

# Phenolic Compounds and Cytotoxic Activities of Methanol Extract of Basil (*Ocimum basilicum* L.)

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## Abstract

Chemical investigation of methanol extract of *Ocimum basilicum* L. resulted the isolation and identification of twelve phenolic compounds as *p*-hydroxy benzoic acid, ferulic acid, gallic acid, *p*-qumaric acid, benzoic acid, kaempferol, catechin, quercetin, chlorogenic acid, caffeic acid, cinnamic acid and ellagic acid. The structures of these compounds were confirmed by Chromatographic, UV, MS, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectral data. The cytotoxic activity of the methanol extract of *Ocimum basilicum* showed strong cytotoxic activity against colon (HCT116) and liver (HEPG2) carcinoma cell line, where IC<sub>50</sub> of the two human cell line were 27 µg/ml and 34.5 µg/ml, respectively.

**Keywords:** *Ocimum basilicum* L.; Phenolic constituents; HCT116; HEPG2; Cytotoxic activity

## Introduction

The *Ocimum basilicum* L. (basil) is an annual, herbaceous, white to purple flowering plant, 20–60 cm tall, that originated in Iran and India [1,2]. The genus *Ocimum* belonging to the Lamiaceae comprises annual and perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa and South America [3]. The taxonomy of *Ocimum* is complex due to interspecific hybridization and polyploidy of the species in the genus. Pushpangadan and Bradu, [4] recognized more than 150 species; however, also Paton [5]; proposed that *Ocimum* had only 65 species and other attributions should be considered as synonyms. Basil is used as a medicinal herb in medical treatments such as for headaches, coughs, diarrhea, worms and kidney malfunctions. Basil essential oil has been utilized extensively in the food industry as a flavoring agent, and in perfumery and medical industries [6]. Basil (*Ocimum basilicum* L.) is an aromatic herb that is used extensively to add a distinctive aroma and flavor to food. The leaves can be used fresh or dried for use as a spice. Basil extract has antimicrobial and antioxidant activities due to its phenolic and aromatic compounds [7,8]. The main phenolics reported in basil are phenolic acids and flavonol-glycosides [9,10]. Also, basil possesses insecticidal [11] properties and it is a promising fungi static [12]. Rosmarinic acid is the most prevalent basil phenolic, [13,14] but other caffeic acid derivatives, such as chicoric acid found in substantial concentrations [14]. In continuation of our studies, [15-17] we report here the chemical composition and antitumor activity of methanol extract of Egyptian *Ocimum basilicum* L., from the family Lamiaceae.

## Materials and Methods

### Plant materials

*Ocimum basilicum* L. was collected Aswan city (South Egypt), during spring season, 2012. The collected plants were identified by Botany Department, Faculty of Science, Zagazig University and by comparison with plant description in flora of Egypt. The plant parts were dried under shade and then grinded to fine powder.

### Chromatographic analysis

Sheets of Whatman paper No 1 or 3 MM were used for two-dimensional, comparative or preparative paper chromatography. The

separation of the phenolic and flavonoid components was performed by column fractionation of the extract or its fractions on one of the following stationary phases as stated in each case. A- Polyamide powder, polyamide 6-S for CC, Riedel-De Haen AG, seelze-Hannover, Germany. B-Sephadex LH-20, (25-100 µm), Pharmacia fine chemicals.

### Spectroscopic analysis

**V spectroscopic:** Investigated material and UV measurements were then carried out. Chromatographically, pure materials dissolved in analytically pure methanol were subjected to UV spectrophotometric investigation in 4 ml capacity quartz cells Zeiss spectrometer PMQ-II. In case of flavonoids, AlCl<sub>3</sub>, AlCl<sub>3</sub>/HCl, fused NaOAc/H<sub>3</sub>BO<sub>3</sub> and NaOMe reagents were separately added to methanolic solution of the investigated material and UV measurements were then carried out [18].

