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Antiproliferative, antioxidant and antimicrobial activities of phenolic compounds from *Acrocarpus fraxinifolius*.

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ABSTRACT

*Eight flavonoids, quercetin (1), quercetin 3-O-β-D-glucopyranoside (**2**), quercetin3-O-α-L-rhamnopyranoside(**3**), myricetin (4), myricetin 3-O-β-D-galactopranoside (**5**), myricetin 3-O-α-L-rhamnopyranoside (**6**), desmanthin-1 (**7**), and naringenin (**8**), in addition to four phenolic acids, brevifolin carboxylic acid (**9**), ellagic acid (**10**) gallic acid (11), and methyl gallate (12) were isolated from the leaves of Acrocarpus fraxinifolius. The chemical structure of the isolated phenolic compounds were established by ^{1}H, ^{13}C-NMR, UV, and HR-ESI-MS. Ellagic acid produced an antiproliferative effect on the triple negative breast cancer cells MDA-MB-231 with IC_{50} of 38.7 µM. The aq. methanolic extract and most of the isolated compounds showed significant antioxidant activity in comparison with the positive control; rutin using TLC-DPPH˙-image processing procedures. Also the methanol extract and some isolated compounds exhibited antibacterial activity against P.aeruginosa L. subtilis E. coli S. aureus, it did not show any antifungal activity.*

Keywords: *Acrocarpus fraxinifolius*, phenolic compounds, triple negative breast cancer, MDA-MB-231, TLC-DPPH˙-image processing, antibacterial.

INTRODUCTION

Since arrival of human beings to the earth, plants were used in many domains including medicine, nutrition, flavoring, beverages, dyeing, cosmetics, and several industries. From the old prehistoric era, plants were used in a wide area in all medicinal therapy until synthetic drugs were developed in the 19nd century [1].

Breast cancer represents the most abundant tumor in women with a ratio of 25% of all discovered tumors worldwide [2]. Triple negative breast cancer (TNBC) represents an incidence rate of about 20% of breast cancer. It has substantial difficulties in the treatment by hormone replacement because the tumor cells have no estrogen receptors. Identifying natural compounds with antiproliferative activities against this type of cancer is of great value [3].

Acrocarpus fraxinifolius Wight &Arn. (family: Fabaceae) is a native wide spread tree around the world especially in Africa and Asia [4]. The anti-inflammatory effects and lipoidal content of different extracts of the leaves of this

plant were studied [5]. Until now, there are no reports about the secondary metabolites identification of *A. fraxinifolius.*

Therefore, in the present study, we tested the isolated flavonoids and phenolic acids for the antiproliferative effects against MDA-MB-231 TNBC cells. Additionally; the isolated phenolics were evaluated for their free radical scavenging and antimicrobial activities.

EXPERIMENTAL SECTION

2.1. General

NMR spectra were recorded on a Varian MR 400 NMR spectrometer (Japan) at 400 MHz for ^{1}H and 100 MHz for ^{13}C-NMR. HR-ESI-MS were recorded by a Bruker APEX II mass spectrometer, Hiroshima University (Japan). UV spectra were measured by using OMM 7070E Shimadzu UV 240 spectrophotometer (Shimadzu, Cairo, Egypt).

2.2. Plant material

The leaves of *A. fraxinifolius* Wight &Arn. were collected from El-Zohria garden and identified by Dr. Mohamed El-Gebally, former Researcher of Botany, National Research Centre, Dokki, Cairo, Egypt, in March 2013. A voucher specimen (Code No: AF-112-023) was deposited at the herbarium of the National Research Centre (Giza, Egypt).

2.3. Extraction and isolation

The air-dried leaves of *A. fraxinifolius* (1400 g) were crushed and macerated in 70% methanol at room temperature then filtered, and dried under vacuum to give dark black gum (73 g). The aq. methanolic extract was dissolved in distilled water and defatted by methylene chloride and concentrated *in vacuo*. The residue (42 g) was subjected to hp 20 diaion column chromatography using H_2O / MeOH mixtures in order of decreasing polarities afforded 7 major sub-fractions (AF-1 : AF-7). The sub-fractions were then subjected to different chromatographic techniques including 3MM preparative paper chromatography and repeated sephadex LH-20 column using eluents of different polarities. This led to the isolation and purification of twelve phenolic compounds (**1-12**).

2.4. Spectroscopic data of isolated compounds

Quercetin 3-*O*-β-D-glucopyranoside (Isoquercetin) (2): was isolated as yellow amorphous powder, HR-ESI-MS at *m/z* 487.07732 $(M+Na)^-$; 463.1 $(M-H)^+$, **^{1}H-NMR** (DMSO-d_6, 400 MHz): δ (ppm), 6.16 (1H, d, *J* = 1.8 Hz, H-6), 6.38 (1H, d, J = 1.8 Hz, H-8), 7.63 (1H, d, J = 2.2 Hz, H-2'), 6.91 (1H, d, *J* = 9.0 Hz, H-5'), 7.53 (1H, dd, J = 2.2, 9.0 Hz, H-6'), 5.25 (1H, d, J = 7.3 Hz, H-1``), 2.91-3.62 (sugar remaining protons); **$^{13}$C-NMR** (DMSO-$d_6$, 100 MHz): δ (ppm), 158.8 (C-2), 136.0 (C-3), 179.4 (C-4), 162.1 (C-5), 98.6 (C-6), 165.3 (C-7), 94.2 (C-8), 158.9 (C-9), 104.5 (C-10), 121.6 (C-1'), 115.8 (C-2'), 144.9 (C-3') , 149.9 (C-4'), 116.7 (C-5'), 122.0 (C-6'), 100.6 (C-1``), 74.3 (C-2``), 75.8 (C-3``), 70.8 (C-4``), 78.7 (C-5``), 61.7 (C-6``)

