

Chemical constituents and cytotoxic activities of the extracts of *Melilotus indicus*Sayed Abdel Kader Ahmed <sup>a</sup> and Mahmoud Al-Refai <sup>b,\*</sup><sup>a</sup> Department of Chemistry, Faculty of Science, Beni-Suef University, Beni-Suef, 62514, Egypt<sup>b</sup> Department of Chemistry, Faculty of Science, Al Al-Bayt University, Mafraq, 25113, Jordan\*Corresponding author at: Department of Chemistry, Faculty of Science, Al Al-Bayt University, Mafraq, 25113, Jordan.  
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## ARTICLE INFORMATION



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## ABSTRACT

The phytochemical investigation of the ethanolic extract of the aerial parts of *Melilotus indicus* resulted in the isolation of eight known flavonoids, namely; catechin (1), epicatechin (2), taxifolin (3), quercetin (4), quercetin 3-O-β-D-glucopyranoside (5), and quercetin 3-O-α-L-rhamnopyranoside (6), isorhamnetin 3-O-β-D-glucopyranoside (7) and isorhamnetin 3-O-β-D-rutinoside (8). These compounds have never been isolated from this species. The cytotoxic activities of the extracts of *Melilotus indicus* was evaluated by MTT assay against six cancer cell lines using doxorubicin as standard drug. The chloroform extract was the most potent cytotoxic agent against HepG-2 cells with an IC<sub>50</sub>: 9.21 μg/mL.

## 1. Introduction

The family leguminosae is one of the largest families of flowering plants, consisting of about 463 genera and 18000 species [1]. *Melilotus* is a genus of plants including 20-25 species that are widely distributed all over the world. *Melilotus indicus* (*M. indicus*) belongs to the family Leguminosae (Fabaceae). It is an annual or biennial herb from 10 to 50 cm in height (rarely to one metre), with yellow flowers native to northern Africa, Europe and Asia, but naturalized throughout the rest of the world [2].

Flavonoids have received an increasing attention in the last decade, especially for their potential protective effects against degenerative diseases linked to oxidative stress [3-6]. Chemoprevention is a rapidly growing practical approach that focuses on cancer prevention by the administration of one or more synthetic or naturally occurring agents to suppress or reverse the process of carcinogenesis. It is becoming increasingly clear that chemopreventive compounds present in diet offer great potential in the fight against cancer by inhibiting the process of carcinogenesis through regulation of cell-defensive and cell death machineries [7]. Dietary chemopreventive substances are regarded as being generally safe, inexpensive and they have been found to contain various phytochemicals which are antioxidant in nature [7]. In Egypt, the leaf extract of *M. indicus* have been utilized as food additives for the prevention of many chronic diseases. The *Melilotus* species have been

found to contain coumarins (Coumarin, dihydrocoumarin and coumarol), acids (*o*-Hydroxycinnamic, melilotic, coumarinic and *o*-coumaric), coumarin glycosides, fatty acids (Linoleic acid) and flavonoids (Quercetin and coumestrol), triterpenes (Melilotoside A2 and adzukisaponin V) as well as xylan [8,9]. Some of *Melilotus* species have been found to possess anti-oxidant and antibacterial properties [10,11]. The aim of the work described herein was to isolate and identify the flavonoid constituents of *M. indicus*, and to evaluate the cytotoxicity of its extracts.

## 2. Experimental

## 2.1. Instrumentation

<sup>1</sup>H and <sup>13</sup>C NMR spectra were registered in a Bruker Advance DRX-400 apparatus. Chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. Mass spectra were registered in a Finnigan MAT90 spectrometer operating with ionization energy of 159 eV, at positive and negative modes. IR and UV-Vis spectra were registered in the spectrophotometers Shimadzu IR-408, in KBr discs, and Shimadzu UV-160A, respectively. Melting points were determined in a MQAPF-301 apparatus.

