Review

Rhamnus alaternus Plant: Extraction of Bioactive Fractions and Evaluation of Their Pharmacological and Phytochemical Properties

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Abstract: Rhamnus alaternus, is a wild-growing shrub, belonging to the Rhamnaceae family. Widely distributed in the Mediterranean basin, R. alaternus is used in the usual medicine in numerous countries, mostly Tunisia, Algeria, Morocco, Spain, France, Italy, and Croatia. A large number of disorders—including dermatological complications, diabetes, hepatitis, and goiter problems—can be treated by the various parts of R. alaternus (i.e., roots, bark, berries, and leaves). Several bioactive compounds were isolated from R. alaternus, including flavonoids, anthocyanins, and anthraquinones, and showed several effects such as antioxidant, antihyperlipidemic, antigenotoxic, antimutagenic, antimicrobial, and antiproliferative. This review summarizes the updated information concerning the botanical description, distribution, extraction processes applied on R. alaternus, and its ethnopharmacology, toxicity, phytochemistry, and pharmacological effects.

Keywords: Rhamnus alaternus; extraction processes; phytochemistry; ethnopharmacology; phytoterapy; toxicity; bioactive compounds

1. Introduction

Rhamnus species are considered as medicinal plants. Indeed, these sources of natural compounds possess pharmacological activities, and are used for their curative effects to treat some symptoms and diseases [1–7]. During the past few years, several studies highlighted the potential efficacy of Rhamnus species in many areas [8–11]. Among these naturally available species, the Rhamnus alaternus plant (R. alaternus) is commonly recognized as a 5-meter-tall shrub, and is distributed throughout the Mediterranean area [12–15] including North Algeria, Tunisia, and Morocco [16,17]. This plant widely grows in a Mediterranean climate with hot and dry summer and winter period is moderate to cold [14,18,19].

The Rhamnus alaternus plant, the so-called "Imlilesse or Safir" in the North of Algeria, has been traditionally used for a long time in various medicine areas as infusion notably for its gastric, hypotensive, purgative, laxative, diuretic, antihypertensive, hepatoprotective, and digestive effects and finally to treat dermatological complications [20]. Such biological activities would be related to the natural presence of beneficial compounds as evidenced by many experimental studies, that pointed out that R. alaternus contains important metabolites—such as flavonoids, coumarins, glycosides, tannins, anthraquinones, and polyphenolic compounds [21,22]. Some of these molecules were isolated from Rhamnus alaternus using various extraction processes (i.e., maceration, decoction, hydrodistil-
lation, soxhlet, ultrasonic extraction) and demonstrated various pharmacological properties including antihyperlipidemic, antioxidant, antigenotoxic, antiproliferative, and antimutagenic activities. Some biological activities, especially antibacterial and antiproliferative effects were reviewed elsewhere [8,20,22–24].

To date, to the author’s knowledge, there are no accurate published reviews concerning *Rhamnus alaternus*. In this current review, we compiled and described various extraction methods of bioactive compounds applied on various parts of this plant. Besides therapeutic effects, phytochemical, and pharmacological activities of *R. alaternus* are presented. The exploitation and investigation of potential beneficial effects of *Rhamnus alaternus* are summarized here in order to guide the research strategy for its industrial application.

2. Botanical Data

2.1. Geographical Distribution

Common in wild, *Rhamnus alaternus* growths generally between evergreen shrubs of the Mediterranean region, especially in a climate with discontinuous rains during winter. With such characteristics, *R. alaternus* is a very important species of the Mediterranean basin, where it is well acclimated to high solar radiation [25,26]. *Rhamnus alaternus* is widely distributed and grows naturally in a large part of the littoral and islands of the Mediterranean. In France, this plant is mainly found in the South departments such as Isère, Ardèche, Aveyron, Maine-et-Loire, in Vienne but also in Brittany [27]. In addition, this plant growths in Corsica, Algeria, and Northern Tunisia [22,28–30].