Quercetin 3-*O*-α-L-rhamnopyranoside (3): was isolated as yellow powder, HR-ESI-MS at *m/z* 471.2374 $(M+Na)^-$; 447.2 $(M-H)^+$,**^{1}H-NMR** (DMSO-d_6, 400 MHz): 5.31 (1H, s, H-1``), 6.21 (1H, s, H-6), 6.39 (1H, s, H-8), 6.84 (1H, d, *J* = 8.2, H-5`), 7.31 (1H, d, *J* = 8.2, H-6`), 7.34 (1H,s, H-2`), 2.96 – 3.61 (Sugar remaining protons). **$^{13}$C-j`uyhNMR** (DMSO-d_6, 100 MHz): 157.5 (C-2), 134.0 (C-3), 177.5 (C-4), 161.1 (C-5), 98.4 (C-6), 163.9 (C-7), 93.8 (C-8), 156.2 (C-9), 103.8 (C-10), 120.4 (C-1`), 115.6 (C-2`), 145.3 (C-3`), 148.2 (C-4`), 116.0 (C-5`), 119.8 (C-6`), 101.2 (C-1``), 70.5 (C-2``), 70.1 (C-3``), 71.3 (C-4``), 69.8 (C-3``), 17.3 (C-6``).

Myricetin 3-*O*-β-D-galactopyranoside (5): was isolated as yellow powder, HR-ESI-MS at *m/z* 503.0789 $(M+Na)^+$; 479.2 $(M-H)^-$, **^{1}H-NMR** (DMSO-d_6, 400 MHz): 5.32 (1H, d, *J* = 7.2, H-1``), 6.18 (1H, d, *J* = 1.9, H-6), 6.43 (1H, d, *J* = 1.9, H-8), 7.11 (1H, s, H-2`, H-6`), 3.13 – 3.62 (Sugar remaining protons), **^{13}C-NMR** (DMSO-d_6, 100 MHz): 156.1 (C-2), 134.1 (C-3), 177.2 (C-4), 161.3 (C-5), 98.7 (C-6), 164.5 (C-7), 94.2 (C-8), 156.3 (C-9), 103.0 (C-10), 120.1 (C-1`), 108.6 (C-2`, C-6`), 145.7 (C-3`, C-5`), 136.8 (C-4`), 102.6 (C-1``), 71.7 (C-2``), 73.8 (C-3``), 68.5 (C-4``), 76.4 (C-5``), 60.5 (C-6``).

Myricetin 3-*O*-α-L-rhamnopyranoside (6): was isolated as white powder, HR-ESI-MS at *m/z* 481.3274 $(M+Na)^+$; 463.1 $(M-H)^-$, **^{1}H-NMR** (DMSO-d_6, 400 MHz): 5.18 (1H, br s, H-1``), 6.20 (1H, d, *J* = 2.0, H-6), 6.39 (1H, d, *J* = 2.0, H-8), 6.95 (1H, s, H-2`, H-6`), 3.01 – 3.21 (Sugar remaining protons), **^{13}C-NMR** (DMSO-d_6, 100 MHz): 156.5 (C-2), 133.8 (C-3), 177.3 (C-4), 161.0 (C-5), 98.6 (C-6), 164.0 (C-7), 93.4 (C-8), 156.3 (C-9), 104.1 (C-10), 119.9 (C-1`), 108.6 (C-2`, C-6`), 145.5 (C-3`, C-5`), 136.9 (C-4`), 101.8 (C-1``), 70.7 (C-2``), 71.1 (C-3``), 71.6 (C-4``), 70.5 (C-5``), 17.8 (C-6``).

Desmanthin-1 (7): was isolated as yellow amorphous powder, HR-ESI-MS at *m/z* 617.1040 $(M+H)^+$; 615.0992 $(M-H)^+$, **^{1}H-NMR** (DMSO-d_6, 400 MHz): ^{1}H-NMR (DMSO-*d6*, 400 MHz), δ: 6.22 (1H, d, *J* = 2.1, H-6), 6.37 (1H, d, *J* = 2.1, H-8), 7.01 (2H, s, H-2'/6'), 5.53 (1H, d, *J* = 1.7, H-1''), 5.61 (1H, dd, *J* = 1.7, 3.5 Hz, H-2''), 4.08 (1H, dd, *J* = 3.5, 8.9 Hz, H-3''), 3.50 (1H, t, *J* = 8.9, H-4''), 3.53 (1H, m, H-5''), 1.09 (3H, d, *J* = 5.7, CH3), 6.93 (2H, s, H-2'''/6'''). ^{13}C-NMR (DMSO-*d6*, 100 MHz), δ: 99.1 (C-6), 94.2 (C-8), 110.1 (C-2'/6'), 100.0 (C-1''), 73.0 (C-2''), 70.3 (C-3''), 73.1 (C-4''), 71.9 (C-5''), 16.6 (CH3), 109.4 (C-2'''/6''').

