Potential Anticancer Activities of *Rhus coriaria* (sumac) extract against human cancer cell lines

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**Sub title:** *Rhus coriaria* (sumac) inhibition activity to carbonic anhydrase isoforms

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Abstract

Therapeutic strategies of plant origin are a better choice as both dietary plant products or its isolated active constituents against the development and progression of cancer. This study aims to evaluate the anticancer activity of sumac (*Rhus coriaria*) against different human cancer MCF-7, PC-3, and SKOV3 cell lines. In addition, the study tries to explore a prospective mechanism of action, assessment of in vitro enzyme-inhibitory capacity of sumac extract against hCA I, II, IX and XII. In this study, the potential antitumor effects of sumac (*Rhus coriaria*) were explored in the human cancer cell lines; MCF-7, PC-3, and SKOV3 using in vitro assays. Apoptotic, cell survival, ELISA immunoassays were also conducted to reveal the inhibitory effects of sumac extract against hCA I, II, IX and XII. In addition, both Clioquinol and Acetazolamide were used as standards to explore the in vitro enzyme-inhibitory capacity of sumac extract against hCA I, II, IX, and XII. The hydro-alcoholic extract of *Rhus coriaria* (Sumac) subjected to phytochemical analysis using GC/MS assays. Sumac at non-cytotoxic doses of 50 µM, and 100 µM significantly modulate the growth of the MCF-7, PC-3, and SKOV3 cancer cell with a higher inhibitory effect and selectivity to carbonic anhydrase (CA) isoforms; hCA I, II, hCA IX, and XII. The data showed that sumac at doses of 50 µM, and 100 µM significantly inhibited the growth, proliferation, and viability of cancer cells by activating the apoptotic process via caspase-3 overexpression and the regulation of Bcl-2 anti-apoptotic protein.

**Key words:** human cancer cells; *Rhus coriaria* L.; Carbonic anhydrase inhibitors; cytotoxic activity; Apoptosis
1. Introduction

Cancer is the most common disease that causes a growing health problem globally. It greatly affects millions of people and significantly continues to increase rapidly for the following years [1].

A steady decline in overall deaths was reported by about 1.5% per year among cancer patients following early diagnosis and treatment interventions [2]. Although current therapeutic strategies are efficient, a transient durability effect was reported during the recurrence of cancer. Thus, growing interest was focused on herbal remedies and nutrition as alternative approaches in treating cancer, particularly to breast cancer patients [3]. Dietary plant products either whole products or isolated active constituents play a potential protective role against the development and progression of cancer disease, including breast cancer (BC) [4–8]. In most studies, phytochemicals showed to reduce the growth and progression of cancer via anti-inflammatory, immunomodulatory, and antioxidant activity as well as a modulation of several cellular processes, particularly the proliferation, apoptosis, and angiogenesis of cancer cells [9-10]. Thus, new therapeutic advances that use plant-derived phytochemicals as a source of clinically active anti-cancer agents are highly appreciated [11].

Sumac (Rhus coriaria L., Anacardiaceae) used as an alternative medicine for several different diseases [12]. In Middle Eastern cuisine, sumac commonly used as sour spice [13-14]. Sumac extracts have used in the treatment of several human diseases [15-18, particularly in cancer [19]. Active compounds present in sumac such as flavonoids, tannins and xanthons [20, 21], reveal its potency as antiviral, antimicrobial, anticancer, antioxidant and radical scavenging activities [15-19].

Human carbonic anhydrase (CA) isoforms showed to be highly expressed in many types of tumors. About 15 isoforms of CA were expressed during the progression and growth of the tumors, the most important CA isoforms were the transmembrane tumor-associated isoforms (hCA IX and hCA XII) that significantly overexpressed during proliferation of cancer cells [22-23]. Many chemotherapeutic agents targeted hCA IX and hCA XII isoforms for the treatment of cancer [22-24].