2.2. Botanical Description

In North Africa, *Rhamnus alaternus* has several names such as Am’Tile’ce, M’liila, Soit-fair, Oud El-khir, or Safir, and is commonly known as Meliles in Berber language [31–33]. *R. alaternus* is also called Buckthorn in English, Nerprun in French, Kreülzdorn in German, Aladierna, Cosco Unia, or Sanguino de Andalucia in Spanish, and Alaterno or Legno Puzzo in Italian [34]. Concerning its botanical classification, *R. alaternus* belongs to the Magnoliophyta division, the Magnoliopsida class, the Rhamnales order, the Rhamnaceae family, the Reynosia Genus and the *Rhamnus alaternus* species [35]. In addition, this plant has various synonyms including *R. a. var angustifolia* DC, *R. a. var balearica* DC, *R. a. var hispanica* DC, and *R. a. var vulgaris* DC [36]. *R. alaternus* is a small shrub of about 5-meter-tall (Figure 1A–D). Its flowers are fecundated by insects or with the help of wind [37,38] and get yellow-green from January until the end of April (Figure 1E), with a top in mid-February [39]. Then puffy and black fruits are produced, and mature between late spring and early summer, each one containing between 2 and 5 red berry seeds with on average 2.5 mm width, 4.6 mm length, and 9.1 mg weight as maximum (Figure 1F) [38,40]. Seeds are surrounded within a pericarp, which opens up once dried [38], and represents an important trophic source for birds and small mammals [41]. Usually germinated in 3 to 4 weeks between 7.5 and 24 °C, the seeds remain viable for several years in storage [40].
Figure 1. (A) *Rhamnus alaternus* plant with a focus on its different aerial parts: (B) leaves, (C) stem, (D) pods, (E) flowers, and (F) berries.

3. Extraction Processes Investigated in *R. alaternus*

3.1. Main Processes Applied

The bioactive compounds present in *Rhamnus alaternus* plant can be extracted by using various techniques described in Figure S1 (see Supplementary Materials) with the critical parameters of each extraction process. Besides, all techniques used for the extraction of bioactive compounds from different parts of *R. alaternus* were reported with the solvents used for isolating the targeted compounds, and their respective pharmacological and biological activities including: antioxidant, antimicrobial, antigenotoxic, and antiproliferative activities (Table 1) [42–46]. Also, the chemical structure of the molecules isolated from this plant are presented in Figure S2 (see Supplementary Materials).
Table 1. Bioactive compounds extracted from different parts of *R. alaternus* according to the process of extraction involved and their pharmacological activities.