**NMR spectroscopic:** <sup>1</sup>H and <sup>13</sup>C chemical shifts (δ) were measured in ppm, relative to DMSO -d<sub>6</sub> and converted to TMS scale. Jeol EC A 500 MHz NMR Spectrometer at 500 MHz, (Institute Fur Chemie, Humboldt Universität zu Berlin, Germany). <sup>1</sup>H chemical shifts were measured in ppm, relative to TMS and <sup>13</sup>C NMR chemical shifts to DMSO-d<sub>6</sub> and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 8 KHz for <sup>1</sup>H and 30 KHz for <sup>13</sup>C, 64 K data points and a flip angle of 45°C.

**Mass spectroscopic:** The isolation of pure compounds were subjected (FAB -MS). The isolated pure compounds were subjected, in most cases to Fast Atom Bombardment (positive and negative) mass spectroscopic analysis (FAB-MS) on MM 7070 E spectrometer (VG analytical). Some other compounds were subjected to electron spray ionization mass spectroscopic analysis (ESI-MS) a Varian Mat1 12-

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ET Spectrometer. All measurements were carried out at Institute Fur Chemie, Humboldt Universitat zu Berlin, Germany.

**Extraction and isolation of the phenolic compounds:** The dried plant (all parts of the plant), was extracted exhaustively with petroleum ether, diethyl ether and methanol (according to polarity). For each extraction, the powder was left 24 h, in a Soxhlet apparatus. After that the methanol extract was concentrated under vacuum and left overnight. This yielded a gummy solid which was separated by filtration. The methanol filtration was shown by paper chromatography used two-dimensional (TDPC), comparative or preparative paper chromatography to determine phenolic acid and flavonoids compounds [16].

**p-Hydroxy benzoic acid:**  $R_f$  values (x 100): 36(HOAc-6), 84(BAW). UV/Vis (MeOH,  $\lambda_{max}$ , nm): 253. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 7.8(*d*, *J*=9Hz) and 6.8(*d*, *J*=9Hz).

**Ferulic acid:**  $R_f$  values (x100): 40(H<sub>2</sub>O), 42(HOAc-6%), 86(BAW). UV UV/Vis (MeOH,  $\lambda_{max}$ , nm): 268, 313. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 3.81(*s*, CH<sub>3</sub>), 6.69(*d*, H- $\alpha$ ), 6.18(*d*, H-5), 7.10(*d*, H-6), 7.30(*s*, H-2), 7.52(*d*, H- $\beta$ ).

**Gallic acid:**  $R_f$  values (x100): 44(H<sub>2</sub>O), 55(HOAc-6), 72(BAW). UV/Vis (MeOH,  $\lambda_{max}$ , nm): 272. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 6.98(*s*, 2H, H-2 and H-6). <sup>13</sup>C-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 120.6(C-1), 108.8(C-2 and C-6), 145.5(C-3 and C-5), 138.1(C-4), 167.7(C=O). MS (EI, *m/z* (%)): 170.9[M<sup>+</sup> + 1, 5.1], 169.0[M<sup>+</sup> - H, 100, ion A], 167.9[M<sup>+</sup> - H<sub>2</sub>, 63.9], 153[M<sup>+</sup> - OH, 11.2], 145[ion A - C<sub>2</sub>, 3.5], 139[ion A - HCHO, 11.3], 126[M<sup>+</sup> - CO<sub>2</sub>, 6.5] and 123[ion A - HCOOH, 27.1]. Anal. Calcd for C<sub>7</sub>H<sub>6</sub>O<sub>5</sub> (170.02): C, 49.42; H, 3.55; O, 47.02. Found: C, 49.50; H, 3.30; O, 47.20.

**p-Coumaric acid:**  $R_f$  values (x100): 43(H<sub>2</sub>O), 45(HOAc-6%), 90(BAW). UV/Vis (MeOH,  $\lambda_{max}$ , nm): 266, 310; (MeOH + NaOMe,  $\lambda_{max}$ , nm): 228, 333. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 6.2 (*d*, *J*=15Hz, H- $\alpha$ ), 6.72(*d*, *J*=8Hz, H-3 and H-5), 7.32 (*d*, *J*=8 Hz, H-2 and H-6), 7.52(*d*, *J*=15Hz, H- $\beta$ ). Anal. Calcd for C<sub>9</sub>H<sub>8</sub>O<sub>3</sub> (164.16): C, 65.85; H, 4.91; O, 29.24. Found: C, 65.80; H, 4.80; O, 29.40.