5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one (Naringenin) (**8**): was isolated as yellow powder, HR-ESI-MS at *m/z* 295.1723 $(M+Na)^+$; 271.3 $(M-H)^+$, **^{1}H-NMR** (DMSO-d_6, 400 MHz): 2.53 (1H, dd, *J* = 17.3, 3.0, H-3β), 3.31 (1H, dd, *J* = 17.1, 12.6, H-3α), 5.36 (1H, dd, *J* = 12.7, 2.7, H-2), 6.12 (2H, s, H-6, H-8), 6.79 (2H, d, *J* = 8.4, H-3`, H-5`), 7.33 (2H, d, *J* = 8.3, H-2`, H6`); **^{13}C-NMR** (DMSO-d_6, 100 MHz):81.0 (C-2), 44.3 (C-3), 198.3 (C-4), 165.4 (C-5), 97.1 (C-6), 168.6 (C-7), 96.5 (C-8), 166.1 (C-9), 105.1 (C-10), 131.8 (C-1`), 129.3 (C-2`; C-6`), 116.9 (C-3`; C-5`), 159.1 (C-4`).

Brevifolin carboxylic acid (9): was isolated as yellow powder, **^{1}H NMR** (DMSO-d_6, 400 MHz): δ 7.15 (1H, s, H-7), 4.35 (1H, d, *J*= 6.4, H-2), 2.99 (1H, d, *J*=17.3, H-3a), 2.62 (1H, d, *J*=17.3, H-3b). **^{13}C NMR** (DMSO-d_6, 100 MHz): 195.3 (C-4) **,** δ 173.1 (C-1), 161.3 (C-6), 148.8 (C-4a),δ 145.7 (C-10), 143.0 (C-8), 142.4 (C-9), 141.4 (C-10a),116.2 (C-4b), 113.0 (C-6a), 108.6 (C-7), 42.6 (C-2), 37.7 (C-3).

2.5. Cell culture

All materials and reagents for cell culture were purchased from LONZA (Germany). Human triple negative breast cancer cell line MDA-MB-231 (a generous gift from Dr. Atanasio Pandiella, University of Salamanca, Spain) were maintained as monolayer culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and100µg/ml streptomycin sulfate. Monolayers were passaged at 70-90% confluence using trypsin-EDTA solution. All cell incubations were maintained at humidified CO_2 incubator with 5% CO_2 at 37°C.

2.6. Cell Proliferation assay (MTT)

MDA-MB-231 cells (5000 cells/well) were seeded onto 96-well plates in a total volume of 200 µl and left overnight to form a semi-confluent monolayer. We employed the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide dye (Carbosynth, UK) that is based on the reduction of the dye by mitochondrial dehydrogenases of metabolically active cells to insoluble formazan crystals (Mosmann, 1983). Briefly, cell monolayers were treated in quadrate with vehicle (DMSO, 0.1% v/v) or test samples for an exposure time of 48 h. At the end of exposure, MTT solution in PBS (5 mg/ml) was added to all well and left to incubate for 90 min. The formation of formazan crystals was visually confirmed using phase contract microscopy. DMSO (100 µl/well) was added to dissolve the formazan crystals with shaking for 10 min after which the absorbance was read at 590 nm against no cell blanks on a FLuo Star Optima microplate reader (BMG technologies, Germany). Cell proliferation was calculated comparing the OD values of the control wells and those of the samples both represented as % proliferation (control proliferation=100%). Dose-response experiment were performed on samples producing > or =50% loss of cell viability using five serial 2-fold dilutions (50, 25, 12.5, 6.25 and 3.125 µM or µg/ml of the compounds or the total extract, respectively. IC_{50} values (concentration of sample causing 50% loss of cell proliferation of the vehicle control) were calculated using the dose response curve fit to non-linear regression correlation using GraphPad Prism® V6.0 software.

2.7. Morphological assessment

Assessment of morphological changes of MDA-MB-231 cells following treatments were performed using phase contrast inverted microscope (Ziess, USA) and photomicrographs were taken using digital camera.

2.8. TLC-DPPH˙-image free radical scavenger activity processing

TLC-DPPH˙ test was used to evaluate the free radical scavenging activity of the methanol extract along with seven isolated phenolics. 5 mL of well determined concentration of *A. fraxinifolius* methanol extract and seven isolated phenolics along with standard of rutin were applied on silica TLC plate spot-wise, with a distance of 8mm between them, and a 10 mm distance from both the left and low edge applied by means of a micropipette with a scale. The plates were eluted and developed in vertical chambers pre-saturated for 15 min with the optimized mobile phase: acetonitrile: water: chloroform: formic acid (60:15:10:5, v/v/v/v).

The tested extract and isolated compounds were compared with the activity of rutin as a standard of antioxidant. ImageJ program was used for different image processing and compared: which has been used most often in published papers [6-7]. The TLC images were performed by ImageJ program, issued by the National Institute of Health in the USA, according to the procedure described in details elsewhere [7-9].

2.9. Antimicrobial activity of methanol extract and isolated compounds

2.9.1. Microorganisms

The used microorganisms in our study were obtained from the culture collection of Microbial and Natural Products Chemistry Department, National Research Centre (NRC), Cairo, Egypt. Gram positive bacteria, *Staphylococcus aureus* ATCC 29213 and *Lactoacillus subtilis*, the gram negative bacteria, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27953 were used. The used fungi were *Aspergillus niger* NRRL-599and *Fusarium solani*. The culture of all bacteria strains were performed in nutrient agar medium at 37°C for 24 h prior while the culture of fungi were occurred on potato dextrose agar at 28°C for 72 h then suspended in potato dextrose broth prior before using.