Acetazolamide (AZM) one of a sulfonamide compounds that reported as CA inhibitors (CAIs), it has a high affinity to bind with human CA isoforms [25]. Recently, CA inhibitors (CAIs) of the sulfonamide origin have profound antitumor effects which significantly proceeds via inhibition of hypoxia-inducible isoforms CA IX and XII, overexpressed in many hypoxic tumors [26]. In addition, Clioquinol (5-chloro-7-ido-8-hydroxyquinoline; CQ) has been recognized as a novel anticancer drug that is able to disrupt proteasome activity [27-30]. The cytotoxicity of Clioquinol was revealed in several cancer models including leukemia, multiple myeloma, and cancer of prostate, bladder, and breast [28–30]. Clioquinol has been demonstrated to induce cancer cell death via several mechanisms including inhibition of lysosome, NF-kappa B, histone deacetylases, and mTOR signaling pathway [31-35]. Investigation of CQ has also been extended to study its efficacy as effective of phenolic compound used as CA inhibitor. Clioquinol showed to be the best phenol inhibitor against all CA isozymes, with inhibition constants in the range of 3.3–16.0 IM [36]. However, little or no data are known about using herbal based trials particularly sumac or its related phytoconstituents as selective hCA IX and hCA XII inhibitors.
Thus, in the current study, the potential antitumor effects of sumac (*Rhus coriaria*) were explored in the human cancer cell lines using in vitro assays. Apoptotic and cell survival assays were also conducted to reveal the inhibitory effects of sumac extract against hCA I, II, IX and XII. In addition, both Clioquinol and Acetazolamide were used as standards to explore the in vitro enzyme-inhibitory capacity of sumac extract against hCA I, II, IX, and XII.

2. Materials and Methods

The proposal of the current study was approved by the Ethics Committee of the experimental animal care society, rehabilitation research chair (RRC), college of applied medical sciences, King Saud University, Riyadh, Saudi Arabia, under file number ID: RRC-2019-085.

2.1. Chemicals

All chemicals used were of analytical reagent grade. Acetonitrile and methanol used for HPLC analysis were of sigma grade (Dublin, Ireland). All chemicals used in this study are of analytical grade.

2.2. *Rhus coriaria* plant extraction

Sumac samples was purchased from local practitioner market in Riyadh city, KSA and identified by the Pharmacognosy Laboratory, pharmacy college, king Saud University. The samples of air-dried sumac fruit (50 g) were treated with 16 ml of the sonicated hydro-alcoholic buffer (EtOH/H2O; 70:20) as previously reported in the literature [21]. The extract was subjected to several centrifugation processes, the supernatant collected, and the solvent evaporated under vacuum to produce pure extract deposits. These deposits re-dissolved with 0.5 ml of EtOH/H2O (70:30, v/v) and filtered within a 0.22 µm syringe filter. The final extract residues stored at −20°C until reused [21].

**GC/MS Analysis of Sumac Phenolic compounds**

In this experiment, active constituents were separated and quantified in the concentrated organic buffers by using UltraFast TRACE GC (A thermo scientific, Co.) [37-38]. In addition, a triple quadrupole mass spectrometer (Waltham, MA, USA), equipped with a Phenomenex Zebon ZB-5MS (5 m × 0.25 mm i.d. × 0.25 µm film thickness or equivalent) column (411 Madrid Avenue, Torrance, CA, USA) was used in this protocol to separate and quantify sumac phenolic compounds [37-38].

2.3. Carbonic anhydrase (CA) inhibition assay

In this experiment, the inhibition of carbonic anhydrase (CA) isoforms was estimated by using an Applied Photophysics stopped flow instrument as previously reported [24]. In this method, the CA catalyzed CO₂ hydration activity and phenol red (at a concentration of 0.2 mM) has been used as indicator with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na₂SO₄ (for maintaining constant the ionic strength). Then, CA enzyme isoforms were incubated with different concentrations of the sumac extracts (10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL) for 15 min at room temperature or 4°C. In addition, both Clioquinol and Acetazolamide were applied...
as standards inhibitors of CA enzyme isoforms. Finally, the inhibition rates of sumac extracts to CA isoforms were measured at the absorbance maximum of 557 nm and compared with applied standards inhibitors [39-40]. The inhibition constants of each sumac concentration were estimated by using a non-linear least squares methods with a PRISM program as previously reported [39-40].