| Part of Plant | Extraction Method | Solvent(s) Used | Bioactive Compounds (or Groups) | Pharmacological Activities | Reference |
|---------------|-------------------|-----------------|---------------------------------|---------------------------|-----------|
| Leaves        | Maceration        | Methanol        | Kaempferol hexoside, rhamnocrin, kaempferol 3-O acetyl-rhamnoside, quercetine, pilosin hexoside, pilosin, apigenin glucoside, rhamnocitinin hexoside, rhamnetin hexoside, kaempferol | Antihyperlipidemic activity | [23] |
|               | Ethanol, distilled water, methanol, ethyl acetate | Emodin, kaempferol | Antioxidant activity | [47] |
|               | Methanol          | Rutin, antraquinones, quercetin-3-rhamnside, kaempferol, gallic acid, p-coumaric acid, ferulic acid, luteolin | Antioxidant activity | [48] |
|               | Maceration/ Decoc- | Distilled water | NA | Hepatoprotective effects | [49] |
|               | tion              |                 |                                  |                           |           |
|               | Maceration        | Methanol, petroleum ether, chloroform, ethyl acetate water/acetone | Rhamnetin-3-O-isorhamninoside, kaempferol 3-O-isorhamninoside, rhamnocitinin-3-O-isorhamninoside | Antioxidant activity | [50] |
|               | Maceration        | Methanol, chloroform, petroleum ether, ethyl acetate water, acetone | Kaempferol 3-O-β-isorhamninoside, rhamnocitinin 3-O-β-isorhamninoside | Antioxidant activity | [24] |
|               | Soxhlet extraction/ | Petroleum ether, chloroform, ethyl acetate, methanol, butanol water, acetone | Coumarins, flavonoids, antraquinones, tannins | Antimicrobial activity | [20] |
|               | Maceration        | Petroleum ether, chloroform, ethyl acetate, methanol, water, acetone | Flavonoids, antraquinones, tannins, sterols, coumarins | Antigenotoxic activity | [51] |
|               | Soxhlet extraction | Ethyl acetate, water, acetone, chloroform | Flavonoids | NA | [52] |
|               | Chloroform, water, petroleum ether, ethyl acetate, dimethyl sulfoxide, butanol | Flavonoids, phenols | Antigenotoxic activity | [53] |
|               | Hydrodistillation extraction | Water | Oxygenated monoterpenes hydrocarbons, oxygenated diterpenes hydrocarbons, oxygenated Sesquiterpenes hydrocarbons, sesquiterpenes hydrocarbons, monoterpenes hydrocarbons, aliphatic hydrocarbons, fatty acids | NA | [54] |
|               | Methanol          | Malvidin, delphinidin 3-rutinoside, cyanidin, petunidin 3-glucoside, petunidin, delphinidin 3-glucoside, pelagonidin, malvidin 3-rutinoside, peonidin 3-ruti- | | NA | [18] |
| Component | Extraction Method | Solvents | Major Constituents | Antioxidant Activity | Antimicrobial Activity |
|-----------|-------------------|----------|-------------------|---------------------|------------------------|
| Berries   | Maceration        | Methanol, water | Flavonoids, delphinidin peonidin 3-glucoside, malvidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-rutinoside, pelargonidin 3-rutinoside, | NA [55] | |
|           |                   |          | Quercetin, rhamnazin-3-O rhamninoside, rhamnatin, quercetin-4′-O-rhamninoside, kaempferol-4′-O-rhamninoside, isorhamnetin, rhamnocitrin, kaempferol, rhamnocitrin-3-O-rhamninoside, quercetin-3-O-rhamninoside, rhamnetin-3-O-rhamninoside, rhamnocitrin-4′-O-rhamninoside, kaempferol-3-O-rhamninoside |          | |
| Bark      | Maceration/Decoction | Methanol, water | Flavonoids, aloe-emodin, rhein, emodin, chrysophanol, physcion | Antioxidant activity [21] | Antioxidant activity Antimicrobial activity [8] |
|           | Ultrasonic extraction | Methanol, ethyl acetate | Flavonoids, aloe-emodin, rhein, emodin, chrysophanol, physcion |          | |
| Roots     | Decoction         | Chloroform, dichloromethane, ethyl acetate | Rhein, physcion, aloe-emodin | NA [57] | |
|          | Maceration        | Methanol, butanol | Flavonoids, coumarins, anthraquinones, sterols | Antiproliferative activity | Antimutagenic activity [22] |
|          |                   |          | Emodin-6-O-α-L-rhamnoside, β-sitosterol, physcion-8-O-rutinoside, kaempferol-7-methylether. 1, 6 dihydroxy-3 methyl 6 [2′-Me (heptoxy)] anthraquinone. β-sitosterol-3-O-glycoside. 1,4,6,8 tetrahydroxy-3 methyl anthraquinone 1-O-β-D-glucopyranosyl-4,6-di-O-α-L rhamnopyranoside. 1,2,6,8 tetrahydroxy-3 methyl anthraquinone 8-O-β-D-glucopyranoside |          | |
|          | Soxhlet extraction/Maceration | Methanol, ethyl acetate, chloroform water, acetone | Flavonoids, tannins | Antigenotoxic activity [58] | |

NA, not available.
3.2. Maceration and Decoction

Among all the reported extraction processes, maceration and decoction are the most commonly applied methods to extract biomolecules [59,60]. The maceration method is vastly used in research domain of medicinal plants. These methods are described and explained by authors in numerous references [60–62]. The decoction method uses the same concept as maceration. Yet, for the decoction, the plant powder is boiled in a specific volume of water for a defined time, then cooled and filtered, while in maceration, the plant powder is mixed with extraction solvent at room temperature [61,62].

In 2015, Boussahel and colleagues investigated the flavonoid profile obtained by decoction and maceration of aqueous and methanolic extracts, obtained from *R. alaternus* bark and determined their antioxidant activity. Former authors observed that the methanolic maceration was better for extracting flavonoids than both aqueous maceration and aqueous decoction [21]. In another study, Berroukche and coworkers compared the same former extraction processes applied on leaves of *Rhamnus alaternus* to study their antioxidant and hepatoprotective effects [49]. The results of their investigation proved that leaves extracts obtained by maceration were better and more effective than those obtained by decoction in terms of bioactivity. Also, Moussi and colleagues investigated the antioxidant activity of the methanolic extract from *R. alaternus* leaves and reported that leaves extract from this plant can be used for formulating pharmaceutical products for various diseases [48]. The composition of *R. alaternus* roots, bark and leaves extracts was extensively investigated using the maceration method [18,20,22,47] [50–52,55,57,58]. The same extraction techniques were used by Tacherfiout and colleagues, who investigated the antihyperlipidemic effect of leaves extracts of *Rhamnus alaternus* [23].

