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CHEMICAL CONSTITUENTS, CYTOTOXIC AND ANTIBACTERIAL ACTIVITIES OF THE AERIAL PARTS OF *BRASSICA NIGRA*

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Abstract: A phytochemical studies on *Brassica nigra* resulted in the isolation of seven flavonoids; kaempferol-3,7-di-O-β-D-glucopyranoside (**1**), 7-O-β-D-glucopyranosyl kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**2**), kaempferol 3-O-β-D-glucopyranoside (**3**), Kaempferol 3-O-β-D-galactopyranoside (**4**), kaempferol 3-O-α-L-rhamnopyranosyl-(1→ 2)-β-D-glucopyranoside (**5**), Kaempferol (**6**) and kaempferol 7-methyl ether (**7**) These compounds is the first time to be isolated from this species. The structure of these compounds was elucidated by chromatography, NMR and Mass spectroscopy. Cytotoxic activity of the ethanolic, ethyl acetate and hexane extracts of *Brassica nigra* was evaluated against five cancer cell lines and it was found to exhibit significant growth inhibitory activities against HepG2, HeLa, HCT, MCF-7 and HEp2 tumor cells. Antibacterial activity was evaluated using the agar diffusion and microwell dilution assays against five strains of bacteria.

Keywords: *Brassica nigra*; cytotoxic activity; Flavonoid glycoside; Antibacterial activity; Kaempferol; Plant phenolic.

INTRODUCTION

The phytochemical and biological studies on the natural crude extracts from plants are considered an important source for discovery of new drugs beneficial to human health. Natural products, mainly the plant-derived constituents, have long been considered as source of drugs, and a great part of the pharmaceuticals available in modern medicine are directly or indirectly derived from natural sources. *Brassica nigra* (Family: Brassicaceae) commonly known as mustard has both edible and medicinal value. This plant has been traditionally used in Africa for treatment of inflammation and rheumatism; also it has been used as simple rubefacient, diuretic, emetic, pneumonia, bronchitis, nerve stimulation and vesicant [1]. The main focus of our study is isolation and characterization of flavonoids from the aerial parts of *Brassica nigra*, as well as evaluation of the antibacterial and cytotoxic activities of its extracts.

MATERIALS AND METHODS

Plant Material:

Brassica nigra was collected from Beni Suef governorate on March 2011. 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.

Extraction and Isolation:

The comminuted air-dried aerial parts (1.5kg) was defatted with chloroform (2 x 3 L) and exhaustively extracted with EtOH: H_2O (7: 3) under reflux over a boiling water bath for 10 hours. The extract was then filtered and the solvent was removed in vacuo at ≈40°C. Finally, the extraction process was repeated to yield 190 g of dark amorphous material. A sample (140 g) dissolved in 400 ml aqueous ethanol (70%); this solution was extracted with hexane followed by ethyl acetate four times each. The ethyl acetate fraction was carefully applied to the top of a column (150 x 4 cm) containing 600 gm of polyamide 6S. Gradient elution started with water followed by H_2O/EtOH mixtures of decreasing polarities at a flow rate 1 ml/minute was then carried out. The bands migrated along the column were traced under UV light during elution to note their characteristics and to control the fractionation process as well. Six fractions were then obtained, individually collected, dried under vacuo at ≈40°C and subjected to detailed investigations by TDPC. Phytochemical investigation of the first and second fraction revealed the presence of trace amounts of phenolic compounds and the majority of this fraction is free sugars which were elucidated by comparative paper chromatography (CoPC) to be glucose, galactose, and rhamnose. The residual material of the third fraction (260mg) obtained after the evaporation of the eluent 40% ethanol was applied on a polyamide column chromatography (80 x 3 cm), and eluted by the solvent system 30 % ethanol-water which gave two successive sub-fractions, the two sub fractions were further purified by Sephadex LH-20 column chromatography (60 x 1.5 cm) and eluted by methanol HPLC to afford the purified samples of compound (**1**) (30 mg) and compound (**2**) (34 mg). The fourth fraction (180 mg) obtained after the evaporation of the eluent 60%

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ethanol was applied on a polyamide column chromatography (80 x 3 cm), and eluted by the solvent system 40 % ethanol-water which gave three successive sub-fractions, the three sub fractions were further purified by Sephadex LH-20 column chromatography (60 x 1.5 cm) and eluted by methanol HPLC to afford the purified samples of compound (**3**) (42 mg), compound (**4**) (55 mg) and compound (**5**) (37 mg). The residual material of the fifth fraction (160 mg) obtained after the evaporation of the eluent (80 % EtOH) was applied on a Sephadex LH-20 column and eluted by using methanol HPLC to afford the purified samples of compound (**6**) (29 mg) and compound (**7**) (25 mg).