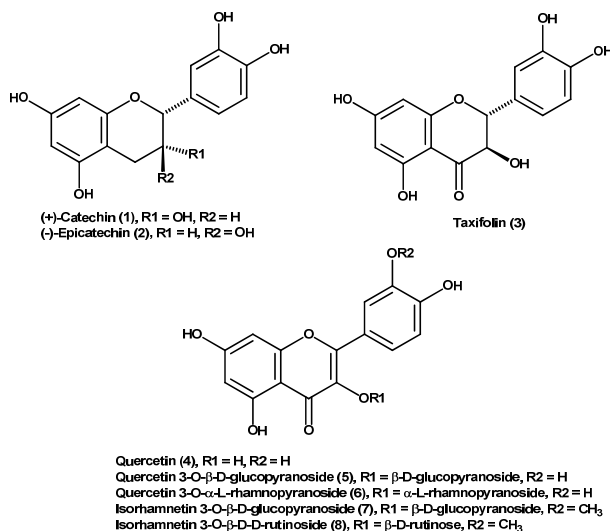


Figure 1. Structures of the isolated flavonoids.

## 2.2. Plant material

The aerial parts of *M. indicus* were collected from the Eastern Nile region, Beni-Suef city on March 2013. Identification of the plant was confirmed by the Department of Flora, Agricultural Museum, Ministry of Agriculture and Herbarium of the Department of Botany, Faculty of Science, Cairo University. Voucher specimen was kept in Herbarium, National Research Center, Giza, Egypt.

## 2.3. Extraction and isolation

The air dried and powdered aerial parts of *M. indicus* (1.8 kg) were macerated in ethanolic solution (70%). The solvent was removed under reduced pressure, and the residue was dissolved in hot water and extracted with chloroform, ethyl acetate and, *n*-butanol, successively. The *n*-butanol extract (47 g), a dark brown gum, was subjected to vacuum liquid chromatography (VLC) on silica gel using a gradient elution system of *n*-hexane:EtOAc (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10; v:v). Twenty-five fractions were obtained and spotted on TLC using the same solvent system. Similar TLC profiles were grouped to yield 8 fractions (S1-S8). Fraction S3 was chromatographed on a polyamide column eluted with water followed by water/methanol solutions with increasing contents of methanol (20-70%) and finishing with methanol. The collected fractions (217, 25 mL each) were combined according to their TLC analysis. Fractions 14-17 (1.3 g; eluted with water:methanol (4:1, v:v) were dissolved in methanol and subjected to a sephadex LH-20 column and eluted with methanol for further purification to afford compounds **1** (43 mg) and **2** (36 mg). Compound **4** (28 mg) was purified from S5 on a silica gel column using a mobile phase of hexane:ethyl acetate (1:1, v:v). The fraction S6 was chromatographed over a silica gel column with CHCl<sub>3</sub>:MeOH (30:1, v:v) as an eluent to give seven sub-fractions (S1<sub>1</sub>-S1<sub>7</sub>). The sub-fraction S1<sub>3</sub> was subjected to a Sephadex LH-20 (methylene chloride:MeOH, 1:1, v:v) and purified with silica gel prep. HPLC (Econosil® RP-18, 10 μ column, 250 × 22 mm, 47% MeOH) to yield compounds **3** (39 mg). The sub-fractions S1<sub>5</sub> and S1<sub>6</sub> was also subjected to a Sephadex LH-20 (methylene chloride:MeOH, 1:1, v:v) and purified with silica gel prep. HPLC (Apollo Silica 5 μ column, 250 × 22 mm; *n*-hexane:CHCl<sub>3</sub>:EtOAc, 7:3:1, v:v:v) to yield compounds **5** (22 mg) and **6** (31 mg). Fraction S8 were further TLC chromatographed on silica gel eluted with EtOAc:MeOH:

H<sub>2</sub>O (10:1:1, v:v:v) to obtain compounds **7** (27 mg) and **8** (20 mg) (Figure 1).

**(+)-Catechin:** Color: Off-white amorphous powder. M.p.: 208-211 °C. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, δ, ppm): 2.62 (dd, 1H, *J* = 16.1 and 8.1 Hz, H-4<sub>ax</sub>), 2.93 (dd, 1H, *J* = 16.1 and 5.4 Hz, H-4<sub>eq</sub>), 3.98 (m, 1H, H-3), 4.64 (d, 1H, *J* = 7.5 Hz, H-2), 5.87 (d, 1H, *J* = 2.3 Hz, H-6), 5.94 (d, 1H, *J* = 2.3 Hz, H-8), 6.73 (dd, 1H, *J* = 8.1 and 2.0 Hz, H-6'), 6.81 (d, 1H, *J* = 8.1 Hz, H-5'), 6.89 (d, 1H, *J* = 2.0 Hz, H-2'). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD, δ, ppm): 83.2 (C-2), 69.1 (C-3), 28.8 (C-4), 157.9 (C-5), 96.7 (C-6), 158.1 (C-7), 95.9 (C-8), 157.3 (C-9), 101.2 (C-10), 132.5 (C-1'), 115.6 (C-2'), 146.3 (C-3'), 146.5 (C-4'), 116.5 (C-5'), 120.4 (C-6'). ESI-MS (*m/z*): 291.0 [M+H]<sup>+</sup>. [α]<sub>D</sub> = + 8.0 (CH<sub>3</sub>OH, *c* 0.23).

