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Tyrosinase Modulation and Antioxidant Activity of Jordanian *Rhus coriaria* L. Fruit Extract

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Abstract

Background: Tyrosinase is an enzyme involved in the melanin synthesis. Hyperactive tyrosinase leads to hyperpigmentation diseases such as melasma, lentigo, and post-inflammatory hyperpigmentation.. Sumac (*Rhus coriaria* L.) is an antioxidant fruit abundant in phenolic compounds, which is native to the Levantine area. **Aim:** to evaluate the potential antibacterial, antioxidant, and anti-tyrosinase activity of sumac fruit extract, and to quantify total phenolic and flavonoid content. **Methods:** Methanolic extract was prepared, and the antibacterial effect was determined by the agar well diffusion method and by Minimal Inhibitory Concentration (MIC). The anticancer effect was tested on glioblastoma U-87 cells by MTT assay. Antioxidant activity was quantified by Diphenyl-Picrylhydrazyl (DPPH) assay. Antityrosinase activity was evaluated by Mushroom Tyrosinase activity enzymatic assay. Finally, total flavonoids and phenolic content were measured using the colourimetric method. **Results:** Phytochemical analysis showed good concentrations of phenolic compounds (14.3±0.44) mg GAE /100Mg and flavonoids (1.71±0.17) mg catechin/100 mg dried extract; a good antibacterial activity against *Escherichia coli* (0.0625 mg/ml), *S. aureus* (0.25 mg/ml) and *S. agalactiae* (0.25 mg/ml). A remarkable DPPH scavenging activity IC_{50} of 17 µcg/ml. Furthermore, tyrosinase activity with 87.24% inhibition at 1mg concentration and an IC_{50} of 17 µg/ml whereas kojac acid IC_{50} of 9.3 µg/ml. MTT assay were % survival was significantly reduced at 5 and 50 ug/ml to 72%, 65.8 % respectively. **Conclusion:** Sumac fruit extract is a potential source of active compounds that can be used as antibacterial, antioxidant, antityrosinase, and anticancer agents due to its richness in phenolic compounds and alkaloids, supporting its use in cosmeceutical and pharmaceutical formulations.

Major Findings: Jordanian *R. coriaria* L. demonstrated potent tyrosinase inhibition (IC_{50} = 17 µg/ml), markedly superior to arbutin (IC_{50} = 92.5 µg/ml), alongside strong antioxidant (IC_{50} = 17 µg/ml) and antibacterial activities, highlighting its promise as a plant-derived candidate for hyperpigmentation management.

Keywords: Antibacterial, Anticancer, Antioxidant, Antityrosinase, *Rhus coriaria* L., Sumac

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1. Introduction

Tyrosinase is a ubiquitous copper oxidoreductase enzyme that facilitates melanogenesis, which is the biochemical process that leads to pigmentation of skin, hair, and eye colour. In the melanogenic biochemical pathway, tyrosinase (EC 1.14.18.1) acts as the key regulatory and limiting enzyme in two

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different processes: first, the conversion of L-tyrosine to L-DOPA, and then the conversion of L-DOPA to dopaquinone, which subsequently polymerises into melanin[1]. Disruptions to tyrosinase function are associated with several important conditions that involve hyperpigmentation, such as melasma, solar lentigo, post-inflammatory hyperpigmentation, and age-related hyperpigmentation. It is also upregulated in melanoma, which makes it a potential therapeutic target in oncology. Many antityrosinase inhibitors are used to treat skin disorders such as hyperpigmentation, lentigo, vitiligo, and skin cancer. As a result of tyrosinase enzyme modulation activity, antityrosinase inhibitors are widely used in the pharmaceutical and cosmetics industry[1]. Consequently, the discovery and development of effective tyrosinase inhibitors represents a priority in both dermatology and cosmeceutical science.