Kaempferol- 3, 7-di-O-β-D-glucopyranoside:
Yellow amorphous powder
^{1}H NMR (500 MHz) (DMSO-d_6), δ (ppm): ***Aglycone moiety;*** 8.2 (2H, d, *J* = 8.9 Hz, H-2', H-6'), 7.2 (2H, d, *J* = 8.9 Hz, H-3', H-5'), 6.98 (1H, d, *J* = 2.4 Hz, H-8), 6.77 (1H, d, *J* = 2.4 Hz, H-6). ***Sugar moiety;*** 6.40 (1H, d, *J* = 7.3 Hz, H-1''), 5.83 (1H, d, *J* = 7.8 Hz, H-1'''), 4.2-4.6 (10H, m, H-2''- H-6'', H-2'''-H-6''')
^{13}C NMR (125 MHz) (DMSO-d_6), δ (ppm): ***Aglycone moiety;*** 155.76 (C-2), 130.97 (C-3), 178.82 (C-4), 161.2 (C-5), 100.82 (C-6), 162.9 (C-7), 95.85 (C-8), 156.79 (C-9), 105.95 (C-10), 120.7 (C-1'), 130.22 (C-2') 114.90, (C-3'), 161.11 (C-4'), 114.90 (C-5'), 130.22 (C-6'). ***Sugar moiety; Glucose at 3- position:*** 99.20 (C-1''), 74.95 (C-2''), 77.50 (C-3''), 70.40 (C-4''), 77.98 (C-5''), 61.60 (C-6''). ***Glucose at 7- position:*** 102.40 (C-1'''), 74.01 (C-2'''), 77.56 (C-3'''), 70.34 (C-4'''), 78.10 (C-5'''), 61.53 (C-6''').
Negative ESI-Mass Data [M-H]$^-$ = *m/z* 609.

7-O-β-D-glucopyranosyl kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside:
Yellow powder
^{1}H NMR (500 MHz) (DMSO-d_6), δ (ppm): ***Aglycone moiety***; 8.1 (2H, d, *J* = 9 Hz, H-2', H-6'), 6.82 (2H, d, *J* = 9 Hz, H-3', H-5'), 6.77 (1H, d, *J* = 1.9 Hz, H-8), 6.50 (1H, d, *J* = 1.9 Hz, H-6). ***Sugar moiety; Glucose at 7- position:*** 5.08 (1H, d, *J* = 7.2 Hz, H-1''), 3.65-4.15 (5H, m, H-2''- H-6''). ***Glucose at 3- position:*** 5.71 (1H, d, *J* = 7.8 Hz, H-1'''), 3.31-3.62 (5H, m, H-2'''- H-6'''***). Rhamnose at 3-position as terminal sugar:*** 1.05 (3H, d, *J* = 6.1, CH3 of rhamnose), 5.26 (1H, br, H-1''''), 3.7-4.1 (4H, m, H-2''''-H-5'''').
^{13}C NMR (125 MHz), (DMSO-d_6), δ (ppm): ***Aglycone moiety;*** 159.79 (C-2), 134.56 (C-3), 179.64 (C-4), 164.30 (C-5), 101.72 (C-6), 164.9 (C-7), 95.97 (C-8), 157.94 (C-9), 107.83 (C-10), 121.98 (C-1'), 132.45 (C-2') 117.34, (C-3'), 163.1 (C-4'), 117.34 (C-5'), 132.45 (C-6').). ***Sugar moiety; Glucose at 7- position:*** 100.52 (C-1''), 74.96 (C-2''), 77.92 (C-3''), 71.45 (C-4''), 78.36 (C-5''), 62.67 (C-6''). ***Glucose at 3- position:*** 100.82 (C-1'''), 80.14 (C-2'''), 79.08 (C-3'''), 71.95 (C-4'''), 78.63 (C-5'''), 62.75(C-6'''). ***Rhamnose at 3-position as terminal sugar:*** 102.74 (C-1''''), 72.52 (C-2''''), 72.43 (C-3''''), 73.98 (C-4''''), 69.97 (C-5''''), 18.0 CH3 of Rhamnose.
Negative ESI-Mass Data [M-H]$^-$ = *m/z* 753.