**(-)-Epicatechin:** Color: Colorless amorphous powder. M.p.: 175-176 °C. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, δ, ppm): 2.74 (dd, 1H, *J* = 16.8 and 2.9 Hz, H-4<sub>ax</sub>), 2.86 (dd, 1H, *J* = 16.8 and 4.3 Hz, H-4<sub>eq</sub>), 4.19 (br. s, 1H, H-3), 4.80 (br. s, 1H, H-2), 5.92 (d, 1H, *J* = 2.3 Hz, H-8), 5.95 (d, 1H, *J* = 2.3 Hz, H-6), 6.76 (d, 1H, *J* = 8.2, H-5'), 6.80 (dd, 1H, *J* = 8.2 and 1.8 Hz, H-6'), 6.98 (d, 1H, *J* = 1.8 Hz, H-2'). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD, δ, ppm): 80.0 (C-2), 67.6 (C-3), 29.4 (C-4), 157.8 (C-5), 96.6 (C-6), 158.1 (C-7), 96.1 (C-8), 157.5 (C-9), 100.3 (C-10), 132.5 (C-1'), 115.5 (C-2'), 146.1 (C-3'), 145.9 (C-4'), 116.1 (C-5'), 119.6 (C-6'). ESI-MS (*m/z*): 291.0 [M+H]<sup>+</sup>. [α]<sub>D</sub> = -29.6 (CH<sub>3</sub>OH, *c* 0.23).

**Taxifolin:** Color: Yellow crystals. M.p.: 242 °C. <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD, δ, ppm): 4.50 (d, 1H, *J* = 11.0 Hz, H-3), 4.92 (d, 1H, *J* = 11.0 Hz, H-2), 5.89 (d, 1H, *J* = 2.0 Hz, H-8), 5.91 (d, 1H, *J* = 2.0 Hz, H-6), 6.81 (d, 1H, *J* = 8.0 Hz, H-5'), 6.86 (dd, 1H, *J* = 2.0 and 8.0 Hz, H-6'), 6.97 (d, 1H, *J* = 2.0 Hz, H-2'). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD, δ, ppm): 85.1 (C-2), 73.6 (C-3), 198.4 (C-4), 164.3 (C-5), 97.3 (C-6), 168.7 (C-7), 96.3 (C-8), 164.5 (C-9), 101.8 (C-10), 129.8 (C-1'), 115.9 (C-2'), 116.1 (C-5'), 147.1 (C-4'), 146.3 (C-3'), 120.9 (C-6'). FAB-MS (*m/z*): 305 [M+H]<sup>+</sup>. [α]<sub>D</sub> = + 17.6 (CH<sub>3</sub>OH, *c* 1.0).

**Quercetin:** Color: Yellow amorphous powder. M.p.: 311-317 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ, ppm): 6.20 (d, 1H, *J* = 1.8 Hz, H-6), 6.42 (d, 1H, *J* = 1.8 Hz, H-8), 6.90 (d, 1H, *J* = 8.4 Hz, H-5'), 7.57 (d, 1H, *J* = 2.1 Hz, and 8.4 Hz, H-6'), 7.69 (d, 1H, *J* = 2.1 Hz, H-2'), 9.33 (s, 2H, OH-3',4'), 9.56 (s, 1H, OH-3), 10.77 (s, 1H, OH-7), 12.50 (s, 1H, OH-5). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, δ, ppm): 147.5 (C-2), 136.4 (C-3), 176.6 (C-4), 161.4 (C-5), 98.9 (C-6), 164.6 (C-7), 94.1 (C-8), 156.8 (C-9), 103.7 (C-10), 122.7 (C-1'), 116.3 (C-2'), 145.8 (C-3'), 148.4 (C-4'), 115.8 (C-5'), 120.7 (C-6'). ESI-MS (*m/z*): 301.2 [M-H]<sup>-</sup>.