Rhus coriaria L. (Family: *Anacardiaceae*, hereafter referred to as sumac) is an evergreen shrub that is widespread within the Mediterranean, Middle Eastern, and Central Asian regions. Its fruit has been used for many years in herbal medicine and cooking practices among the Levant population[2]. Studies have shown that the fruit contains high levels of hydrolysable tannins, especially gallotannins and ellagitannins, as well as flavonoids, organic acids, and anthocyanins. Gallic acid, ellagic acid, and their analogues are the main phenolic components found in its composition, with recognised copper chelation activity against tyrosinase[2].

Furthermore, the medicinal values of *R. coriaria* L. have been assessed via cell-based tests, animal assays, and a few clinical trials, which have revealed their potential as antioxidants, anti-inflammatories, hypoglycemics, hypolipidemics, and neuroprotection[3]. Regarding antityrosinase activity, past research work has identified the potential of tyrosinase inhibition using extracts of *R. coriaria* L. obtained from Turkey, Lebanon, and Iran, though information on Jordanian origin samples has not been much reported. The influence of regional differences and seasonal variations of phenols in sumac is well recognised and can significantly affect their biological activity[2, 3]. It is for this reason that the objective of this study is to conduct an in-depth investigation of the antityrosinase, antioxidative, antimicrobial, and anti-cancer potentialities of a fruit extract of *R. coriaria* L., which has been extracted using methanol and harvested from Ajloun, northern Jordan. The results will be interpreted in light of the ongoing phytotherapy studies, while considering the underlying mechanisms behind the biological properties of this plant extract, with the purpose of recommending it for use in cosmeceutical/pharmaceutical applications.

2. Materials and Methods

2.1 Plant Collection

Fresh fruits of *R. coriaria* L. (family: *Anacardiaceae*) were collected in autumn from Ajloun, northern Jordan. The taxonomic identity of the plant was confirmed by Prof. Mohammad Al-Gharaibeh, Department of Plant Science, Faculty of Agriculture, JUST University, Irbid, Jordan.

A voucher specimen No. 1 SUM 2022 has been deposited at the Faculty of Pharmaceutical Sciences, Hashemite University (Pharmacognosy and Phytochemistry laboratory).

2.2 Preparation of Plant Extraction

The fruit of *R. coriaria* L. was separated from hard seeds and made into a dry powder using a mixer. Then the powder was weighed to have (500g). The powder wasn't defatted before being subjected to hot methanolic extraction in a Soxhlet Apparatus. A rotary evaporator was applied for the extract to evaporate under reduced pressure to yield an extract sample. % yield of the methanolic extract was 30% w/w.

2.3 Determination of Total Polyphenolic Content

A modified Folin-Ciocalteu reagent assay described by Hossain *et al.*,[4] was followed to find the total phenolic content in the sumac extract. 600µL of 2 mg/ml of sumac extract was added to 3000µL of bright yellow Folin-Ciocalteu reagent (1:10 dilution) and kept for 7 minutes in a dark place. Then, 7.5% sodium carbonate (2400 µL) was added to it. This was followed by measuring the absorbance at 765 nm for the developed blue colour after it was left for 90 minutes in darkness. The experiments were carried out in triplicate. To construct the standard curve (10-100µL), Y = 0.009X + 0.0519; R2= 0.9809; gallic acid was used. Using mg of Gallic acid that is equivalent per 100mg of dried

extract (mgGAE/100 mg plant extract) to express the concentration of the total phenolic compounds found in methanolic *R. coriaria* L. fruit extract.

2.4 DPPH Radical Scavenging Activity

The method described by Hossain *et al.*,[4] with minor modification, was carried out to measure the radical scavenging activity quantitatively. Briefly, 500µL of *R. coriaria* L. extract was prepared with concentrations ranging from 10 µg/ml to 3 mg/ml, then added to 5ml of a 0.004% w/v solution of DPPH in 80% methanol. The standard was catechin, and 80% of the blank was methanol, whereas the negative control was DPPH solution in the absence of *R. coriaria* L. extract. The absorbance was measured at 517nm after 30 minutes of incubation for the mixture in the dark at 37 ºC. The following equation was used to calculate the DPPH scavenging effect:

$$\% \text{ DPPH scavenging effect} = (A_o - A_1) \times 100/A_o$$

Where A_o is the absorbance of the negative control, and A_1 is the absorbance of DPPH in the presence of plant extract minus the absorbance of plant extract in 80% methanol.