Kaempferol 3-O-β-D-glucopyranoside:
Greenish yellow amorphous powder
^{1}H NMR (500 MHz) (DMSO-d_6), δ (ppm): ***Aglycone moiety;*** 8.1 (2H, d, *J* = 8.8 Hz, H-2', H-6'), 6.98 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 6.54(1H, d, *J* = 2.0 Hz, H-8), 6.31 (1H, d, *J* = 2.0 Hz, H-6). ***Sugar moiety; Glucose at 3- position:*** 5.74 (1H, d, *J* = 7.8 Hz, H-1''), 3.20-3.65 (5H, m, H-2''- H-6'').
^{13}C NMR (125 MHz) (DMSO-d_6), δ (ppm): 159.76 (C-2), 135.57 (C-3), 179.82 (C-4), 162.97 (C-5), 104.10 (C-6), 165.98 (C-7), 95.44 (C-8), 158.76 (C-9), 107.34 (C-10), 123.28 (C-1'), 133.24 (C-2') 116.47, (C-3'), 161.61 (C-4'), 116.47 (C-5'), 133.24 (C-6'). ***Sugar moiety; Glucose at 3-position:*** 100.59 (C-1''), 79.01 (C-2''), 75.98 (C-3''), 71.80 (C-4''), 78.83 (C-5''), 62.63 (C-6'').
Negative ESI-Mass Data [M-H]$^-$ = *m/z* 447.

Kaempferol 3-O-β-D- galactopyranoside:
Yellow amorphous powder
^{1}H NMR (500 MHz) (DMSO-d_6), δ (ppm): ***Aglycone moiety;*** 8.09 (2H, d, *J* = 8.8 Hz, H-2', H-6'), 6.90 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 6.47 (1H, d, *J* = 1.9 Hz, H-8), 6.25 (1H, d, *J* = 1.9 Hz, H-6). ***Sugar moiety; Galactose at 3-position:*** 5.43 (1H, d, *J* = 7.6 Hz, H-1''), 3.21-3.68 (5H, m, H-2''- H-6'').
^{13}C NMR (125 MHz) (DMSO-d_6), δ (ppm): 156.54 (C-2), 133.48 (C-3), 177.73 (C-4), 161.38 (C-5), 100.12 (C-6), 164.98 (C-7), 98.88 (C-8), 156.41 (C-9), 103.98 (C-10), 120.96 (C-1'), 130.99 (C-2') 115.45 (C-3'), 159.97 (C-4'), 115.45 (C-5'), 130.99 (C-6'). ***Sugar moiety; Galactose at 3-position:*** 101.65 (C-1''), 71.83 (C-2''), 73.42 (C-3''), 68.10 (C-4''), 75.89 (C-5''), 60.62 (C-6'').
Negative ESI-Mass Data [M-H]$^-$ = *m/z* 447.