2.4. **Cell cultures**

Human tumor breast cell lines (MCF-7), prostate adenocarcinoma (PC-3), and ovary adenocarcinoma (SKOV3) human cell lines were purchased from the American type culture collection (ATCC, USA). The cells were cultured in Dulbecco’s modified Eagle medium (DMEM, Thermo Fisher Scientific). Then, the cells incubated at 37°C in a humidified CO₂ (5%) incubator in flasks supplied with fetal bovine serum (FBS; 10% v/v), 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) [41-46]. The cells at 80% confluence were sub cultured into 96 well plates, 6 well plates and 25 cm² flasks and were performed in triplicates according to designed experiments previously mentioned [41-46].

2.5. **Anti-cancer activity**

2.5.1. **3-(4, 5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide (MTT) Cytotoxicity Assay**

In this study, the cytotoxic activity of *Rhus coriaria* (sumac) extract was measured in vitro by using different human cancer cell lines; MCF-7, PC-3, and SKOV3 as previously reported [24,44-46]. The reduction of MTT into formazan crystal in MCF-7, PC-3, and SKOV3 cells was determined to find out the potential cytotoxicity of sumac and related Clioquinol and Acetazolamide as standards inhibitors of CA enzyme isoforms in MCF-7, PC-3, and SKOV3 cells. Human cancer cells; MCF-7, PC-3, and SKOV3 cells (1×10⁴ per well) have been cultured in 96-culture plate and exposed to different concentration of sumac and related standards inhibitors (0, 10, 25, 50 and 100 μg/mL) for 24 h. After 24 h, culture media with tested sumac and standard were discarded from 96 well plates and new culture media containing MTT powder (5 mg/mL) were filled (100 μL/well) and incubated for 4 h at 37°C. The produced formazan crystal was dissolved in dimethyl sulfoxide (DMSO) and optical density (OD) was determined at 570 nm using a micro-plate reader (Synergy-H1; BioTek, Winooski, VT, USA).

2.5.2. **Lactate Dehydrogenase Enzyme Assay (LDH) Assay**

The activity of sumac extract was performed by using LDH cytotoxicity ELISA assay (601170 Cayman chemical kit, 1180 E. Ellsworth Rd, Ann Arbor, MI, USA). In this experiment, human target cancer cells are cultured with different concentrations of sumac extract (0, 10, 25, 50 and 100 μg/mL) to induce cell death and subsequent release of LDH to the culture medium. In addition, 10% of Triton X-100 solution provided in the test as negative control. The supernatant containing LDH was transferred to a new wells of ELISA plate and mixed with the LDH reaction solution, and incubated for 30 min in room temperature. For each sumac concentration, the corresponding absorbance was estimated by using ELISA plate reader at 490 nm (A490). Finally, the cytotoxic activity of sumac was calculated as a percentage of the total amount of LDH contained with the target human cancer cells as described by manufacturer.
2.5.3. Apoptotic assay

2.5.3.1. Evaluation of Caspase-3

Caspase-3 enzymes as significant biological apoptotic parameters were estimated in sumac-treated and non-treated human cancer cells as previously reported [47]. In this experiment, Bio-Vision colorimetric assay kits were used to estimate the expression of Caspase-3 enzymes in both sumacs treated and non-treated human cancer cells (MCF-7, PC-3, and SKOV3). The cells exposed to different concentrations of sumac extract, Clioquinol and Acetazolamide inhibitors of CA enzyme isoforms. In each sample, a spectrophotometer with a 100-µl micro quartz cuvette (Sigma) was used to estimate the concentration of Caspase-3 enzymes at 400- or 405-nm.

2.5.3.2. Evaluation of Bcl-2

Immunoassay techniques were performed to estimate Bcl-2 concentrations using a commercially available ELISA kits (Cat# QIA23, Oncogene Research Products, Germany) [48]. In this experiment, Bcl-2 concentrations were estimated in sumacs treated and non-treated human cancer cells (MCF-7, PC-3, and SKOV3) and compared with the results of both Clioquinol and Acetazolamide inhibitors of CA enzyme isoforms.