IC_{50} values were determined by non-linear regression analysis fitted to a four-parameter logistic (4PL) sigmoidal dose-response model using GraphPad Prism. Results are expressed as the mean ± SD of triplicate experiments.

2.5 Determination of Total Flavonoid Content

Al-Matani *et al.*, method[5] with minor modifications, was applied to measure total flavonoid content. (1.0ml of 1 mg/ml) of the extract was added to 4ml of distilled water. First, 5%w/v $NaNO_2$ (0.15ml) was added. The solution was left to stand for 8 min. Next, 10% $AlCl_3$ (0.15ml) was added to it. A 2ml of 4% w/v NaOH and 0.2ml of distilled water were also added, and at 510nm the absorbance was measured.

The calibration curve was plotted using different concentrations of catechin ($Y = 0.0045 + 0.0027X$, $R^2 = 0.9996$). Flavonoid content was estimated as mg of catechin/100mg of the dried extract[5].

2.6 Mushroom Tyrosinase Activity of *R. coriaria* L. Methanolic Extract

The plant extracts were prepared as 20 mg/ml stock solution in Dimethyl Sulfoxide (DMSO), and dilutions of different concentrations in the assay buffer (100mM potassium phosphate, pH 6.5) were prepared. Zhu *et al.'s* method with some modifications was carried out. In brief, using a flat-bottom, transparent 96-well microplate, 20µLof different dilutions of *R. coriaria* L. methanolic extract (10-700 µg/ml), 100µL of L-tyrosine (0.5mM), 60µL of phosphate buffer (100µM, pH 6.5) were added. Finally, a volume of µLof mushroom tyrosinase (10U/well) was added to start the reaction. A microplate reader was used to measure the absorbance at 490nm. The negative control was without plant extract; the plant without enzyme was used as a blank, and kojic acid and arbutin were used as a standard[6]. IC_{50} values were determined by non-linear regression (4PL sigmoidal model).

2.7 Antibacterial Activity of *R. coriaria* L. Methanolic Extract

2.7.1 Microorganisms

Gram-positive bacteria, *Staphylococcus saprophyticus* (ATCCBAA750), *Streptococcus pyogenes* (ATCC19615), *Staphylococcus aureus* (ATCC29213), and *Streptococcus agalactiae* (ATCC13813) were observed. Also, two Gram-negative bacteria, *E. coli* (ATCC11775) and *Pseudomonas aeruginosa* ATCC27853 were found. To measure the Antibacterial activities of *R. coriaria* L. extract, the Clinical and Laboratory Standards Institute (CLSI) [7, 8, 9] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were followed[10,11].

2.7.2 Sample Preparation

10mg and 20 mg stock solutions of the methanolic extract were dissolved in 1.0mL DMSO. Filtration was used to sterilise the samples, and then stored at 4ºC[12].

2.7.3 Agar Well Diffusion Method

The methanolic *R. coriaria* L. extract antibacterial activity was tested by the agar well diffusion method against the test microorganisms. Positive control was carried out by using Amoxicillin (20mg) and Gentamicin (10mg), and the negative control was sterile distilled water. The zones of growth inhibition were measured in millimetres (mm) after 24 hours of incubation at 37ºC, and values <8 mm were considered as being not active against the tested bacterial strains[13].

2.7.4 Determination of Minimal Inhibitory Concentration (MIC)

To determine the MIC, the micro broth dilution method was applied (CLSI 2012)[14], using pure bacterial cultures of the same tested microorganisms in the study. Here, MIC was determined by measuring the absorbance at 630nm using an Enzyme-Linked Immunosorbent Assay (ELISA) reader[15]. The lowest concentration with Optical Density (OD) < 0.1 signifies inhibition and is considered the MIC.