**Benzoic acid:** <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 12.9 (*s*, H<sub>Carboxylic</sub>), 8.20(*d*, H-2 and H-6), 7.83(*m*, H-3 and H-5) and 7.4(*m*, H-4).

**Kaempferol:**  $R_f$  values (x100): 00(H<sub>2</sub>O), 10(HOAc-6), 85(BAW). UV/Vis (MeOH,  $\lambda_{max}$ , nm): 268, 369; (MeOH + NaOAc,  $\lambda_{max}$ , nm): 270, 310, 375; (NaOAc + H<sub>3</sub>BO<sub>3</sub>,  $\lambda_{max}$ , nm): 270, 320, 372; (MeOH + AlCl<sub>3</sub>,  $\lambda_{max}$ , nm): 270, 305, 360, 430; (AlCl<sub>3</sub> + HCl,  $\lambda_{max}$ , nm): 278, 316, 413. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 6.4 (*d*, *J*=2.5, H-8), 6.18 (*d*, *J*=2.5, H-6), 8.14 (*d*, *J*=8, H-2' and H-6'), 6.89 (*d*, *J*=8, H-3' and H-5'). <sup>13</sup>C-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 146.8(C-2), 135.4(C-3), 175.9(C-4), 161.0(C-5), 98.6(C-6), 164.2(C-7), 93.8(C-8), 156.4(C-9), 103.7(C-10), 121.9(C-1'), 129.9(C-2' and C-6'), 115.8(C-3' and C-5') and 159.5(C-4'). MS (EI, *m/z* (%)): 285.1 [M<sup>+</sup> - H, 100, ion A], 242.1 [M<sup>+</sup> - CO<sub>2</sub>, 5.3], 203.1 [ion A - C<sub>4</sub>H<sub>2</sub>O<sub>2</sub>, 4.6, ion B] and 169.1[ion B - H<sub>2</sub>O<sub>2</sub>, 5.7].

**Catechin acid:**  $R_f$  values (x 100): 0.33(H<sub>2</sub>O), 0.54(HOAc-6), 0.60(BAW). UV/Vis (MeOH,  $\lambda_{max}$ , nm): 278. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 4.51 (*d*, *J*= 7.3, H-2), 3.84 (*m*, H-3) 2.38 (*ax*, *dd*, *J*=16.0, 7.9, H-4), 2.68 (*eq*, *dd*, *J*= 16.0, 5.3, H-4), 5.90 (*d*, *J*= 2.2, H-6), 5.72 (*d*, *J*=2.2, H-8), 6.74 (*d*, *J*= 1.9, H-2'), 6.7 (*d*, *J*= 8, H-5'), 6.61(*dd*, *J*= 8, 1.9, H-6'). <sup>13</sup>C-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 81.0(C-2), 66.4(C-3), 27.7(C-4), 156.1(C-5), 95.3(C-6), 156.4(C-7), 94.0(C-8), 155.3(C-9), 99.2(C-10), 130.7(C-1'), 114.5(C-2'), 144.8(C-3'), 144.8(C-4'), 115.1(C-5'), 118.4(C-6').