**Table 1.** Cytotoxicity (IC<sub>50</sub> in µg/mL) of the extracts of *M. indicus* <sup>a</sup>.

Extract	IC <sub>50</sub> (µg/mL)					
	MCF-7	Hep-2	HepG-2	HeLa	HCT-116	Caco-2
Chloroform extract	22.52±1.32	25.47±2.1	9.21±0.92	15.52±1.597	11.24±1.44	13.83±1.71
Ethylacetate extract	42.53±0.47	17.44±1.20	34.81±3.88	41.898±2.860	21.58±1.83	26.14±1.56
Methanolic extract	25.65±1.90	10.86±1.15	18.79±1.55	25.41±0.88	19.36±1.96	29.14±1.10
Doxorubicin (µM)	0.48±0.04	0.41±0.09	0.67±0.07	1.84±0.19	1.32±0.11	0.32±0.03

<sup>a</sup> The data shown are mean±SD obtained from three independent experiments.

**Quercetin 3-O-β-D-glucopyranoside:** Color: Yellow amorphous powder. M.p.: 224-226 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ, ppm): 3.28-3.65 (m, 10H, Glc), 5.37 (d, 1H, *J* = 7.63 Hz, H-1''), 6.20 (d, 1H, *J* = 1.8 Hz, H-6), 6.40 (d, 1H, *J* = 1.8 Hz, H-8), 6.82 (d, 1H, *J* = 8.62 Hz, H-5'), 7.53 (d, 1H, *J* = 2.12 Hz, H-2'), 7.67 (dd, 1H, *J* = 2.12 and *J* = 8.62 Hz, H-6'), 9.14 (s, 1H, OH-4'), 9.65 (s, 1H, OH-3'), 10.81 (s, 1H, OH-7), 12.60 (s, 1H, OH-5). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, δ, ppm): 156.8 (C-2), 133.6 (C-3), 177.5 (C-4), 161.6 (C-5), 98.9 (C-6), 164.6 (C-7), 93.8 (C-8), 156.6 (C-9), 104.0 (C-10), 121.6 (C-1'), 115.8 (C-2'), 145.8 (C-3'), 148.8 (C-4'), 116.2 (C-5'), 122.0 (C-6'), 101.2 (C-1''), 71.6 (C-2''), 74.4 (C-3''), 70.0 (C-4''), 77.7 (C-5''), 61.5 (C-6''). EI-MS (*m/z*): 462.9 [M-H].

**Quercetin 3-O-α-L-rhamnopyranoside:** Color: Yellow amorphous powder. M.p.: 174-179 °C. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, δ, ppm): 1.03 (d, 3H, *J* = 6.15 Hz, CH<sub>3</sub> Rha), 3.16-3.94 (m, 4H, Rha), 5.29 (d, 1H, *J* = 1.41 Hz, H-1''), 6.23 (d, 1H, *J* = 2.0 Hz, H-6), 6.42 (d, 1H, *J* = 2.0 Hz, H-8), 6.90 (d, 1H, *J* = 8.5 Hz, H-5'), 7.28 (dd, 1H, *J* = 2.0 and *J* = 8.5 Hz, H-6'), 7.33 (d, 1H, *J* = 2.0 Hz, H-2'). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, δ, ppm): 157.34 (C-2), 134.27 (C-3), 177.79 (C-4), 161.35 (C-5), 98.78 (C-6), 164.34 (C-7), 93.70 (C-8), 156.86 (C-9), 104.16 (C-10), 121.17 (C-1'), 115.52 (C-2'), 145.25 (C-3'), 148.50 (C-4'), 115.72 (C-5'), 120.80 (C-6'), 101.87 (C-1''), 70.43 (C-2''), 70.63 (C-3''), 71.25 (C-4''), 70.11 (C-5''), 17.54 (C-6''). ESI-MS (*m/z*): 447.1 [M-H].