2.8 MTT Cytotoxicity Assay

The cytotoxicity of sumac extract was determined *in vitro* by using the U-87 glioma cell line. Cells were cultured in 96-culture plate at a density of 3000 cells/well and then treated with different concentrations of sumac (50, 5, 0.5, 0.05 µg/ml) for 72 h. Then, 20µl of a 5-mg/ml solution (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tetrazolium substrate was added to each well. The plates were then analysed on a microplate reader (Glowmax) to determine OD at 450nm and 570nm. The test was repeated in quadruplicate in three independent experiments[16].

2.9 Statistical Analysis

Determination of total phenolics and total flavonoids for each fraction was carried out in triplicate. SPSS 10.0 for Windows statistical software package was used for statistical analyses. Analysis of Variance (ANOVA) procedures were also used for analysis of variance followed by Tukey's pairwise comparison. IC_{50} values obtained from DPPH and tyrosinase inhibitory activities were determined by non-linear regression fitting through a four parameter logisitic model (4PL) dose-response curve (GraphPad Prism). A probability value at a level of $p < 0.05$ was statistically considered significant.

3. Results and Discussion

3.1 Total Phenolic Content

Gallic acid (Table 1) was used to estimate total phenolic content and expressed as mg Gallic Acid Equivalent (GAE)/100mg of extract. The analytical data for both total flavonoid and phenolic content of the methanolic extract of *R. coriaria* L. are shown in Table 1. As shown in the table, a substantial amount of total flavonoid content and total phenolic content in the methanolic extract of *R. coriaria* L. is found. Such values are in accordance with the phytochemistry of sumac reported by Sakhr and El Khatib[3], where sumac from Lebanon was found to be especially rich in hydrolysable tannins, gallic acid, and ellagic acid. The role of gallic acid and its derivatives as main components of the phenol fraction of *R. coriaria* L., regardless of origin, has been well recognised. They are strong antioxidants due to hydrogen-donor and metal-ion-chelating abilities.

3.2 DPPH Assay

The radical scavenging activity of *R. coriaria* L. methanolic extract determined by the DPPH test is illustrated in Figure 1. This extract showed good ability as a free radical scavenger with an IC_{50} value of 17 µg/ml, while ascorbic acid has an IC_{50} of 21.4 µg/ml, showing that the extract has a higher potency than ascorbic acid in this test.

3.3 *In Vitro* Antityrosinase Assays

The antityrosinase activity of *R. coriaria* L. methanolic extract is summarised in Table 2 and Figure 2. The extract demonstrated remarkable tyrosinase inhibitory activity, achieving 87.24% inhibition at 1 mg/ml, with an IC_{50} of 17 µg/ml. This compares favourably to the positive controls kojic acid (IC_{50} = 9.3 µg/ml) and arbutin (IC_{50} = 92.5 µg/ml), demonstrating that the extract is substantially more potent than arbutin.

The mechanistic explanation behind this enzyme inhibition involves the chelating ability and the blocking of active sites due to the predominant phenolics found in the *R. coriaria* L. plant. Gallic and ellagic acids, which make up the predominant phenolics found in the sumac, have ortho-dihydroxy (catechol) groups that resemble the substrate, L-DOPA, and are known to

Table 1. Total phenolic and flavonoid contents of *R. coriaria*

Extract	Total Flavonoid Content mg catechin/100 mg Dry Extract	Total Phenolic Content mg Gallic acid/100mg Dry Extract
R. coriaria	1.71±0.17	14.3±0.44