2.9.2. Chemicals

The used nutrient agar medium and potato dextrose agar medium were purchased from Lab M limited (Lancashire BL9 6AS, UK), Becton_Dickinson and Sparks (MD 21152, USA).

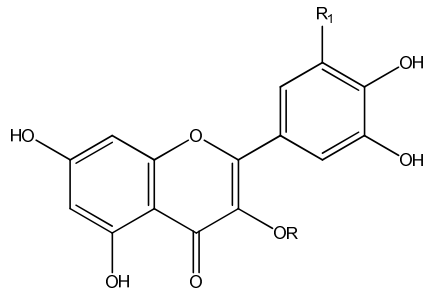
2.9.3. Antimicrobial assay

Agar disc diffusion method was used for antimicrobial and minimum inhibitory concentration (MIC) assays [10]. Briefly, bacterium or fungus microbial suspension was swabbed and spread on nutrient agar or potato dextrose agar, respectively and adjusted to McFarland No. 0.5 standard turbidity. The sterilized paper discs (6 mm D) with the required doses of the aq. methanolic extract and isolated compounds (5, 10, 20, 50 and 100 µg/disc) were placed on the surface of inoculated plates. The positive controls, thiamphenicol and nystatin (50 µg/disc), were used for determination of the sensitivity of bacteria and fungi, respectively. The incubation of the plates was performed at 37°C for 24 h for bacteria and at 28ºC for 48 h for yeast and fungi. MIC (µg/ml) is the minimum concentration of sample that produces visible growth inhibition zone after incubation.

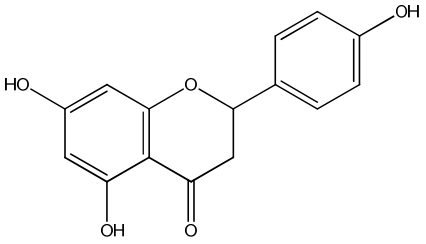
RESULTS AND DISCUSSION

3.1. Phenolic compounds of *A. fraxinifolius*

The phenolic constituents were isolated for the first time from *A. fraxinifolius* leaves using different chromatographic techniques. Structure elucidation of the pure isolated compounds were performed using different spectroscopic techniques; UV, 1D, 2D-NMR and HR-ESI-MS spectrometry. The structure of these isolated phenolics were confirmed by comparing their data with those published data. They were identified to be three flavonoid aglycones: quercetin (**1**), myricetin (**4**), naringenin (**8**) [11-12], four flavonoid glycosides, quercetin 3-*O*-β-D-glucopyranoside (**2**) [13-14], quercetin 3-*O*-α-L-rhamnopyranoside (**3**) [14], myricetin 3-*O*-β-D-galactopyranoside (**5**), myricetin-3-*O*-α-L-rhamnopyranoside (**6**) [11-16], in addition to one acylated flavonoid glycoside, desmanthin-1 (**7**) and four phenolic acids: brevifolin carboxylic acid (**9**), ellagic acid (**10**), gallic acid (**11**) and methyl gallate (**12**) [16] as shown in Fig. 1.



1 -- R = R_1 = H; 2 -- R = β-D-glucopyranoside, R_1 = H; 3 -- R = α-L-rhamnopyranoside, R_1 = H;4 -- R = H, R_1 = OH; 5 -- R = β-D-galactopyranoside, R_1 = OH; 6 -- R = α-L-rhamnopyranoside, R_1 = OH; 7 -- R =2''-Galloyl-α-L-rhamnopyranoside, R_1 = OH



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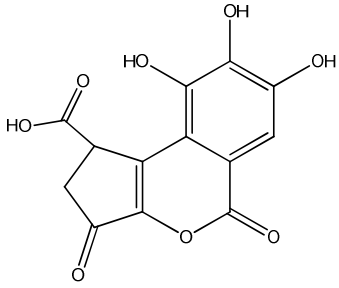

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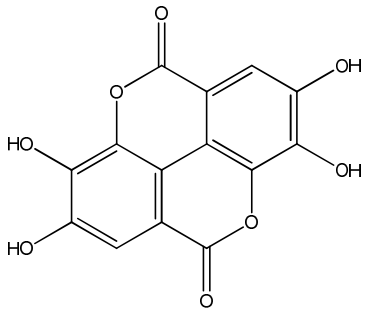

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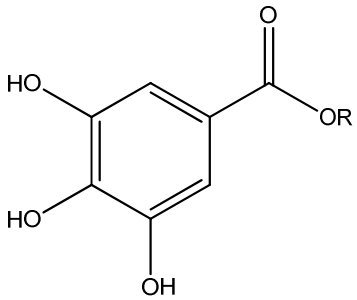

11 – R = H; 12 – R = CH_3

Fig. 1: Chemical structures of isolated compounds from *A. fraxinifolius*

3.2. Effect of compounds on the cell proliferation of MDA-MB-231

The total extract and isolated flavonoids were tested for their antiproliferative activity on the TNBC cells MDA-MD-231. In a prescreen (data not shown), cells were treated with 0.1% (v/v) DMSO (vehicle), total extract (50 µg/ml) or 50 µM from isolated compounds; quercetin 3-*O*-β-D-glucopyranoside (**2**), myricetin 3-*O*-β-D-galactopranoside (**5**), myricetin 3-*O*-α-L-rhamnopyranoside (**6**), desmanthin-1 (**7**), and naringenin (**8**), brevifolin carboxylic acid (**9**), and ellagic acid (**10**). The prescreen revealed that only three samples among tested samples cause pronounced antiproliferation effect on cells, namely compounds quercetin 3-*O*-β-D-glucopyranoside (**2**), myricetin 3-*O*-β-D-galactopranoside (**5**), and ellagic acid (**10**). Further dose-dependent effect were tested to reveal the IC_{50} of those three compounds (concentration causing a 50% loss of cell proliferation, compared to vehicle-treated control) in addition to the total extract (TE). The three tested compounds (**2**, **5** and **10**) displayed dose dependent loss of MDA-MB-231 cell proliferation with IC_{50} of 50.0, 47.6 and 38.7 µM, respectively (Fig. 2). However, treating the cells with increasing concentrations of the total extract caused lower loss of cell proliferation (IC_{50}>50 µg/ml).