3.3. Soxhlet Extraction

Soxhlet extraction is one of the reference methods used for extraction of bioactive compounds [45,46]. This technique is known for its simplicity, a low cost with a minimal solvent consumption compared to other conventional methods. In this technique, the ground sample is placed in a ‘thimble’ made from cellulose, itself located in a thimble chamber of the Soxhlet system, in the presence of extraction solvents. These latter ones are heated in a distillation flask, and evaporated inside the sample thimble. After the liquid reaches the siphon arm, the liquid content empties back into the distillation flask and the operation is continued until extraction is complete [61,63].

Numerous studies focused on the Soxhlet extraction of some bioactive molecules from different parts of *R. alaternus*. To date, various solvents were used in this extraction method such as ethanol, ethyl acetate, methanol, chloroform, etc. [64]. By using Soxhlet extraction and fractionation, Ben Ammar et al., succeeded in isolating three bioactive substances from leaves extracts of this plant endowed with high antioxidant and free radical scavenging capacities [50]. In another study, Bhouri and colleagues investigated the antioxidant and antigenotoxic activities of three bioactive compounds extracted from the leaves of *R. alaternus* using soxhlet extraction method [24]. The results of their investigation proved that compounds obtained from *R. alaternus* leaves are phytopharmaceutical molecules of interest. Other studies revealed that different extracts of *Rhamnus alaternus* obtained by soxhlet has antigenotoxic, antimutagenic, and antimicrobial activities [20,51–53,56,58].

3.4. Ultrasonic Assisted Extraction

Ultrasonic assisted extraction (UAE) has the main advantage to extract bioactive compounds from plants without impacting on their functional properties [65]. This technique depends on the use of ultrasounds power ranging from 20 kHz to 2000 kHz [61,66]. UAE is an important extraction method applied in food processing. It uses ultrasonic waves to create cavitation bubbles in order to increase the mass transport between solvent
and plant cells, and leads to fast and effective extraction of compounds from plants [46,61,67].

In 2013, Kosalec and co-workers used ultrasonic extraction technique and proved that *R. alaternus* was an important source of anthraquinones and other bioactive substances [8]. In addition to three other *Rhamnus* species investigated, former authors reported the anthraquinone profile of the *R. alaternus* bark extracts, characterized with antioxidant and antimicrobial activities.

3.5. Hydrodistillation

Hydrodistillation (HD) is a frequently used method for extraction of volatile compounds. In a reactor, the plant material is immersed in boiling water mostly at atmospheric pressure; the volatile compounds are moved away with the water steam up to the condenser and collected after decantation [68,69]. This technique depends mainly on the distillation time, which impacts the solvent penetration into the plant matrix, and favors the diffusion of substances into the solvent of extraction [70,71].

In their study, Berka and colleagues attempted to isolate essential oil from aerial parts of *R. alaternus* using hydrodistillation extraction [54]. This work was the only one to focus on the essential oil of *Rhamnus alaternus*, which is constituted of a complex mixture of 94 constituents.

4. Phytochemistry

4.1. Generalities

The phytochemical investigations of *Rhamnus alaternus* extracts led to the isolation of various classes of natural bioactive compounds, and evidenced the richness in secondary metabolites of this medicinal plant. These bioactive compounds included flavonoids, tannins, anthraquinones, anthocyanins, anthocyanidins, and other compounds [48,72], isolated from various parts of *R. alaternus* such as barks, leaves, roots, and berries extracts. The investigation of these compounds was limited to qualitative studies. The most important classes of phytochemicals identified in *R. alaternus* are summarized (Table 2) with their main chemical compounds, whose structures are presented in Supplementary Data. Each class of compounds is reviewed hereafter.