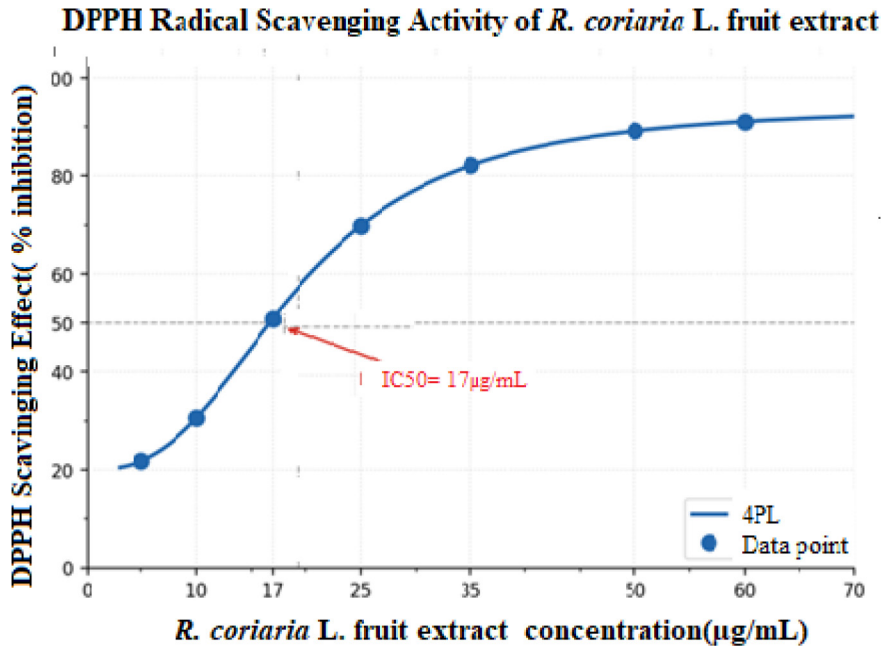


Figure 1. DPPH scavenging effect vs. different concentrations of *R. coriaria* L. extract.

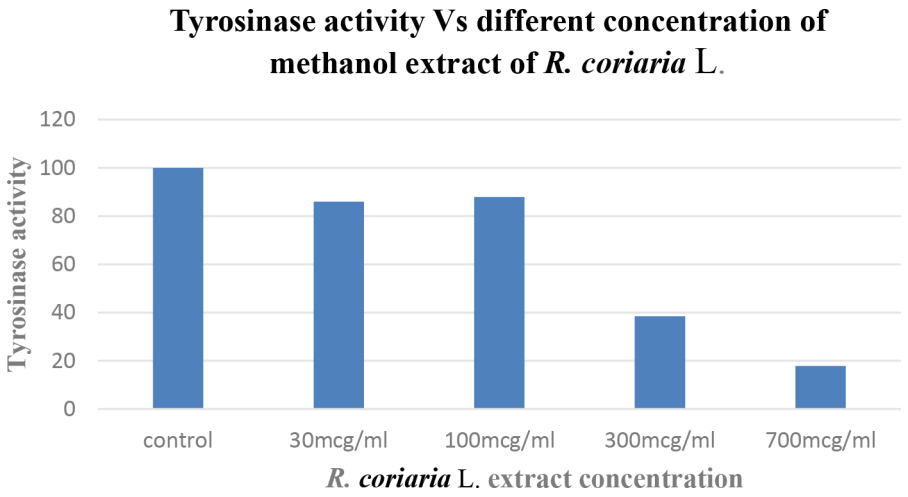


Figure 2. Effect of methanol extract of *R. coriaria* L. at different concentrations on tyrosinase activity.

have a tendency to bind or chelate the binuclear copper ions (CuA and CuB), hence inhibiting the enzyme. This supports the Structure-Activity Relationship (SAR) studies, which indicate that phenolics with vicinal hydroxyl groups are more effective as tyrosinase inhibitors than para-substituted phenols[1].

Regarding the current literature on Jordanian and regional sumac, the IC_{50} value of 17 µg/ml is one of the most active values documented for phenolics from plants against mushroom tyrosinase. Gabr and Alghadir[17] showed anticancer activity against many types of cancer cell lines for extracts of *R. coriaria* L.

Table 2. Tyrosinase inhibitory activity % inhibition for *R. coriaria* L. extract

Plant	Mushroom Tyrosinase Inhibitory Activity	
	%inhibition (1 mg/ml)	*IC_{50} (mg/ml)
R. coriaria L. extract	87.24%	17µg/ml
Kojic acid**	96.3%	9.3µg/ml
Arbutin**	88.5%	92.5 µg/ml

* IC_{50}: half the minimal inhibitory concentration.
**Positive control.