**Isorhamnetin 3-O-β-D-glucopyranoside; 5,7-dihydroxy-2-(4-hydroxy-3-methoxy-phenyl)-3-(3,4,5-trihydroxy-6-hydroxy methyl-tetrahydro-pyran-2-yloxy)-chromen-4-one:** Color: Yellow amorphous powder. M.p.: 267-269 °C. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, δ, ppm): 3.07-3.83 (m, 6H, Glc), 3.90 (s, 3H, OCH<sub>3</sub>-3'), 5.40 (d, 1H, *J* = 8.5 Hz, H-1''), 6.20 (d, 1H, *J* = 2.0 Hz, H-6), 6.39 (d, 1H, *J* = 1.8 Hz, H-8), 7.05 (d, 1H, *J* = 8.4 Hz, H-5'), 7.57 (d, 1H, *J* = 2.0 Hz, H-2'), 7.69 (dd, 1H, *J* = 1.8 and 8.4 Hz, H-6'). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, δ, ppm): 156.2 (C-2), 133.0 (C-3), 177.5 (C-4), 161.1 (C-5), 98.8 (C-6), 164.0 (C-7), 93.8 (C-8), 156.4 (C-9), 104.2 (C-10), 121.7 (C-1'), 111.5 (C-2'), 149.9 (C-3'), 146.4 (C-4'), 115.6 (C-5'), 122.3 (C-6'), 55.7 (OCH<sub>3</sub>-3'), 101.0 (C-1''), 74.2 (C-2''), 77.2 (C-3''), 70.0 (C-4''), 76.6 (C-5''), 61.2 (C-6''). ESI-MS (*m/z*): 479 [M+H].

**Isorhamnetin 3-O-β-D-rutinoside; 5,7-dihydroxy-2-(4-hydroxy-3-methoxy-phenyl)-3-[3,4,5-trihydroxy-6-(3,4,5-trihydroxy-6-methyl-tetrahydro-pyran-2-yloxymethyl)-tetrahydro-pyran-2-yloxy]-chromen-4-one:** Color: Yellow amorphous powder. M.p.: 186-187 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ, ppm): 0.98 (d, 1H, *J* = 5.6 Hz, Rha-H-6), 3.07-3.40 (m, 16H, Rut-H), 3.83 (s, 3H, OCH<sub>3</sub>-3'), 4.41 (d, 1H, *J* = 10.8 Hz, Rha-H-1), 5.44 (d, 1H, *J* = 7.3 Hz, Glc-H-1), 6.19 (d, 1H, *J* = 1.9 Hz, H-6), 6.41 (d, 1H, *J* = 1.8 Hz, H-8), 6.92 (d, 1H, *J* = 8.4 Hz, H-5'), 7.53 (dd, 1H, *J* = 2.0, 8.4 Hz, H-6'), 7.86 (d, 1H, *J* = 2.0 Hz, H-2'), 9.83 (s, 1H, OH-4'), 10.89 (s, 1H, OH-7), 12.50 (s, 1H, OH-5). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, δ, ppm): 156.7 (C-2), 133.2 (C-3), 177.4 (C-4), 161.4 (C-5), 99.1 (C-6), 165.0 (C-7), 94.1 (C-8), 156.5 (C-9), 103.9 (C-10), 121.2 (C-1'), 113.5 (C-2'), 149.6 (C-3'), 55.9 (OCH<sub>3</sub>-3'), 147.1 (C-4'), 115.5 (C-5'), 122.5 (C-6'), 101.5 (glc-C-1), 74.5 (glc-C-2), 76.6 (glc-C-3), 70.8 (glc-C-4), 76.1 (glc-C-5), 67.0 (glc-C-6), 101.1 (rha-C-1), 70.5 (rha-C-2), 70.8 (rha-C-3), 72.0 (rha-C-4), 68.5 (rha-C-5), 17.8 (rha-C-6). ESI-MS (*m/z*): 623 [M-H], 647 [M+Na].

## 2.4. Cell culture

MCF-7 (breast adenocarcinoma), Hep-2 (human epithelial laryngeal carcinoma), HepG-2 (hepatocellular carcinoma), HeLa (human cervix adenocarcinoma), HCT-116 (colorectal adenocarcinoma) and Caco-2 (colon adenocarcinoma) cells were obtained from American Type Culture Collection (ATCC). HeLa and Hep-2 cells were cultured in Dulbecco's modified essential medium, and the other cells were maintained in a RPMI-1640 medium in a humidified 5% CO<sub>2</sub> atmosphere at 37 °C. All media were supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL) and 10% heat-inactivated fetal bovine serum (FBS).

