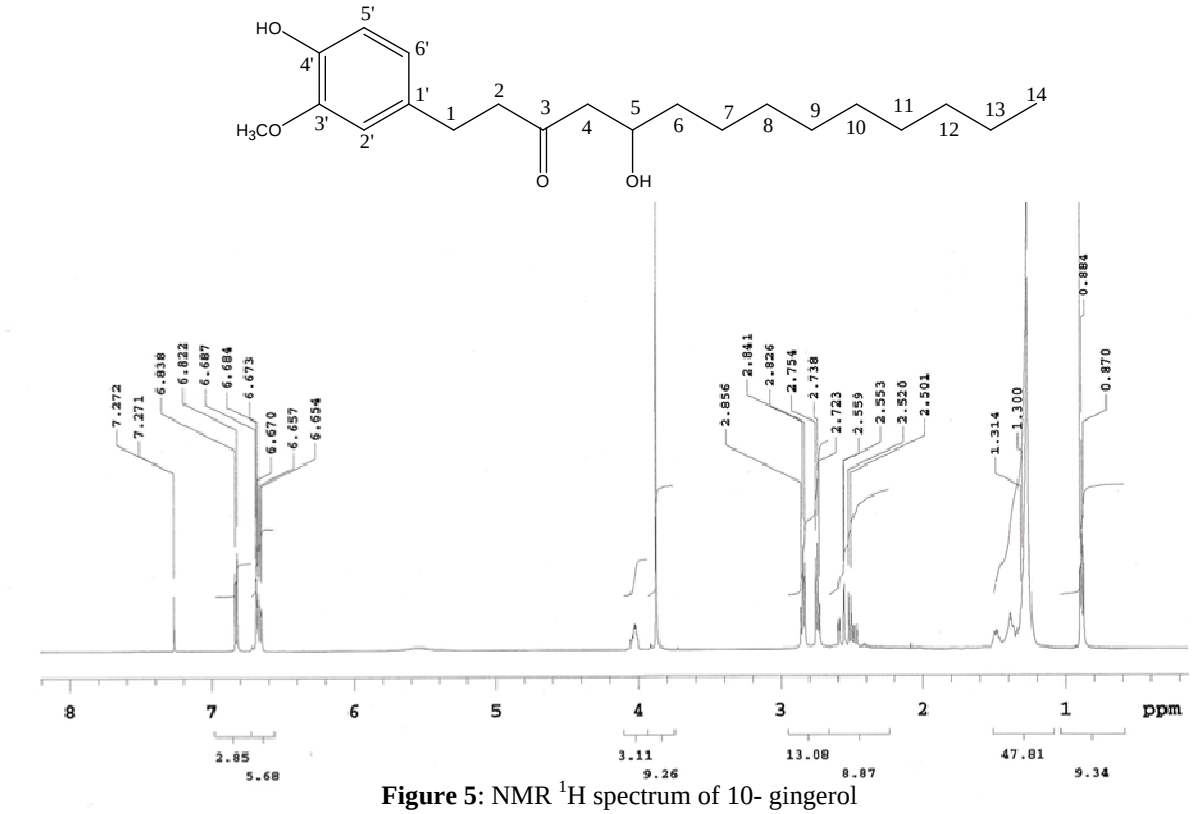

Figure 5: NMR ^{1}H spectrum of 10- gingerol

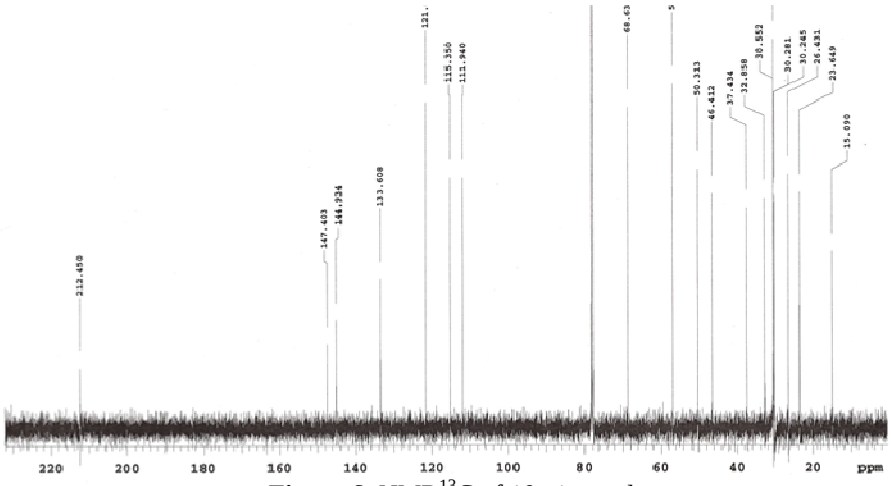

Figure 6: NMR^{13}C of 10-gingerol

3.3.2 Water extract

Water extract (3.4 g)was fractionated by SEPHADEX LH-20 M column, eluted with water-MeOH mixtures of increasing polarity (90%MeOH to 100% MeOH, flow rate of 1 mL/min). The column yielded a total of three 3 fractions (Fr. 1: 0.2 g, Fr. 2: 2.8 g, Fr. 3: 0.4 g). Fr.2 was MTT antioxidant active and it was further purified. This fraction is considered in this paper as polysaccharides fraction (Ps). The crude polysaccharides were precipitated two times from water solution with methanol and kept overnight at 4°C in the refrigerator. The colorless precipitate was washed three times with cold methanol, dissolved in water and lyophilized (240 mg). The NMR spectra (^{1}H and ^{13}C) confirmed the fraction as polysaccharides.