2.6. Statistical analysis

All the assays were conducted in triplicate, and three different microplate wells were used for each concentration. A linear regression analysis was performed to calculate IC50 values. Data from the experiments were statistically analyzed by one-way analysis of variance (ANOVA) followed by a post hoc Dunnett’s test using SPSS statistics version 17.0 for Windows (SPSS Inc. 233, Chicago, IL 60606–6412, USA). P value of <0.05 was considered statistically very significant.

3. Results

3.1. GC/MS analysis of sumac phytochemical compounds

A potential nine bioactive compounds were estimated in the hydro-alcoholic extract of sumac Table (1), Figure (1), and Figure (2). In this experiment, gallic acid, isohyperoside, dihydroxy methyl xanthone, β-Sitosterol-hexoside, α-Tocopherol, and linoleic acid were identified at their respective retention times in the sumac fruit extract as shown in Figure (1) and Table (1). In addition, quercitrin (RT; 5.39), myricetin glucuronide (RT; 7.73), and myricetin rutinoside (RT: 9.35) were identified as flavonoid derivatives in the sumac fruit extract as in Table (1) and Figure (1). There was significant variability in the chemical formula of the identified as shown in Figure (2). These compounds proposed to potentially involved in the sumac effect on cancer cells and inhibition activity to CA isoforms of the selected human cancer cell lines MCF-7, PC-3, and SKOV3.

3.2. Rhus coriaria inhibition activity to carbonic anhydrase (CA) isoforms

The CA inhibitory ability of Rhus coriaria extract was measured at different concentrations; 10 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL as shown in Table (2). Stopped-flow assay method was performed to estimate...
the inhibition and selectivity of sumac extracts against cytosolic CA isoforms (hCA I and II) and the membrane-associated CA isoforms (hCA IX and XII) respectively (Table 2). In addition, acetazolamide (AZA) and Clioquinol (CQ) as the reference inhibitors of CA isoforms were used in this experiment (Table 2). Compared to the effect of AZA and CQ drugs, sumac extract at doses of 10 μg/mL, 25 μg/mL, 50 μg/mL, and 100 μg/mL significantly produced a strong inhibition activity against all carbonic anhydrase (CA) isoforms (Table 2). However, sumac extract at concentrations of 50 μg/mL and 100 μg/mL showed higher inhibition activity compared to lower values (10 μg/mL, 25 μg/mL) and the reference inhibitor controls (AZA & CQ) (table 2).

The cytosolic isoform hCA I was inhibited by the sumac extract with ki values in the range of 16.8–31.8 nM. The most active sumac doses against hCA I were 50 μg/mL, and 100 μg/mL which showed the least ki values (21.8 and 16.8) compared to the moderate active doses; 10 μg/mL, 25 μg/mL with the higher ki values (31.8 and 29.5) respectively. However, all the sumac concentrations were more active than the reference CAI drugs (AZA &CQ) (table 2).

Regarding, the inhibitory activity of sumac against hCA II, all tested sumac concentrations were active with at a ki values with a range of 3.7–8.5 nM. Sumac at concentrations of 50 μg/mL and 100 μg/mL were the most active with ki values (4.5 and 3.7) compared to lower sumac concentrations 10 μg/mL, 25 μg/mL which showed a moderate inhibition activity with ki values (8.5 and 6.7) respectively. Again all tested sumac concentrations being more active than AZA and CQ. Similarly, all sumac concentrations showed moderate to strong inhibition activity against both the tumor-associated target isoforms hCA IX (ki values; 3.1-11.5) and hCA XII (ki values; 0.98-4.9) (Table 2). The most active doses were at 50 μg/mL, and 100 μg/mL compared to the inhibition activity proposed to the reference drugs (AZA & CQ) (Table 2).