Table 3. Growth inhibition zones (mm) produced by methanolic *R. coriaria* L. extract (10 and 20 mg/ml) against different bacterial strains

		Zone of Inhibition (mm)					
Compound	**Concentration (mg/ml)**	***E. coli***	***P. aeruginosa***	***S. aureus***	***S. saprophyticus***	***S. agalactiae***	***S. pyogenes***
R. coriaria	10	28	-	20	-	-	-
	20	10	15	13	18	20	18
Amoxicillin (+ve)	(20 mg)	19	-	20	38	22	32
Gentamicin (+ve)	(10 mg)	20	16	20	26	25	22
Sterile D.W. (-ve)		-	-	-	-	-	-

(-): no inhibition, (+ve): positive control, (-ve): negative control, D.W.: distilled water

Table 4. The MIC values (mg/ml) of methanolic *R. coriaria* L. extract against pathogenic bacterial strains

	E. coli	*P. aeruginosa*	*S. Aureus*	*S. saprophyticus*	*S. agalactiae*	*S. pyogenes*
MIC	0.0625	-	0.250	-	0.250	-

MIC: minimum inhibitory concentration, (-): not detected

Also, Turkish and Lebanese sumac were found to possess antioxidant activity, as indicated by similar IC_{50} values[2,3]. The observed antityrosinase activity clearly shows that Jordanian sumac could be considered a promising candidate for further development as a natural skin-lightening agent, taking into consideration its high degree of safety when compared with synthetic skin-lightening agents such as hydroquinone. Using Lineweaver-Burk analysis and molecular docking can fully characterise the mode of inhibition in the future.

3.4 *In Vitro* Antibacterial Activity

The agar well diffusion results and MIC values are presented in Tables 3 and 4, respectively. Inhibition zones >8 mm were recorded against all susceptible strains. Among gram-negative bacteria, *E. coli* was the most sensitive at 10 mg/ml (zone = 28 mm). Among Gram-positive bacteria, *S. aureus* was most sensitive at 10 mg/ml (zone = 20 mm). MIC was lowest for *E. coli* (0.0625 mg/ml), followed by *S. aureus* and *S. agalactiae* (both 0.25 mg/ml). *P. aeruginosa*, *S. saprophyticus*, and *S. pyogenes* showed no susceptibility.

In general, gram-positive bacteria were more susceptible to the *R. coriaria* L. extract than gram-negative bacteria, and this observation is typical of phenolic extracts from plants due to the structural difference in the cell wall of bacteria: Gram-positive bacteria have a thick peptidoglycan wall without an outer membrane that increases their susceptibility to hydrophilic polyphenolic substances while the presence of the outer membrane in gram-negative bacteria serves as another permeability obstacle. The exceptional antimicrobial activity exhibited by the Jordanian sumac extract against *E. coli* (with the MIC being equal to 0.0625 mg/ml) deserves special mention, as it is comparable with data on the MICs of *R. coriaria* L. in other publications where the MIC ranged between 0.06 and 2.5 mg/ml depending on the solvent used and the pathogen involved[2]. The increased prevalence of drug-resistant strains of bacteria in medicine creates the need to find new natural antimicrobial agents, and the demonstrated effect of the Jordanian sumac makes it a promising candidate.

3.5 MTT Assay (Cytotoxicity Assay)

The cytotoxic potential of *R. coriaria* L. methanolic extract in U-87 glioblastoma cells is shown in Figure 3. Cell survival was significantly reduced at 5 µg/ml (72%) and 50 µg/ml (65.8%) ($p < 0.05$). The results above are in line with the known anticancer effects of extracts from *R. coriaria* in Michigan Cancer Foundation (MCF), Sloan Kettering Ovarian (SKOV3) cancer, and PC-3 prostate cancer cells, as shown by Gabr and Alghadir[17]. The selective cytotoxicity exhibited by sumac extract in the U-87 glioblastoma model, an especially lethal and difficult-to-treat cancer, is worth mentioning. The individual effect of phenolic components in sumac (*e.g.*, gallic acid and ellagic acid) against cancer cells through