**Quercetin:**  $R_f$  values (x 100): 00(H<sub>2</sub>O), 07(HOAc-6), 75(BAW). UV/Vis

(MeOH,  $\lambda_{max}$ , nm): 255, 268, 370; (MeOH + NaOAc,  $\lambda_{max}$ , nm): 254, 276, 375; (NaOAc + H<sub>3</sub>BO<sub>3</sub>,  $\lambda_{max}$ , nm): 272, 388; (MeOH + AlCl<sub>3</sub>,  $\lambda_{max}$ , nm): 270, 360, 440; (AlCl<sub>3</sub> + HCl,  $\lambda_{max}$ , nm): 258, 400. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 6.19 (*d*, *J*=2.5, H-6), 6.4 (*d*, *J*=2.5, H-8), 7.64 (*d*, *J*=2.5, H-2'), 6.88(*d*, *J*=8.5, H-5'), 7.53 (*dd*, *J*=2.5 and 8.5, H-6'). <sup>13</sup>C-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 147.0(C-2), 135.8(C-3), 176.2(C-4), 160.5(C-5), 99.2(C-6), 164.0(C-7), 93.7(C-8), 156.4(C-9), 103.5(C-10), 122.2(C-1'), 115.3(C-2'), 145.1(C-3'), 148.0(C-4'), 115.6(C-5') and 120.2(C-6'). MS (EI, *m/z* (%)): 300.8 [M<sup>+</sup> - H, 100, ion A], 299.8 [M<sup>+</sup> - H<sub>2</sub>, 28.3], 270.9 [ion A - C<sub>2</sub>H<sub>6</sub>, 8.9], 162.9 [ion A - C<sub>7</sub>H<sub>6</sub>O<sub>3</sub>, 10.1, ion B] and 117.3[ion B - HCOOH, 5.3]. Anal. Calcd. for C<sub>15</sub>H<sub>10</sub>O<sub>7</sub> (302.04): C, 59.61; H, 3.33; O, 37.06. Found: C, 59.30; H, 3.40; O, 37.30.

**Chlorogenic acid:**  $R_f$  values (x100): 67 (H<sub>2</sub>O), 65 (HOAc-6%), 59 (BAW). UV/Vis (MeOH,  $\lambda_{max}$ , nm): 220, 245, 300, 330; (MeOH + NaOMe,  $\lambda_{max}$ , nm): 229, 275, 400. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): Caffeic acid moiety : 7.42 (*d*, *J*= 16Hz,  $\beta$ -H), 7.05(*d*, *J*= 2Hz, 6-H), 6.96 (*dd*, *J*=7.5Hz and *J*=2Hz, 2-H), 6.79 (*d*, *J*= 7.5Hz, 3-H), 6.19 (*d*, *J*= 16Hz,  $\alpha$ -H); Quinic acid moiety: 5(*m*, 1'-H), 1.88(*m*, 2'-H and 6'-H), 3.85(*m*, 3'-H and 5'-H), 3.5 (*m*, 4'-H). <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): Caffeic acid moiety: 126.1(C-1), 115.2(C-2), 144.9(C-3), 148.5(C-4), 116.29(C-5), 121.5(C-6), 146.2(C-7), 115.2(C-7), 115.2(C-8), 116.5(C-9); Quinic acid moiety: 76.6(C-1'), 68.6(C-3'), 71.8(C-4'), 71(C-5'), 180(C-7').

**Caffeic acid:**  $R_f$  values (x 100): 25(H<sub>2</sub>O), 45(HOAc-6%), 81(BAW). UV/Vis (MeOH,  $\lambda_{max}$ , nm): 218, 245, 298, 325. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 6.2 (*d*, *J*= 16Hz,  $\beta$ -H), 6.76(*J*=7.5Hz, 5-H), 6.88 (*dd*, *J*=7.5Hz and *J*=2.5Hz, 6-H), 6.98 (*d*, *J*= 2.5Hz, 2-H), 7.48 (*d*, *J*= 16Hz,  $\alpha$ -H).

**Cinnamic acid:**  $R_f$  values (x100): 30(H<sub>2</sub>O), 41(HOAc-6%), 80(BAW). <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 6.46 (*d*, H- $\alpha$ ), 7.40 (*t*, H-3 and H-5), 7.42(*t*, H-4), 7.557 (*d*, H-2 and H-6), 7.806 (*d*, H- $\beta$  and C-6'). <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 159.2(C-7 and C-7').

**Ellagic acid:**  $R_f$  values (x 100): 0(H<sub>2</sub>O), 9(HOAc-6), 48(BAW). UV/Vis (MeOH,  $\lambda_{max}$ , nm): 255, 362. <sup>1</sup>H-NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 7.48 (*s*, H-5 and H-5'). <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 112.3(C-1 and C-1'), 136.4(C-2 and C-2'), 140.2(C-3 and C-3'), 153(C-4 and C-4'), 111.4(C-5 and C-5'), 107.6(C-6').