| Compound Class | Compound | Compound Number | Reference |
|----------------|----------|----------------|-----------|
| Flavonoids     | Quercetin-3-O-rhamninoside | 1 | [48,55] |
|                | Kaempferol-3-O-rhamninoside | 2 | [55] |
|                | Quercetin-4′-O-rhamninoside | 3 | [55] |
|                | Kaempferol-4′-O-rhamninoside | 4 | [55] |
|                | Rhamnetin-3-O-rhamninoside | 5 | [55] |
|                | Rhamnositrin-3-O-rhamninoside | 6 | [55] |
|                | Rhamnocitrin-3-O-rhamninoside | 7 | [55] |
|                | Kaempferol | 8 | [21,23,47,48,55] |
|                | Quercetin | 9 | [21,23,55] |
|                | Isorhamnetin | 10 | [21,55] |
|                | Rhamnetin | 11 | [21,55] |
|                | Rhamnazin | 12 | [55] |
|                | Kaempferol-3-O-isorhamninoside | 13 | [23,24,50] |
|                | Rhamnositrin-3-O-isorhamninoside | 14 | [24,50] |
|                | Rhamnetin-3-O-isorhamninoside | 15 | [50] |
| Anthraquinones | Emodin | 16 | [8,47,56] |
|                | Rhein | 17 | [8,57] |
|                | Chrysophanol | 18 | [8] |
|                | Physcion | 19 | [8,57] |
|                | 1,4,6,8 tetrahydroxy-3 methyl anthraquinone | 20 | [16] |
1-O-β-D-glucopyranosyl-4,6-di-O-α-L-rhamnopyranoside 1,2,6,8 tetrahydroxy-3 methyl anthraquinone 8-O-β-D-glucopyranoside 1, 6 dihydroxy-3 methyl 6 [2'-Me (heptoxy)] anthraquinone Physcion-3-O-β-rutinoside Emodin-6O-α-L-rhamnopyranoside β-sitosterol β-sitosterol-3-O-3β-D-glycopyranoside

| Anthocyanins |
|--------------|
| Cyanidin 3-rutinoside 27 [18] |
| Petunidin 3-rutinoside 28 [18] |
| Delphinidin 3-rutinoside 29 [18] |
| Pelargonidin 3-rutinoside 30 [18] |
| Peonidin 3-rutinoside 31 [18] |
| Malvidin 3-rutinoside 32 [18] |
| Delphinidin 3-glucoside 33 [18] |
| Cyanidin 3-glucoside 34 [18] |
| Petunidin 3-glucoside 35 [18] |
| Pelargonidin 3-glucoside 36 [18] |
| Peonidin 3-glucoside 37 [18] |
| Malvidin 3-glucoside 38 [18] |
| Delphindin 39 [18] |
| Cyandin 40 [18] |
| Petunidin 41 [18] |
| Pelargonidin 42 [18] |
| Peonidin 43 [18] |
| Malvidin 44 [18] |

*These numbers refer to the chemical structures plotted in Supplementary Data (Figure S2).

4.2. Flavonoids

Flavonoids, whose skeleton is based on about 15-carbon and is composed of two benzene rings [73,74], gather the bioactive compounds with low molecular weight (286-610 g/mol).

These flavonoids are the most active constituents of *Rhamnus alaternus*. Among these isolated compounds, literature reports quercitin-3-0-rhamninoside, kaempferol-3-0-rhamninoside, quercitin-4'-0-rhamninoside, kaempferol-4'-0-rhamninoside, rhamnetin-3-0-rhamninoside, rhamnocitrin-3-0-rhamninoside, and rhamnocitrin-4'-0-rhamninoside (Figure S2(1)–(7)), identified from green fruits of *Rhamnus alaternus* [55]. Other flavonols —including quercitin, kaempferol, isorhamnetin, rhamnetin, rhamnazin (Figure S2(8)–(12))—were extracted by maceration, from methanolic and aqueous extracts from Algerian *Rhamnus alaternus* barks [21,55]. More, flavonols such as kaempferol 3-0-β-isorhamninoside, rhamnocitrine 3-0-β-isorhamninoside, and rhamnetin-3-0-isorhamninoside (Figure S2(13)–(15)) were also isolated from *Rhamnus alaternus*’s leaves by Soxhlet extraction method [24,50]. Furthermore, other valuable bioactive compounds were isolated from *R. alaternus’* leaves such as kaempferol 3-O acetyl-rhamnosoide and quercetin-3-rhamnosoide [23,48].