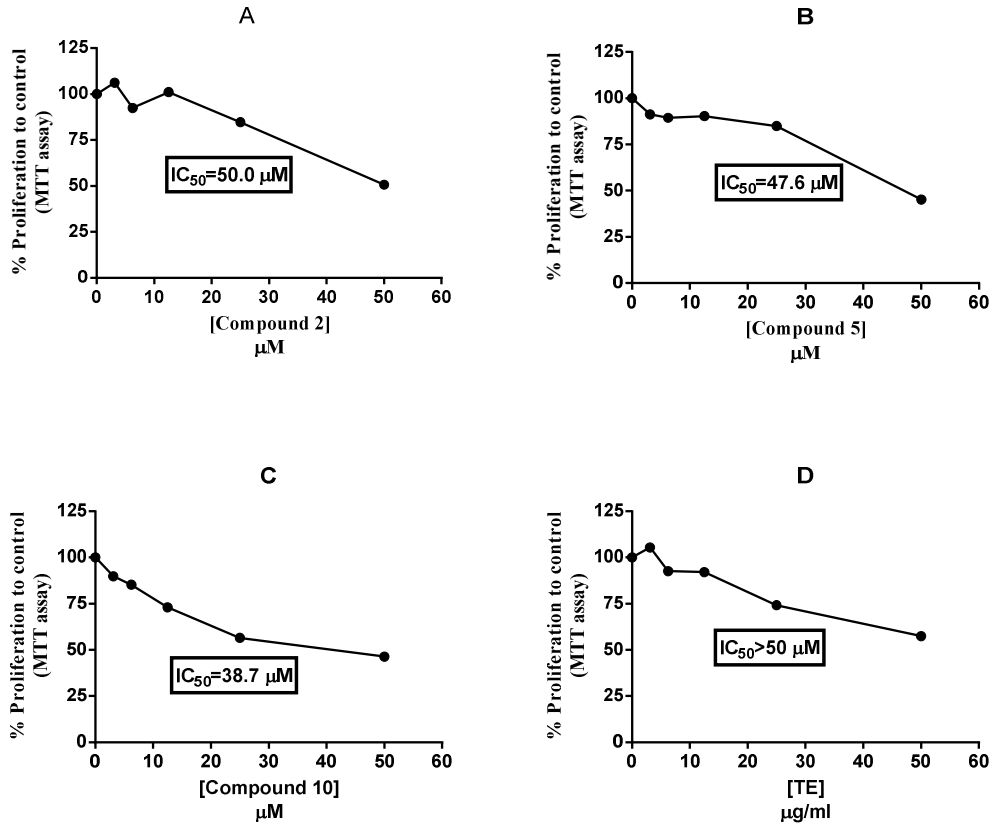

Fig. 2: Dose-response curves showing the Effect of Q-3-O-Glu (A), M-3-O-Gal (B), Ellagic acid (C) and Total Extract (D) from *A.fraxinifolius* on the proliferation of MDA-MB-231 cells

Cells were treated with indicated amounts of each samples for 48 h after which MTT assay was performed as described in the Materials and Methods section. Data for cell viability are means of quadruplicate tests.