## SRB assay of cytotoxic activity

Measurement of potential cytotoxic activity of methanol extract of *O. basilicum* against the liver (HEPG2) and colon carcinoma cell line (HCT116) was tested by SRB (Sulphorhodamine-B) assay according to the method of Skehan et al. [19]. Human tumor cell lines were obtained frozen in liquid nitrogen (-180°C) from the American Type Culture Collection. The tumor cell lines were maintained in The National Cancer Institute, Cairo, Egypt, by serial sub-culturing. This experiment was conducted in the National Cancer Institute, Cairo, Egypt.

## Results and Discussion

### Results of phenolic constituents in methanol extract of *O. basilicum*

Investigation of the phenolic compounds was done by fractionation of the extract, over polyamide column and elution with methanol/bi-distilled water mixtures of decreasing polarities for gradient elution led to the desorption of sub fractions which were dried, individually, in vacuum, and then subjected to re-chromatography for several times led to the separation of twelve pure phenolic compounds. The structure

of these compounds was confirmed by comparison of their physical and spectral data with those of reported compounds. Compounds were identified as *p*-hydroxy benzoic acid, ferulic acid, gallic acid, *p*-quaric acid, benzoic acid, kaempferol, catechin, quercetin, chlorogenic acid, caffeic acid, cinnamic acid and ellagic acid.