Kaempferol 3-O-α-L-rhamnopyranosyl- (1→ 2)-β-D-glucopyranoside:
Yellow powder
^{1}H NMR (500 MHz) (DMSO-d_6), δ (ppm): ***Aglycone moiety***; 7.99 (2H, d, *J* = 8.4 Hz, H-2', H-6'), 6.81 (2H, d, *J* = 8.4 Hz, H-3', H-5'), 6.15 (1H, d, *J* = 2 Hz, H-8), 5.98 (1H, d, *J* = 2 Hz, H-6). ***Sugar moiety; Glucose at 3- position:*** 5.68 (1H, d, *J* = 7.8 Hz, H-1''), 3.19-3.70 (5H, m, H-2''- H-6''). ***Rhamnose at 3-position as terminal sugar:*** 1.03 (3H, d, *J* = 6.6, CH3 of rhamnose), 5.24 (1H, br, H-1'''), 3.23-4.10 (4H, m, H-2'''-H-5''').
^{13}C NMR (125 MHz), (DMSO-d_6), δ (ppm): ***Aglycone moiety;*** 158.41 (C-2), 134.64 (C-3), 179.49 (C-4), 163.32 (C-5), 99.83 (C-6), 165.91 (C-7), 94.86 (C-8), 158.84 (C-9), 105.96 (C-10), 123.20 (C-1'), 132.22 (C-2'), 116.24 (C-3'), 161.35 (C-4'), 116.24 (C-5'), 132.22 (C-6').). ***Sugar moiety; Glucose at 3- position:*** 100.51 (C-1''), 79.91 (C-2''), 78.94 (C-3''), 71.85 (C-4''), 78.36 (C-5''), 62.67 (C-6''). ***Rhamnose at 3-position as terminal sugar:*** 102.73 (C-1'''), 72.45 (C-2'''), 72.39 (C-3'''), 74.58 (C-4'''), 70.07 (C-5'''), 17.60 CH3 of Rhamnose.
Negative ESI-Mass Data [M-H]$^-$ = *m/z* 593.

Kaempferol:
Yellow crystals
^{1}H NMR (500 MHz) (DMSO-d6), δ (ppm): 8.03 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.93 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.17 (1H, d, *J* = 2.0 Hz, H-6).
^{13}C NMR (125 MHz) (DMSO-d6), δ (ppm): 146.80 (C-2), 135.84 (C-3), 176.20 (C-4), 161.60 (C- 5), 98.60 (C-6), 164.59 (C-7), 93.85 (C-8), 156.40 (C-9), 103.70, (C-10), 121.90 (C-1'), 129.90 (C-2', C-6'), 115.80 (C-3', C-5'), 159.5 (C-4').
Negative ESI-Mass Data [M-H]$^-$ = *m/z* 285.

Kaempferol 7-methyl ether:
Yellow crystals
^{1}H NMR (500 MHz) (DMSO-d6), δ (ppm): 8.23 (2H, d, *J* = 8.6 Hz, H-2', H-6'), 7.33 (2H, d, *J* = 8.6 Hz, H-3', H-5'), 6.65 (1H, d, *J* = 1.8 Hz, H-8), 6.41 (1H, d, *J* = 1.8 Hz, H-6), 3.79 (3H, S, OMe).
^{13}C NMR (125 MHz) (DMSO-d6), δ (ppm): 146.95 (C-2), 136.24 (C-3), 176.19 (C-4), 161.75 (C- 5), 97.98 (C-6), 165.78 (C-7), 91.95 (C-8), 156.51 (C-9), 103.91 (C-10), 124.94 (C-1'), 131.56 (C-2', C-6'), 115.90 (C-3', C-5'), 158.57 (C-4').
Negative ESI-Mass Data [M-H]$^-$ = *m/z* 299.

Determination of antibacterial activity:

Agar diffusion assay: Antibacterial activity was performed against five strains of bacteria by agar diffusion method [2]; cotton swabs are used for each bacterial suspension (10^6/ml) and inoculated onto plates where the bacteria were spread uniformly on the agar surface. Agar surface was perforated with 6-mm-diameter holes, aseptically cut and filled with the various extracts. The extracts were used in the concentration of 10 mg extract/ml of dimethylsulphoxide (DMSO) 10% because this concentration of DMSO does not inhibit bacterial growth. The plates were incubated at 37°C for 18 h and then examined to evaluate inhibition.