*3.4. Biological assays*The assays, MTT [17], LPO [18], anti-inflammatory [19] and human tumor cell proliferation inhibition, described in the manuscript were performed according to previously published procedures form our laboratory [13].

4. Conclusion

Four compounds were isolated from organic ginger *Z. officinale*. These compounds have good lipid peroxidation (37-78%) and COX-2 (26-96%) inhibition activities. Their tumor cells proliferation inhibition activity is weak. Water extract showed strong MTT at 25 µg/mL (0.26 of absorbance) activity but very weak lipid peroxidation (5%), COX-1 (0%) and COX-2 (36%) inhibition. However, isolation of water yielded mainly polysaccharides type compounds.

Organic and conventional ginger extracts have the same TLC profile. The MTT activity of water and methanol extracts were same, except the content of the extracts. Organic ginger contained three times more active compounds than non-organic ginger.

References

[1]. Gupta, R.K. Ginger – a wonderful spice: An overview. Vegetos, (2008), 21, 1–10.
[2]. Nilesh, P.;Sandeep, P.;Mansingraj, N.; Ghansham, D. Food Chemistry, (2011)126, 1330–1336.
[3]. Sang, S.G.; Hong, J.; Wu, H.; Liu, J.; Yang, S.C.; Pan, M-H.;Badmaev, V.; Ho, C.T. Journal of Agricultural and Food Chemistry, (2009)57, 10645–10650.
[4]. Afshari, A.T.;Shirpoor, A.;Farshid, A.; Saadatian, R.; Rasmi, Y.;Saboory, E.;Ilkhanizadeh, B.;Allameh, A. Food Chemistry, (2007)101, 148–153.
[5]. vanBreemen, R.B.; Tao, Y.; Li, W. (Zingiberofficinale).Fitoterapia, (2011)82,38–43.
[6]. Ficker, C.; Smith, M.L.;Akpagana, K.;Gbeassor, M.; Zhang, J.; Durst, T.;Assabgui, R.;Arnason, J.T. Phytotherapy Research, (2003)17, 897–902.
[7]. Shukla, Y.; Singh, M.Food and Chemical Toxicology, (2007)45, 683–690.

[8]. Bowen-Forbes, C.S.;Mulabagal, V.; Liu, Y.; Nair, M.G. Food Chemistry, (2009)116, 633–637.
[9]. Han, Q.; Yu, Q-Y.; Shi, J.;Xiong, C-Y.; Ling, Z-J.; He, P-M Journal of Food Science, (2011)76, 462-471.
[10]. Li, S.P.; Zhao, K.J.;Ji, Z.N.; Song, Z.H.; Dong, T.T.X.; Lo, C.K.; Cheung, J.K.H.; Zhu, S.Q.;Tsim, K.W.K. Life Sciences, (2003)73, 2503–2513.
[11]. Qi, H.;Zhang, Q.;Zhao, T.;Hu, R.;Zhang, K.; Li, Z Bioorganic & Medicinal Chemistry Letters, (2006), 16, 2441–2445.
[12]. Yuan, Y.V.; Bone, D.E.; Carrington, M.F. Food Chemistry, (2005), 91, 485-494.
[13]. Jayaprakasam, B.; Zhang, Y.; Nair, M.G.Journal of Agricultural and Food Chemistry, (2004), 52, 6939–6943.
[14]. Yamagishi, T.; Hayashi, K.;Mitsuhashi, H. Chemical & Pharmaceutical Bulletin, (1972), 20, 2291–2292.
[15]. Lee, S.W.; Lim, J-H.; Kim, M.S.;Jeong, J.H.; Song, G-Y.; Lee, W.S.; Rho, M-C. Food Chemistry, (2011)128, 778–782.
[16]. Li, F.; Wang, Y.;Parkin, K.L.;Nitteranon, V.; Liang, J.; Yang, W.; Li, Y.; Zhang, G.; Hu, Q. Food Research International, (2011)44, 1597-1603.
[17]. Liu, Y.; Nair, M.G. .Journal of Natural Products,(2010)73, 1193–1195.
[18]. Arora, A.; Nair, M.G.; Strasburg, G.M. Free Radical Biology & Medicine, (1998)24, 1355–1363.
[19]. Wang, H.; Nair, M.G.; Strasburg, G.M.; Chang, Y-C.;Booren, A.M.; Gray, J.I.; DeWitt, D.L. Journal of Natural Products,(1999)62, 294-296.