## 2.5. Cytotoxicity assay

Cytotoxic activities were measured *in-vitro* the human cell lines with the MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) assay [12]. Briefly, a limited number of human cancer cells (5000/well) were seeded onto a 96-well microplate and became attached to the bottom of the well overnight. On the second day of the procedure, the original medium was removed and 200 µL new medium containing the tested extracts was added. After an incubation period of 24 h, the living cells were assayed by the addition of 20 µL 5 mg/mL MTT solution. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4 h contact period. The medium was then removed and the precipitated crystals were dissolved in 100 µL DMSO during a 60 min period of shaking. Finally, the reduced MTT was assayed at 545 nm using a microplate reader, wells with untreated cells being taken as the control. All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Doxorubicin was used as a positive control. Stock solutions of 10 mg/mL of the tested extracts were prepared with dimethyl sulphoxide (DMSO). The highest DMSO concentration (0.3%) of the medium did not have any significant effect on cell proliferation. The dose-response curves of the compounds were fitted by means of the computer program GraphPad Prism 4.0 (Graph Pad Software, San Diego, CA, USA), and IC<sub>50</sub> values were calculated.

## 3. Results and discussion

The ethanolic extract of *M. indicus* was fractionated and purified by various chromatographic techniques. Eight known flavonoids (**1-8**) were isolated for the first time from this species (Figure 1). The structural elucidation of the isolated flavonoids was achieved through chemical and spectroscopic analysis, including HRMS, UV, <sup>1</sup>H, <sup>13</sup>C and 2D NMR, and by comparison with reported data. The isolated flavonoids were identified as catechin (**1**) [13,14], epicatechin (**2**) [15,16], taxifolin (**3**) [17,18], quercetin (**4**) [19-21], quercetin 3-O-β-D-glucopyranoside (**5**) [21-23], and quercetin 3-O-α-L-rhamnopyranoside (**6**) [21,24-26], isorhamnetin 3-O-β-D-glucopyranoside (**7**) [21,27-29] and isorhamnetin 3-O-β-D-rutinoside (**8**) [21,25,30].

The anti-proliferative activity of three extracts from aerial parts of *M. indicus* was determined against Hep-2, HepG-2, HeLa, HCT-116 and Caco-2 cells by MTT assay using doxorubicin as a positive control. The IC<sub>50</sub> values are presented in Table 1.

Phytochemicals have been shown to be effective in preventing malignant transformation of cells in culture and experimentally induced tumorigenesis in various animal models. Mechanistically, chemoprevention with dietary phytochemicals could be achieved by stimulating inactivation of potential carcinogens, inhibition of abnormal cell proliferation, induction of apoptosis and delaying angiogenesis [31]. The present study was undertaken to investigate the potential of *M. indicus* chloroform, ethyl acetate and methanol extract as a chemopreventive agent by evaluating its effect on viable cell number and by evaluating the sensitivity of different cancer cell lines to the extract. The results of the present study demonstrated that, the cytotoxicity of chloroform extracts was superior to that of the methanol and water extracts, it exhibited a dose-dependent growth inhibitory effect after a continuous exposure during a 24 h period. The cytotoxic effect was greater on HepG-2 and HCT-116 cell lines. The most effective cytotoxic agent was the chloroform extract against HepG-2, HCT-116, HeLa and Caco-2 cells with an IC<sub>50</sub> 9.21, 11.24, 15.52 and 13.83 µg/mL, respectively. In contrast, the most potent cytotoxic agent against Hep-2 cells was the methanolic extract with an IC<sub>50</sub> 10.86 µg/mL.

#### 4. Conclusion

Four flavonoid aglycons and four flavonoid glycosides were isolated from ethanolic extract of *Melilotus indicus*. Their structures were confirmed by comparison of their chromatographic properties, chemical and spectroscopic data (UV, <sup>1</sup>H and <sup>13</sup>C NMR) with those reported in the literature. The antiproliferative assay of chloroform, ethyl acetate and methanol extract have been evaluated by MTT assay using doxorubicin as a positive control, and it has been shown that the methanol extract was the most potent antitumor agent against Hep-2 cell line.

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