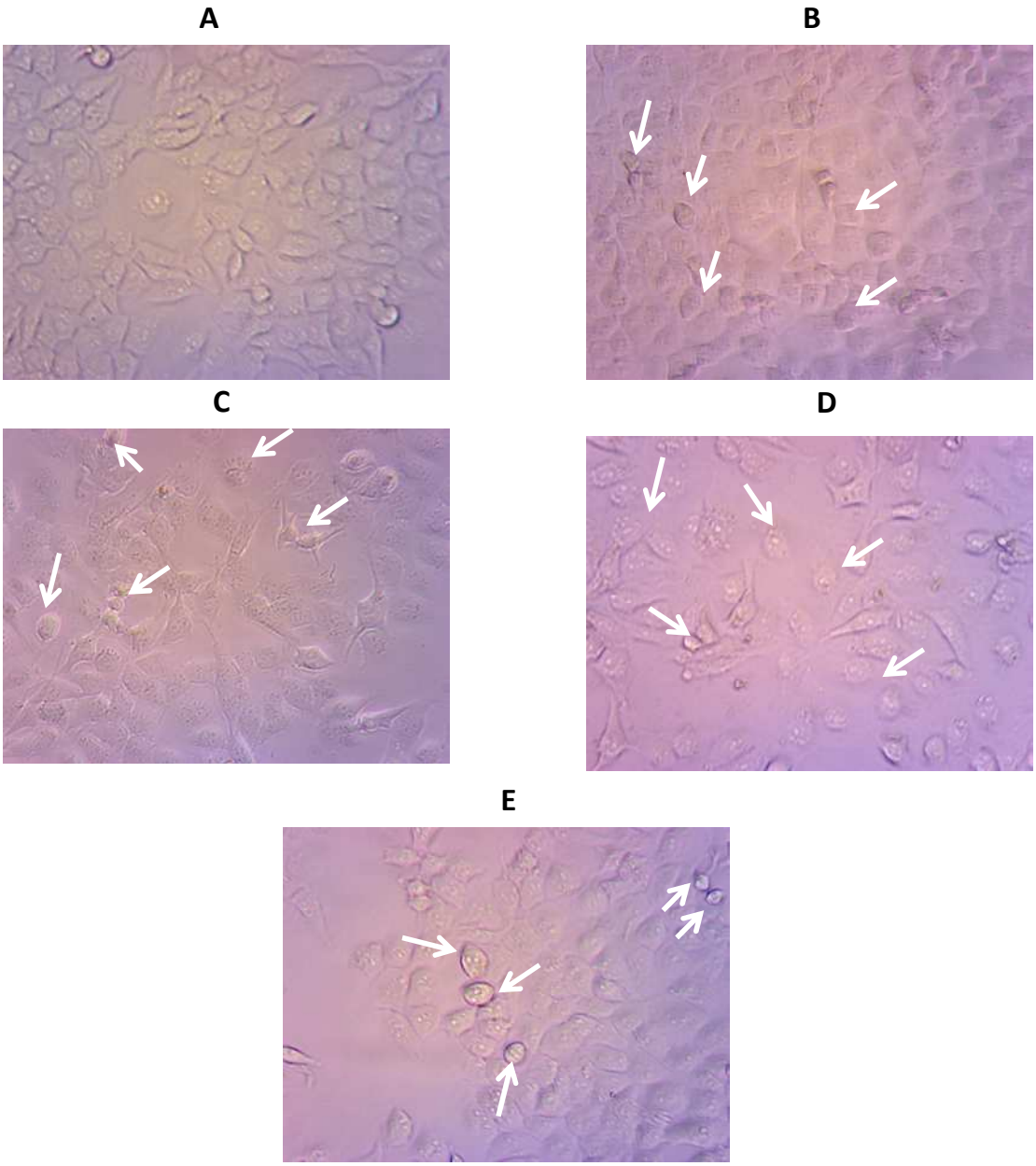

Fig. 3: Photomicrographs showing morphological assessments of MDA-MB-231 cell monolayers

*Cells were treated with vehicle (DMSO, **A**) or50 µM of Q-3-O-Glu (B), M-3-O-Gal (**C**) or Ellagic acid (**D**) isolated from A. fraxinifolius. Total extract of the plant were also tested in parallel at 50µg/ml (**E**). Arrows indicate morphological signs of cytotoxicity including cell rounding and/or disintegrated monolayer compared to the vehicle control (A). Photomicrographs were taken using Zeiss® Primovert (Carl Zeiss Group, USA) equipped with a digital camera. Total magnification is 150×.*

The morphological assessment of cellular antiproliferation changes revealed a parallel results to the MTT assay as displayed in (photomicrographs of Fig. 3). As shown in the photomicrographs, ellagic acid (**10**) caused morphological signs of cytotoxicity including cell rounding and loss of monolayer integrity. Gomes and coworkers (2003) have reviewed the structure–anticancer activity of natural and synthetic phenolic acids and justified the toxicity to their number of –OH groups. To the best of knowledge, no previous reports exist about the toxicity of ellagic acid on the TNBC cells and our findings warrant further investigation to identify the mechanism (s) of its toxicity in MDA-MB-231 cells such as apoptosis and inhibition of cell cycle progression [17].

3.3. Free radical scavenging activity

The free radical scavenging activity of aq. methanolic extract and the isolated phenolic compounds; quercetin 3-*O*-β-D-glucopyranoside (**2**), quercetin 3-*O*-α-L-rhamnopyranoside (**3**), myricetin 3-*O*-β-D-galactopyranoside (**5**), myricetin 3-*O*-α-L-rhamnopyranoside (**6**), desmanthin-1 (**7**), naringenin (**8**), brevifolin carboxylic acid (**9**), were evaluated using TLC-DPPH$^{\bullet}$-image processing procedures with two concentrations 10 and 20 µg/ml in relation to

rutin's activity. The aq. methanolic and the most of the isolated compounds showed significant antioxidant activity (**Fig. 4).**