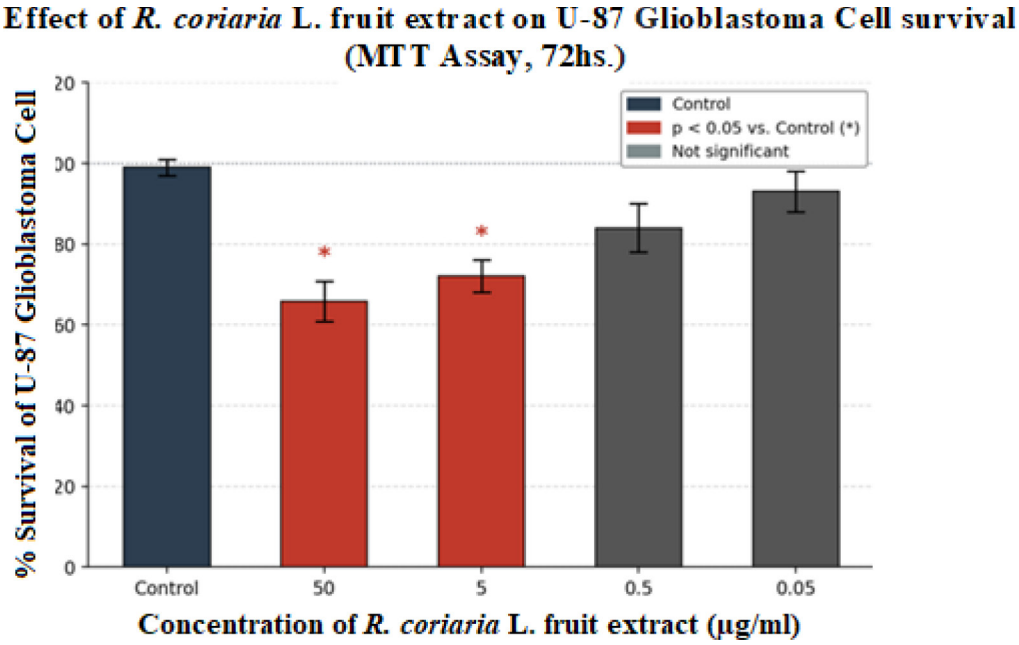


Figure 3. MTT assay using U-87 cells. Each point represents the mean value ± SD (n = 3).

mechanisms of oxidative damage, mitochondrial membrane breakdown, and blocking survival pathways (such as NF-κB and Mitogen-Activated Protein Kinase (MAPK) has been established. Hence, the results contribute to existing data on the anticancer properties of *R. coriaria* L. extract, and the U-87 glioblastoma cancer model should be considered for future studies based on sumac fractions and apoptosis mechanisms.

4. Conclusion

This study demonstrates that the methanolic fruit extract of Jordanian *R. coriaria* L. possesses potent antityrosinase, antioxidant, antibacterial, and anticancer activities underpinned by a rich profile of phenolic compounds and flavonoids. The antityrosinase IC_{50} of 17 µg/ml markedly surpasses that of the established inhibitor arbutin (92.5 µg/ml), positioning Jordanian sumac as a highly promising plant-derived candidate for cosmeceutical and pharmaceutical formulations targeting hyperpigmentation disorders.

The mechanism of action is probably due to the chelating property of gallic acid and ellagic acid derivatives, which are the main phenolic compounds present in *R. coriaria* L. Future investigations are needed to conduct enzymatic inhibition studies, molecular docking analysis, and bioactivity-based isolation of active components.

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Author Contribution

Dr Lubna Swellmeen: Conceptualisation, writing (original draft), and plant extraction; Dr Ashraf Qasim: antioxidant assay; Dr Haneen Basheer: MTT cytotoxicity assay; Dr Iman Mansi: antityrosinase activity assay; Dr Amal Uzrail: antibacterial assay; Dr Heba A. Khader: phytochemical assay; Dr Farah Al-Mamoori: manuscript revision and editing. All authors have read and agreed to the published version of the manuscript.