Micro-well dilution assay: Minimal inhibitory concentration (MIC) values were determined for the bacterial strains by microdilution method [3]. Tested bacteria strains were suspended in the Nutrient Broth by adjusting to the 0.5 McFarland standard. The ethanolic, hexane and ethyl acetate extracts were firstly dissolved in 10% DMSO and then diluted to the highest concentration (20 mg/ml) to be tested, and then serial two-fold dilutions were made in 10 ml sterile test tubes containing nutrient broth. The 96-well plates were prepared by dispensing into each well 95 µl nutrient broth and 5 µl inoculums. A 100 µl from the stock solutions of each extract initially prepared at the concentration of 20 mg/ml were added to the first wells. Then, 100 µl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl nutrient broth without compound and 5 µl inoculums on each strip was used as negative control. The final volume in each well was 200 µl. The plate was covered with a sterile plate sealer and then incubated for 18 h at 37 °C. MIC values were determined by visual inspection and defined as the lowest concentration of the extract to inhibit growth of microorganisms after incubation.

Cancer cell lines:

All cell lines were purchased from the American Type Culture Collection (ATCC). Five cell lines were used in this study; HepG2 cells (Human cell line of a well differentiated hepatocellular carcinoma isolated from a liver), HeLa (Cervical carcinoma cells), HCT (Colon carcinoma cells), MCF-7 (Breast carcinoma cells), HEp2 (Human epidermoid larynx carcinoma cells) and Vero cell line (Normal kidney cells) to evaluate the cytotoxic effect of extracts of *Brassica nigra*. Cells were grown or maintained upon arrival at 37°C in a humidified incubator with 5% CO_2 and 95% atmosphere as recommended by ATCC. All media were supplemented with penicillin (100 U/ml), streptomycin (100µg/ml) and 10% heat-inactivated fetal bovine serum (FBS).

Cytotoxicity assay:

Cytotoxicity was determined by nonradioactive, colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[4]. The HeLa, HepG2, HCT, HEp2 and MCF-7 cells seeded in 96-well plates at a concentration of 1×10^4 cells per well were treated with various concentrations of tested extracts and incubated in a humidified 5% CO_2 atmosphere at 37°C. After an incubation period for 72 hours, 10µl of 5 mg/ml MTT was added to each well and the incubation was continued for another 4 hours. After removal of the supernatant, formazan crystals were dissolved in 100µl DMSO (The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment) and the optical density values were determined at 570 nm with a microplate reader. Deguelin was used as the positive control.

RESULTS AND DISCUSSION

Characterization of constituents:

In this study, the ethyl acetate fraction of the aerial parts of *Brassica nigra* was subjected to chromatographic separation to afford seven flavonoids (**1-7**), By comparison of their spectral data with those reported, these isolated compounds were identified as: kaempferol- 3,7-di-O-β-D-glucopyranoside (**1**) [5], 7-O-β-D-glucopyranosyl kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**2**)[6], kaempferol 3-O-β-D-glucopyranoside (**3**)[7], kaempferol 3-O-β-D-galactopyranoside (**4**)[8], kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**5**)[7], Kaempferol (**6**) [9,10] and kaempferol 7-methyl ether (**7**) [11]. All of these compounds were isolated for the first time from this species.

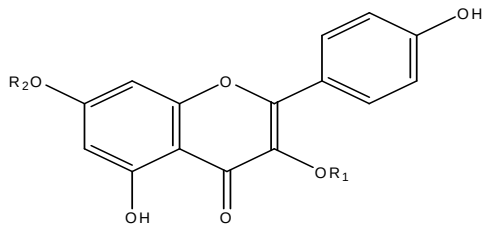

Figure 1: Structure of the isolated compounds.

Compound		
1	R1 = β-D-glucopyranose	R2 = β-D-glucopyranose
2	R1 = α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranose	R2 = β-D-glucopyranose
3	R1 = β-D-glucopyranose	R2 = H
4	R1 = β -D-galacotpyranose	R2 = H
5	R1 = α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranose	R2 = H
6	R1 = H	R2 = H
7	R1 = H	R2 = CH_3

Antibacterial activity:

According to the results given in Table 1, the ethanolic extract of *Brassica nigra* exhibited the best antibacterial activity against all tested bacteria; it was the most effective extract with the lowest MIC value (0.81 and 0.83 mg/ml) for *Streptococcus pyogenes* and *Escherichia coli* inhibition. The ethyl acetate and hexane ether extracts exhibited conservative antibacterial activity.

Table.1: Antibacterial activity of *Brassica nigra* extracts.