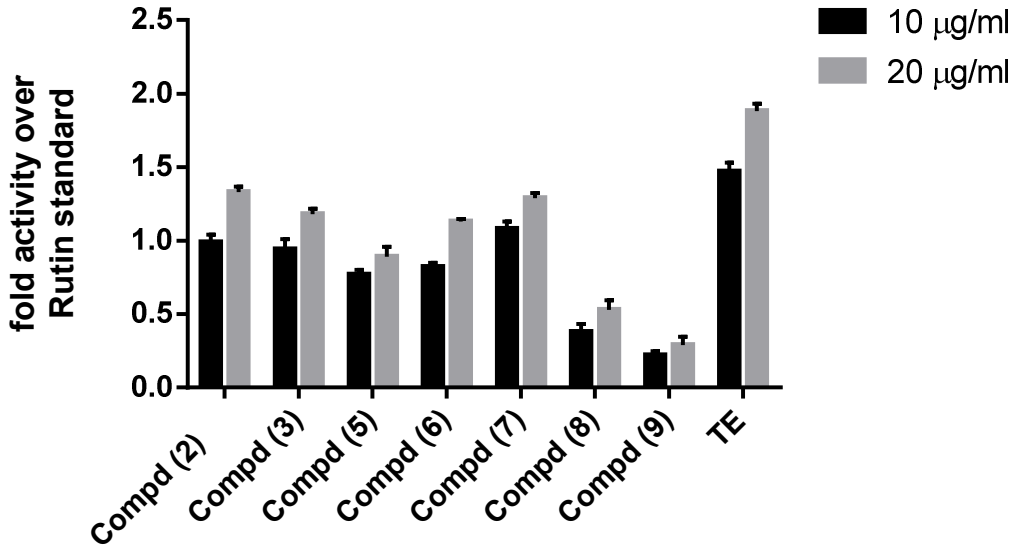

Fig. 4: Antioxidant activity of isolated phenolics from *A. fraxinifolius* in relation to rutin's activity
Data are means±SD.

It is well known that flavonoids and phenolic acids are potent antioxidant agents [8]. Phenolic compounds such as flavonoids have been stated to act as potent antioxidants in plants. The diverse chemical structure of flavonoids strongly affects their corresponding antioxidant activity, for example, the position of hydroxyl groups, carbon double bonds, and modifications such as glycosylation, prenylation or methylation [18-19].

The antioxidant results showed that aq. methanolic extract have very strong antioxidant activity in the two tested concentrations with quantitative values at 1.47 ± 0.061 and 1.88 ± 0.052. By the same, all the tested flavonoid, quercetin-3-*O*-β-D-glucopyranoside (**2**), quercetin-3-*O*- α-L-rhamnopyranoside (**3**), myricetin-3-*O*-β-D-galactopyranoside (**5**) and myricetin-3-*O*-α-L-rhamnopyranoside (**6**) exhibited strong antioxidant activity. The isolated flavonoids, quercetin-3-*O*-β-D-glucopyranoside (**2**) and naringenin (**7**), showed strongest antioxidant activity between all tested compounds with quantitative values at [0.99 ± 0.05 and 1.33 ± 0.037] and [1.08 ± 0.05 and 1.29 ± 0.032], respectively. Also, the other, flavonoids quercetin 3-*O*-α-L-rhamnopyranoside (**3**)**,** myricetin 3-*O*-α-L-rhamnopyranoside (**6**) and myricetin 3-*O*-β-D-galactopyranoside (**5**), exhibited significant antioxidant activity in the two selected concentrations with values at [0.94 ± 0.07 and 1.18 ± 0.038]; [0.82 ± 0.03 and 1.13 ± 0.018] and [0.77 ± 0.03 and 0.89 ± 0.067], respectively. In the other side, the results revealed that the two phenolics, naringenin (**8**), brevifolin carboxylic acid (**9**), have moderate antioxidant activity with the values at [0.38 ± 0.054 and 0.53 ± 0.064] and [0.22 ± 0.028 and 0.29 ± 0.055], respectively.

3.4. Antimicrobial Activity

In the present study, the alcoholic extract along with the isolated phenolic compounds, quercetin 3-*O*-β-D-glucopyranoside (**2**), quercetin 3-*O*-α-L-rhamnopyranoside (**3**), myricetin 3-*O*-β-D-galactopyranoside (**5**), myricetin 3-*O*-α-L-rhamnopyranoside (**6**), desmanthin-1 (**7**), naringenin (**8**), brevifolin carboxylic acid (**9**), were evaluated for their antibacterial activity against 6 microbes, 4 bacteria, *Staphylococcus aureus* ATCC 29213, *Lactoacillus subtilis*, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27953. In addition, the antifungal activity against two fungi namely *Aspergillus niger* NRRL-599 and *Fusarium solani* was also tested. The results as described in Table 2 showed that the alcoholic extract and most of the tested compounds exhibited significant antibacterial activity with approximately no antifungal activity.

The net results of minimum inhibitory concentration (MIC, Table 2) showed that the alcoholic extract and all of tested isolated compounds have antibacterial activity against the tested bacterial strains, with *P. aeruginosa* and *L. subtilis* being the most sensitive strains, and the activity was comparable to with the reference antibiotics thiophenicol and nystatin. The antibacterial results revealed that the alcoholic extract exhibited significant antibacterial activity against *P. aeruginosa* and *L. subtilis* moderate antibacterial activity against *S. aureus* with MIC value of 50 µg/disc and 10 µg/disc, respectively with no activity against *E. coli*.

Myricetin 3-*O*-β-D-galactopyranoside (**5**) exhibited strongest antibacterial activity against all the used bacteria strains, *L. subtilis (*MIC 30 µg/disc), *S. aureus*, *E. coli*, and *P.aeruginosa* (MIC 50 µg/disc). Also quercetin 3-*O*-α-L-rhamnopyranoside (**3**) showed strong antibacterial activity against all the used bacteria strains, *S. aureus* (MIC 30 µg/disc), *E. coli*, and *P.aeruginosa* (MIC 50 µg/disc) and no activity against *L. subtilis*. Myricetin 3-*O*-α-L-rhamnopyranoside (**6**) exhibited significant antibacterial activity against two bacteria strains, *L. subtilis* and *P.aeruginosa* (MIC 20 µg/disc), moderate activity against *S. aureus* (MIC 5 µg/disc) and no activity against *E. coli*. The antibacterial test revealed that naringenin (**8**) produced a potent inhibition of the growth of bacterial strains *L. subtilis* and *P.aeruginosa* with the same MIC values at 20 µg/disc. As shown in the results, a strong inhibition of the two bacterial strains *P. aeruginosa* and *E. coli* with the MIC value of 50 µg/disc was exhibited by the isolated phenolic acid, brevifolin carboxylic acid (**9**). On the other hand, both of quercetin 3-*O*-α-L-rhamnopyranoside (**3**) and desmanthin-1 (**7**) exhibited strong antibacterial inhibition against only one bacterial strain, *P. aeruginosa*, with MIC value at 50 µg/disc and with no activity with the other strains.