In this experiment, the selectivity index (SI) was calculated for each sumac concentration as hCA I/hCA XII and hCA II/hCA XII respectively. All tested sumac extract concentrations showed high selectivity for the transmembranal tumor-associated isoform hCA XII than hCA I especially at higher dose100 μg/mL with SI value more than 286. In addition, higher sumac concentrations; 50 μg/mL, and 100 μg/mL showed high selectivity for hCA XII than hCA II with SI values more than 123 and 365 respectively (Table 2). The selectivity of tested sumac concentrations was evaluated to reduce the unwanted side effects as the inhibition of the cytosolic isoforms hCA I and hCA II will lead to potential diuresis. The selectivity SI values of tested sumac at 100 μg/mL concentrations showed to be of more selectivity compared to both AZA and CQ CAI inhibitors (Table 2)

3.3. Cytotoxic activity

The cytotoxic activity of Rhus coriaria extract (RCE) at different concentrations on human cancer cells (MCF-7, PC-3, and SKOV3) was assessed by MTT and LDH assays as shown in Figure (3A, 3B & 3C). Rhus coriaria extract (RCE) at higher doses 25, 50, and 100 μg/mL showed more adverse effects on human cancer cells SKOV3, PC-3, and MCF-7 in a dose-dependent manner compared to the reference CAI drugs inhibitors (AZA &CQ) and respective non-treated cancer cells (control) as shown in Figure (3A). In addition, a variation in LDH activity was reported in the studied human cancer cells SKOV3, PC-3, and MCF-7 (figure 3B &3C). In
cells treated with higher doses of sumac extract, the release of LDH enzyme was significantly higher in SKOV3 and PC-3 with lower levels of LDH in MCF-7 cell lines was reported which supports that the release of LDH is a dose-dependent manner. Also, the release of LDH was significantly increased at higher doses of (50 and 100 μg/mL) of CAI drug inhibitors (AZA &CQ) which supports the mechanistic role of sumac as a potential CAI inhibitor (figure 3C). Thus it was confirmed that RCE was cytotoxic for the studied human cell lines and the result of the LDH test was in agreement with the finding of the MTT test (Figure 3B&3C).

3.4. Apoptosis in human cancer cells

The activity of caspase-3 and Bcl-2 protein expression as apoptotic parameters were estimated in all sumac treated and non-treated human cancer cells as shown in Figure (4A&4B). The activity of caspase-3 was found more in SKOV3 cells than both PC-3 and MCF-7 cells respectively (Figure 4A). The activity of caspase-3 was significantly more in 50 μg/mL and 100 μg/mL RCE exposed SKOV3, PC-3, and MCF-7 cells (Figure 4A) as compared to CAI drug inhibitors (AZA &CQ) exposed cells and non-treated control cells (Figure 4A), respectively. Also, the expression of Bcl-2 protein as an anti-apoptotic parameter was evaluated in RCE- treated and non-treated cancer cells (control) (Figure 4B).

In all studied human cancer cells, RCE induced dose-dependent decline in Bcl-2 protein expression (Figure 4B) the results significantly compared to both CAI drug inhibitors (AZA &CQ) exposed cells and non-treated cancer cells (control) as shown in Figure (4B). As in observation, there was a more reduction in the expression of the anti-apoptotic protein Bcl-2 in SKOV3 cells treated with RCE at higher doses of 50 μg/mL and 100 μg/mL than PC-3 and MCF-7 cells respectively (Figure 4B).

4. Discussion

The use of herbal-based therapy as an alternative strategy for chemo therapeutics drugs is highly appreciated in many human diseases [15-18]. Sumac (Rhus coriaria L., Anacardiaceae) one of the most commonly used spices was recommended in the treatment of several human diseases including cancer [12-14,19].

In this study, a potential nine bioactive compounds were estimated in the hydro-alcoholic extract of sumac. The characterized compounds were gallic acid, isohyposide, dihydroxy methyl xanthone, β-Sitosterol-hexoside, α-Tocopherol, and linoleic acid. In addition, quercitin, myricetin glucuronide, and myricetin rutinoside were identified as flavonoid derivatives in the extract of the sumac fruit. These compounds proposed to potentially involved in the sumac effect on cancer cells and inhibition activity to CA isoforms of the studied human cancer cell lines; MCF-7, PC-3, and SKOV3.