Bacteria	Ethanolic extract		Hexane extract		Ethylacetate extract	
	Diameter of inhibition	MIC	Diameter of inhibition	MIC	Diameter of inhibition	MIC
Staphylococcus aureus (RCMB 000106)	17.4 ± 0.5	6	11.2 ± 0.4	11	13.3 ± 0.2	9
Escherichia coli (RCMB 000103)	19.2 ± 0.3	0.83	9 ± 1.2	3.1	10.1 ± 0.8	2.9
Bacillis subtilis (RCMB 000101)	24 ± 0.03	0.97	11.3 ± 0.7	1.45	12.4 ± 0.4	2.6
Streptococcus pyogenes (RCMB 000109)	27.8 ± 0.4	0.81	10 ± 0.6	2.2	10.8 ± 0.4	1.9
Salmonella typhimurium (RCMB 000104)	19.4 ± 0.2	3.1	14.2 ± 0.3	5.2	16.8 ± 0.1	4.9

RCMB: The Regional Center for Mycology and Biotechnology Culture Collection, El-azhar University.
Data are expressed in the form of mean ±SD.

This study showed that, ethanolic extract from *Brassica nigra* has a potent antibacterial activities and this is probably reveals why the plant is widely used in traditional medicine. The ethanolic extract from this plant has a broad spectrum activity since it is effective against both Gram positive and Gram negative bacteria. The extract was found to be active against *Streptococcus pyogenes* which is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases[12]. The extracts were also active against *Salmonella typhimurium* which is the cause of gastroenteritis in humans and other mammals. This observation is of particular interest since many Egyptian traditional healers are using this plant for treating stomach discomfort.

Screening of chemical constituents in the ethanolic extract of *Brassica nigra* revealed presence of flavonoids known for their good antibacterial activity[13]. Based on the results of chemical composition, we conclude that, antibacterial activity of ethanolic extract is apparently related to the presence these compounds. MIC determination was carried out to calculate the lowest concentration of the antibacterial agent for inhibition of different microbes.

Cytotoxic activity:

The Brassica nigra extracts were tested for cytotoxic activity against five cancer cell lines, namely HEPG2 (Human cell line of a well differentiated hepatocellular carcinoma isolated from a liver), HeLa (Cervical carcinoma cells), HCT (Colon carcinoma cells), MCF-7 (Breast carcinoma cells) and HEp2 (Human epidermoid larynx carcinoma cells), using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) dye assay (Table 2).

Table.2: Cytotoxic activity of extracts of *Brassica nigra*

Extracts	IC_{50} value (µg/ml)				
	HEPG2	HEp2	HeLa	HCT	MCF-7
Ethyl acetate extract	19.6 ± 0.8	8.3 ± 0.6	13.5 ± 1.2	18.4 ± 0.9	11.6 ± 0.2
Hexane extract	23 ± 2.4	36 ± 2.3	24 ± 1.8	21.3±3.2	22.8 ± 1.7
Ethanolic extract	21 ± 0.6	15 ± 2.1	18 ± 0.6	25 ± 2.3	23.5± 2.9

Cytotoxicity screening models provide important preliminary data to help selecting plant extract with potential antineoplastic properties for future work [14]. In view of the present data, *Brassica nigra* extracts showed a potent cell growth inhibition activity on all tested cancer cell lines, in a dose dependent manner, with more potent antiproliferative activity for the ethyl acetate extract against the human epidermoid larynx carcinoma cells (Hep2 cell line) with IC_{50} of 8.3 µg/ml, as compared to the control cells. Flavonoids were identified in the current tested plant. This observation is of particular importance since flavonoids are ingredients of many vegetables and fruits and the association of vegetable and fruit consumption with reduced cancer risk has been reported [15,16]. Flavonoids

have been shown to exhibit a series of biological effects among which stand out the inhibition of lipid peroxidation and platelet aggregation, due to their antioxidant properties and their ability of removing free radicals and chelating divalent cations[17]. Data of the phytochemical screening showed the presence of the flavonoid kaempferol and its derivatives which have received special attention as dietary constituents during the last few years. The epidemiological studies pointed out to their possible role in preventing cardiovascular diseases and cancer [18].

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