Using the same methods, antifungal activity of the tested extract and isolated compounds were studied against two strains of fungi, *A. niger* and *F. solani*. The results showed that all of the studied samples were inactive against the two strains. Our present results of antimicrobial activity of isolated compounds are compatible with previously reported [20].

Table (2): Antimicrobial activity of MeOH extract and isolated phenolic compounds

Sample	Minimum inhibitory concentration MIC (µg/disc)					
	L. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***F. solani***	***A. niger***
MeOH extract	50	10	-	50	-	-
Compd **2**	-	50	50	30	-	-
Compd **3**	-	-	-	50	-	-
Compd **5**	30	50	50	50	-	-
Compd **6**	20	5	-	20	-	-
Compd **7**	-	-	-	50	-	-
Compd **8**	50	-	-	50	-	-
Compd **9**	0	-	50	50	-	-
Thiamphenicol	5	5	2.5	2.5	nd	Nd
Nystatin	nd	nd	nd	Nd	>5	>5

CONCLUSION

Taken together, our obtained results uncover the usefulness of *A. fraxinifolius* plant as a new source for pharmacological compounds including antiploriferative, antioxidants and antibacterial agents.

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REFERENCES

[1] V Exarchou; N Nenadis; M Tsimidou; IP Gerothanassis; A Troganis; D Boskou, *J Agric Food Chem.* **2002**, 50(19), 5294-5299.
[2] C Natal; M Caicoya; M Prieto; A Tardón, *Medicina Clínica (English Edition).* **2015**, 144(4), 156-160. [3] S Krishnamurthy; R Poornima; VR Challa; YGB Goud, *Indian Journal of Surgical Oncology.* **2012**, 3(1), 12-16. 10.1007/s13193-012-0138-2.
[4] T Suirezs; O Arenhardt; A Stehr; E Bobadilla; E Weber; A Bragañolo, *Procedia Materials Science.* **2015**, 8(802-806. [5] AH Abou Zeid; FM Soliman; RS Mohammed; AA Sleem; YM El Dakrory, *Planta Med.* **2011**, 77(12), PL8. 10.1055/s-0031-1282657.
[6] K Sorbfil TLC Videodensitometer obtained from Sorbpolymer JSC, Russian Federation, 2006. http://www.sorbfil.com, accessed 15.02.2013.
[7] I Kowalska; Ł Cieśla; T Oniszczuk; M Waksmundzka-Hajnos; W Oleszek; A Stochmal, *Journal of Liquid Chromatography & Related Technologies.* **2013**, 36(17), 2387-2394. 10.1080/10826076.2013.790776.
[8] M Olech; Ł Komsta; R Nowak; Ł Cieśla; M Waksmundzka-Hajnos, *Food Chemistry.* **2012**, 132(1), 549-553.
[9] L Ciesla; I Kowalska; W Oleszek; A Stochmal, *Phytochem Anal.* **2013**, 24(1), 47-52. 10.1002/pca.2379.
[10] A Sokmen; M Gulluce; H Askin Akpulat; D Daferera; B Tepe; M Polissiou; M Sokmen; F Sahin, *Food Control.* **2004**, 15(8), 627-634.
[11] G Ye; C Huang, *Chemistry of Natural Compounds.* **2006**, 42(2), 232-234. 10.1007/s10600-006-0089-3.
[12] G Fortes; A Carvalho; R Ramalho; A Silva; P Ferri; S Santos, *Records of Natural Products.* **2015**, 9(2), 251-256.

[13] A Hasler; G-A Gross; B Meier; O Sticher, *Phytochemistry*. **1992**, 31(4), 1391-1394. [14] H Liu; Y Mou; J Zhao; J Wang; L Zhou; M Wang; D Wang; J Han; Z Yu; F Yang, *Molecules.* **2010**, 15(11), 7933-7945. 10.3390/molecules15117933.
[15] SM Ghanadian; AM Ayatollahi; S Afsharypour; S Hareem; OM Abdalla; J Jules Kezetas Bankeu, *Iranian Journal of Pharmaceutical Research : IJPR.* **2012**, 11(3), 925-930.
[16] N Thitilertdecha; A Teerawutgulrag; JD Kilburn; N Rakariyatham, *Molecules.* **2010**, 15(3), 1453.
[17] CA Gomes; TG da Cruz; JL Andrade; N Milhazes; F Borges; MP Marques, *J Med Chem.* **2003**, 46(25), 5395-5401. 10.1021/jm030956v.
[18] C Rice-Evans; N Miller; G Paganga, *Trends in Plant Science.* **1997**, 2(4), 152-159.
[19] CA Rice-Evans; NJ Miller; G Paganga, *Free Radical Biology and Medicine.* **1996**, 20(7), 933-956.
[20] R Hendra; S Ahmad; A Sukari; MY Shukor; E Oskoueian, *Int J Mol Sci.* **2011**, 12(6), 3422-3431. 10.3390/ijms12063422.