The phytochemicals identified were significantly described recently in leaves and fruits Rhus coriaria [49-51]. Quercitin, myricetin rutinoside, dihydroxy methyl xanthone, β-Sitosterol-hexoside, α-Tocopherol, and linoleic acid are being reported in the fruits of Rhus coriaria [49-51]. In addition, sumac possesses showed to have a wide range of pharmacological properties, particularly antioxidant and cellular free radical scavenging activity, antiviral, and anticancer [52-54]. This owing to its rich bioactive substances regarded in terms of phenolic
compounds and flavonoids [50-54]. Moreover, active compounds present in sumac such as flavonoids, tannins and xanthons [20, 21], reveal its potency as antiviral, antimicrobial, anticancer, antioxidant and radical scavenging activities [15-19].

In this study, sumac extract (RCE) at non-cytotoxic doses of 50 μg/mL, and 100 μg/mL strongly affect on the growth of the studied human cancer cell lines (MCF-7, PC-3, and SKOV3). In addition, the applied RCE doses showed a higher inhibitory effect with selectivity to overexpressed carbonic anhydrase (CA) isoforms from cancer cells in comparison with the effect of the selected CAI drug inhibitors (AZA &CQ). Moreover, both cytosolic isoforms (hCA I and II) and the membrane-associated isoforms (hCA IX and XII) were significantly inhibited at sumac concentrations; 50 μg/mL, and 100 μg/mL respectively. Previously, it was reported that Rhus coriaria extract significantly reduced cancer cell migration in cancer cell lines particularly breast cancer cell lines [12,21,49].

In this study, the selectivity of the sumac fruits extract (RCE) toward the expressed carbonic anhydrase (CA) isoforms was estimated. The results showed that sumac extract at higher doses; 50 μg/mL, and 100 μg/mL significantly inhibited the overexpressed CA isoforms (hCA I; IIhCA; IX; and XII). Sumac showed high selectivity to hCA XII than hCA II with selectivity index (SI) values more than 123 and 365 respectively. The selectivity SI values of tested sumac at 100 μg/mL concentrations showed to be more selective to expressed CA isoforms compared to traditionally used AZA and CQ CAI inhibitors. This potential selectively performed by sumac extract significantly helps in the reduction of unwanted side effects during therapeutic applications as the inhibition of the cytosolic isoforms hCA I and hCA II will lead to potential diuresis [23-24].

Several studies reported that Phenols, polyphenols, and flavonoids present in plants were reported to be a competitive inhibitor of human (h)Carbonic Anhydrase isoforms, particularly II (hCA II) isoform [55-57]. Also, several flavonoids showed a promising inhibitory effects against human CA I, II, IV, VI and bovine CA III isoforms [58-61]. These studies support the presence of the catechol moiety in the polyphenols and flavonoids, which enhances the selectivity index and inhibition activity to CA isoforms.

In this study, the potential cytotoxic activity of sumac extract (RCE) was investigated in MCF-7, PC-3, and SKOV3 cells by using MTT and LDH assays. Cell viability was significantly reduced with the release of cellular LDH in higher quantities following exposure to different doses of RCE extract. More adverse effects on human cancer cells SKOV3, PC-3, and MCF-7 were reported at higher doses of Rhus coriaria extract (RCE; 25, 50, and 100 μg/mL) compared to the reference CAI drugs inhibitors (AZA &CQ) and respective non-treated cancer cells (control). RCE extract at higher doses induced more cytotoxic effects on the SKOV3 than on the PC-3 and MCF-7 cells respectively. This may be related to the anti-proliferation effects of the sumac exerted on treated cancer cells [13,62-63]. Previously, it was reported that rich secondary metabolites components present R. coriaria L showed to responsible for the anticancer and growth inhibitory effects of sumac [21,64]. The co-similarity in the biological activity between sumac and the reference CAI drugs inhibitors (AZA &CQ) as cytotoxic and as competitive inhibitors of human (h)Carbonic Anhydrase isoforms might relate the phenolic properties of both
the phytoconstituents of sumac and respective CAI drugs inhibitors (AZA &CQ which provide best phenolic inhibition activities against all CA isozymes [25-36].