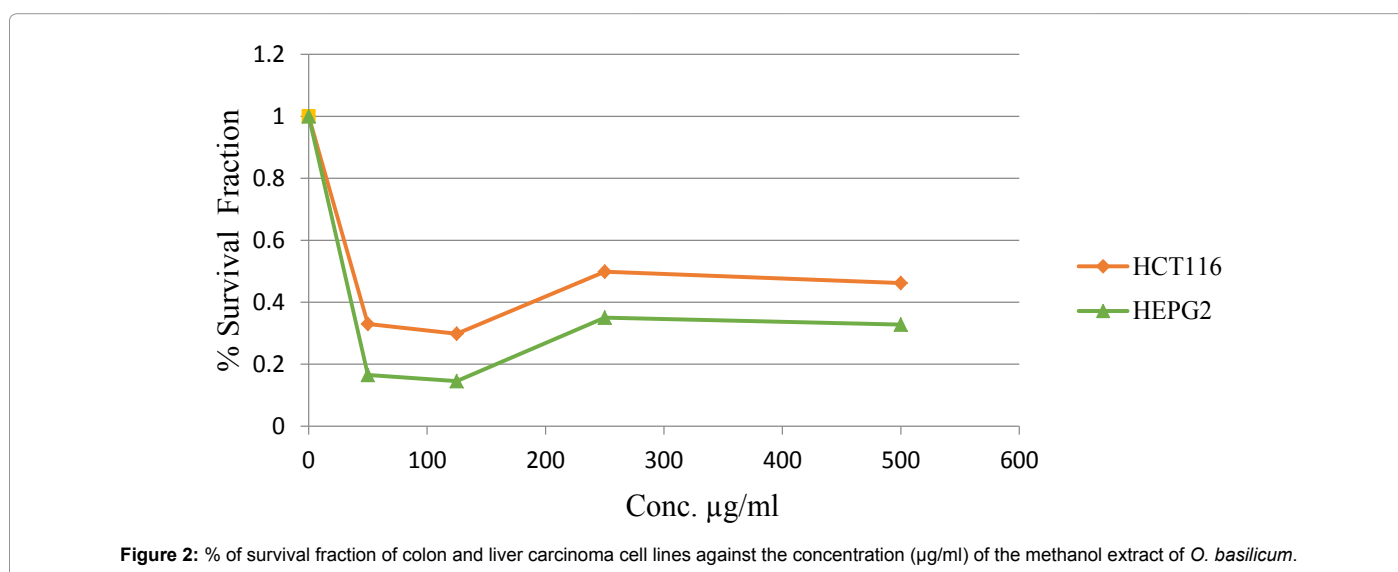
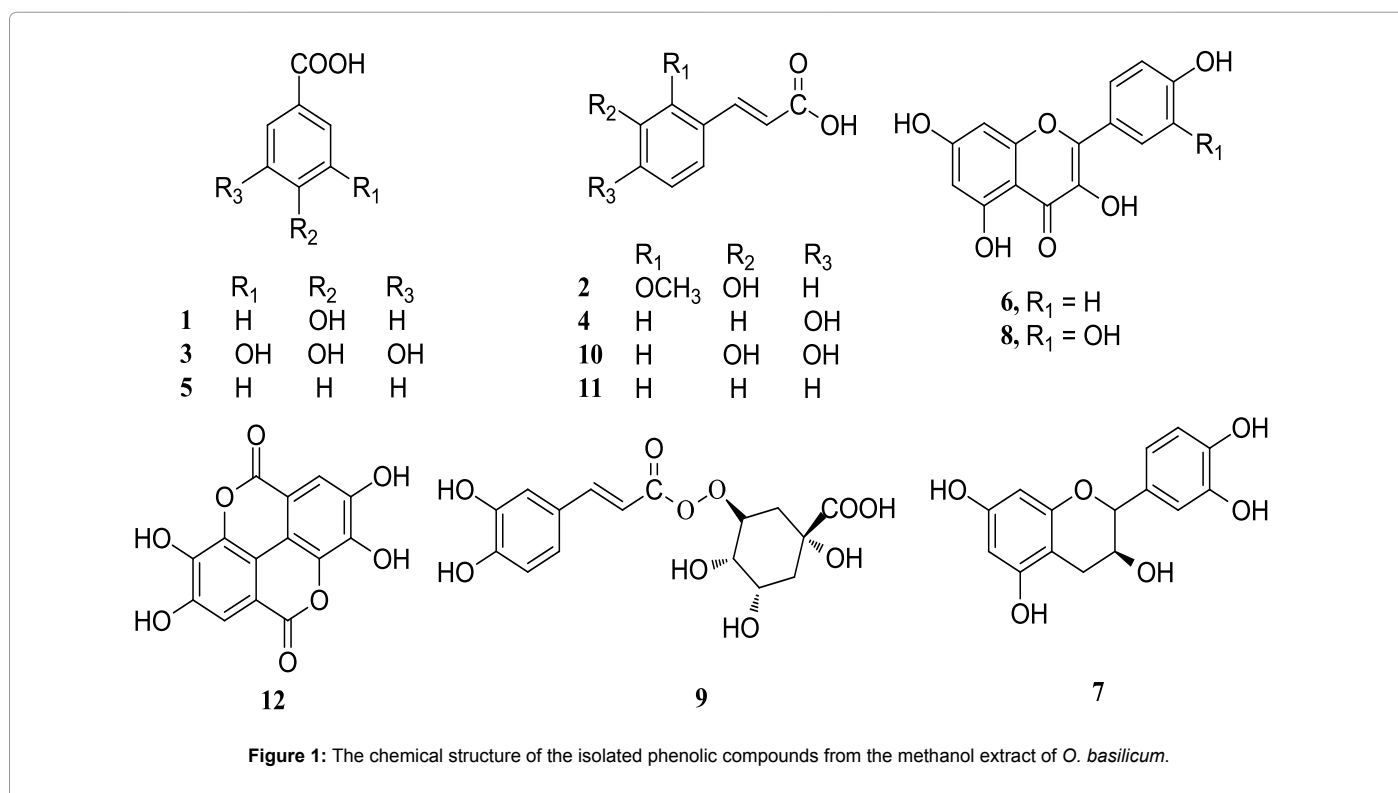
### Anti-tumor activity

The methanol extract of *O. basilicum* were tested against liver (HEPG2) and colon carcinoma cell lines (HCT116), the results showed the strong efficient cytotoxic activity of the methanol extract

of *O. basilicum* against the tested human cell lines, the results were summarized in Figure 1,2. The IC<sub>50</sub> values were calculated for colon and liver carcinoma cell lines as 34.5 and 27 µg/ml, respectively.

### Conclusion

The methanol extract of *O. basilicum* was found to include twelve active phenolic compounds. Also the extract was found to have strong efficient cytotoxic activity against liver (HEPG2) and colon carcinoma cell lines (HCT116).



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