Moreover, cellular apoptosis as an inhibition mode for the proliferation of cancer cells was investigated in this study. The activation of caspase-3 and Bcl-2 anti-apoptotic protein as parameters of apoptosis were identified following exposure to Rhus coriaria extract (RCE). RCE extract at higher doses of 50 µg/mL and 100 µg/mL induced cellular apoptosis via increasing the activity of caspase-3 and the down-regulation of Bcl-2 respectively. Also, cellular apoptosis induced in all cells treated with CAI drugs inhibitors (AZA &CQ). It was significant to note that SKOV3 cells were more susceptible to RCE extract than the PC-3 and MCF-7 cells. Our results matched with others who recently different extracts of Rhus spp. significantly inhibited the growth, proliferation, and viability of cancer cells by activating the apoptotic process via caspase-3 overexpression and the regulation of Bcl-2 anti-apoptotic protein [65-68]. However, this is the first evaluation of in vitro anticancer activity of from R. coriaria L. based upon inhibition activity and selectivity to carbonic anhydrase (CA) isoforms which significantly overexpressed during the growth of some human cancer cell lines.

Conclusion

The potential anticancer activity of R. coriaria L. has fully discussed in the bases of inhibition activity and selectivity to carbonic anhydrase (CA) isoforms expressed from different human cancer cell lines. The data showed that sumac at doses of 50 µM, and 100 µM significantly inhibited the growth, proliferation, and viability of cancer cells by activating the apoptotic process via caspase-3 overexpression and the regulation of Bcl-2 anti-apoptotic protein. In addition, the strong inhibition activity and more selectivity of sumac towards carbonic anhydrase (CA) isoforms hCA I and hCA II provide a potential use of this herbal plant as CAI inhibitor in the treatments of cancer cells.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements:

The authors are grateful to the Deanship of Scientific Research, King Saud University for funding through Vice Deanship of Scientific Research Chairs.

Data Availability

All data generated or analyzed during this study are presented in the manuscript. Please contact the corresponding author for access to data presented in this study.
Authors’ contributions

Research idea, design, and practical work, were proposed by GSA. Data collection and analysis was executed by GSA. All authors, GSA and AAH did reformatting, drafting, and preparing of the revised manuscript. Finally, GSA did manuscript preparation and submission.

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Table 1: List of identified phenolic compounds in Rhus coriaria extract (RCE) by using GC/MS analysis

| Peak | RT(min) | UV max | m/z [M-H] (*) | MS² fragments | Chemical formula |
|------|---------|--------|---------------|---------------|-----------------|
| 1    | 4.20    | 220-295| 169.0         | 125.0         | Gallic Acid [C₆H₁₂(OH)₃COOH] |
| 2    | 5.39    | 260-357| 447.1         | 301.0         | Quercitrin [C₁₅H₁₀O₇] |
| 3    | 5.91    | 257-358| 463.2         | 301.0         | Isohyperoside [C₂₁H₂₀O₁₂] |
| 4    | 7.73    | 261-353| 493.1         | 317.0         | Myricetin glucuronide [C₂₀H₁₈O₁₄] |
| 5    | 9.35    | 365    | 625.1         | 317.0         | Myricetin rutinoside [C₃₃H₄₀O₂₂] |
| 6    | 10.2    | 220    | 241.1         | 195.2         | Dihydroxy methyl xanthone [C₂₇H₂₆O₇] |
| 7    | 10.85   | 204-231| 575.1         | 413.7         | β-Sitosteryl-hexoside [C₃₃H₅₆O] |
| 8    | 11.52   | 295    | 429.6         | 280           | α-Tocopherol [C₂₉H₅₀O₄] |
| 9    | 11.86   | 215.0  | 279.1         | 280           | Linoleic Acid [C₁₈H₃₀O₂] |

* In the negative ion detection mode; RT, retention time.
Table 2: *Rhus coriaria* extract (RCE) inhibition activity to carbonic anhydrase (CA) isoforms; hCA I, II, IX, and XII

| *Rhus coriaria* extract (RCE) | Ki (nM)<sup>a</sup> | SI<sup>b</sup> |
|-------------------------------|------------------|----------------|
|                               | hCA I | hCA II | hCA IX | hCA XII | hCA I/XII | hCA II/XII |
| 10 μg/mL                       | 31.8  | 8.5    | 11.5   | 4.9     | 42.8      | 3.1        |
| 25 μg/mL                       | 29.5  | 6.7    | 9.3    | 2.8     | 46.8      | 8.4        |
| 50 μg/mL                       | 21.8  | 4.5    | 5.8    | 1.9     | 158.7     | 123.3      |
| 100 μg/mL                      | 16.8  | 3.7    | 3.1    | 0.98    | 286.7     | 365.8      |

Standard CAI inhibitors

| AZA (μg/mL) | CQ (μg/mL) |
|-------------|-----------|
| 96.1        | 98.6      |
| 13.7        | 15.6      |
| 27.8        | 36.7      |
| 5.8         | 11.3      |
| 48.98       | 259.7     |
| 3.8         | 136.7     |

AZA (acetazolamide), CQ (Clioquinol) well-known CAI inhibitors, was used as a standard for comparison. <sup>a</sup> ki presented is the mean from 3 different assays; errors are in the range of ±5–10% of the reported values. CAI: carbonic anhydrase inhibitors. <sup>b</sup> SI (selectivity index) is a ratio between the ki values observed for two hCA isoforms; low value index is indicative of weak selectivity.
Figure 1:
Figure 2:
Figure 3:
Figure 4:
Figures title / legends

Fig. 1: GC/MS analysis of the major identified phenolic compounds in *Rhus coriaria* extract. The identified compounds ordered according to their elution time: (1) Gallic acid, (2) Quercitrin, (3) Isohyperoside, (4) Myricetin glucuronide, (5) Myricetin rutinoside, (6) Dihydroxy-methyl xanthone, (7) β-Sitosterol-hexoside, (8) α-Tocopherol, and (9) Linoleic acid.

Fig. 2: Chemical structures of the identified phenolic compounds in *Rhus coriaria* extract. (1) Gallic acid, (2) Quercitrin, (3) Isohyperoside, (4) Myricetin glucuronide, (5) Myricetin rutinoside, (6) Dihydroxy-methyl xanthone, (7) β-Sitosterol-hexoside, (8) α-Tocopherol, and (9) Linoleic acid.

Fig. 3: Evaluation of cytotoxicity of *Rhus coriaria* extract (RCE) at doses of 10 μg/mL, 25 μg/mL, 50 μg/mL, and 100 μg/mL and related CAI enzyme isoforms inhibitors (Clioquinol and Acetazolamide) on human cancer cells MCF-7, PC-3, and SKOV3 for 24 hrs, as evaluated by MTT (A) and LDH assays (B&C). In MTT assay (A): the sumac extract (RCE) at higher doses of 25 μg/mL, 50 μg/mL, and 100 μg/mL significantly reduced the viability of the cancer cells; MCF-7, PC-3, and SKOV3 more than related inhibitors of CAI enzyme isoforms (Clioquinol and Acetazolamide). In LDH assays (B&C), LDH as a marker of cancer cell viability was significantly more released from the cancer cells in a dose-dependent manner form following treatment with sumac extract (RCE) (B) and CAI enzyme isoforms inhibitors (Clioquinol and Acetazolamide) (C) respectively. Each value represents the mean ±SE of three experiments. n= 3, a p < 0.05, b p < 0.01, c p < 0.001 vs control (cancerous non treated cell lines).

Figure 4: Evaluation of cancer cell apoptosis. Cellular apoptosis was identified by estimating both caspase-3 activity [A] and Bcl-2 expression [B] respectively. Caspase-3 activity [A] significantly increased in cancer cells MCF-7, PC-3, and SKOV3 treated with *Rhus coriaria* extract at doses of 50 μg/mL, and 100 μg/mL compared to related CAI enzyme isoforms inhibitors (Clioquinol and Acetazolamide) for 24 hrs. In addition, the expression of Bcl-2 protein [B], significantly reduced in cells treated with *Rhus coriaria* extract at doses of 50 μg/mL, and 100 μg/mL compared to related CAI enzyme isoforms inhibitors (Clioquinol and Acetazolamide) for 24 hrs. The results signify that the anticancer activity of *Rhus coriaria* extracts proceeds via a cellular apoptotic mechanism. Each value represents the mean ±SE of three experiments. a p < 0.05 (compared to control) and b p < 0.001 vs CAI standard control inhibitors (Clioquinol and Acetazolamide).