4.3. Anthraquinone Compounds

Anthraquinones are aromatic organic compounds with the 9,10-anthracenedione core [75]. These ones, including three new Anthraquinones, were isolated from the extracts of various parts collected from *Rhamnus alaternus* (i.e., leaves, barks, and roots). Among anthraquinones, rhein, chrysophanol, and physcion (Figure S2(17)–(19)) were also isolated from the bark extract of *R. alaternus* by ultrasonic extraction [8]. Furthermore, 1,4,6,8 tetrahydroxy-3 methyl anthraquinone 1-O-β-D-glucopyranosyl-4,6-di-O-α-L-rhamnopyranoside, 1,2,6,8 tetrahydroxy-3 methyl anthraquinone 8-O-β-D-glucopyranoside and 1, 6 dihydroxy-3 methyl 6 [2'-Me (heptoxy)] anthraquinone (Figure S2(20)–
were identified from various parts of *Rhamnus alaternus* such as leaves, bark and roots [16].

### 4.4. Anthocyanin Constituents

Anthocyanins are structurally related to anthocyanidins (parent class of flavonoids) and are also derived from the 2-phenylbenzopyrilium ion [76,77].

The extracts of *Rhamnus alaternus*’ berries showed many compounds of high nutritional values and were rich in diverse anthocyanins and anthocyanidins constituents, such as delphinidin 3-rutinoside, delphinidin 3-glucoside, delphinidin, cyanidin 3-rutinoside, cyanidin 3-glucoside, cyanidin, pelargonidin 3-rutinoside, pelargonidin 3-glucoside, pelargonidin, petunidin, peonidin, and malvidin [18](Figure S2(27)–(44)).

### 5. Biological Properties

#### 5.1. Ethnopharmacology

*Rhamnus alaternus* has extensively been used in medicine in Algeria and many other North African countries [22,58]. This plant was empirically used as a laxative, purgative, diuretic, antihypertensive, and depurative [22,50]. In North African countries, the decoction of the aerial parts of *R. alaternus* such as bark is used against certain dermatological and hepatic diseases [22]. This plant is also used for the treatment of diabetes [57]. More recently, Zeouk and colleagues nicely reviewed the traditional uses and pharmacological aspect of *R. alaternus* [78].

#### 5.2. Pharmacological Activities

##### 5.2.1. Antihyperlipidemic Activity

Lipids, among them cholesterol, play a fundamental role in the structure of membranes and in many biological activities in the human heart’s health. Yet, an increase in the concentration of lipids—and particularly of plasma cholesterol beyond the dose required daily—presents a major risk of progression of heart and vascular diseases, including coronary heart diseases and strokes [79,80]. Hyperlipidemia is characterized by decreased levels of high-density lipoprotein cholesterol (HDL-cholesterol) and elevated levels of total cholesterol (TC), phospholipids, triglyceride (TG), low density lipoprotein cholesterol (LDL-cholesterol) and very low density lipoprotein cholesterol (VLDL-cholesterol) into the bloodstream [81–83]. According to a World Health Organization report, the cardiovascular diseases caused by hyperlipidemia—including high blood cholesterol—are responsible of about 4.4 million deaths per year [84].

The antihyperlipidemic activity of *Rhamnus alaternus* was first investigated by Tachefiout et al. [23]. According to these authors, the leaves’ extract of *R. alaternus* contains flavonoids that possess the ability to reduce the intracellular lipids concentration and to increase the oxidation of fatty acids in HepG2 cells. Similarly, the flavonoids and flavonoids derivatives from *R. alaternus* leaves showed a similar positive impact on murine preadipocyte 3T3-L1 cellular model.

##### 5.2.2. Antioxidant Activity

Natural antioxidants are bioactive substances extracted from plants that reduce damages due to reactive oxygen species, in both food and human body. Antioxidants might reduce the cancer risks and protect against different diseases. The antioxidant capacity is a generic term that gathers various mechanisms such as radical-scavenging activity, that can be evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyle) or ABTS assay (2,2’-azinobis (3-ethylbenzthiazoline-6-sulfonic acid)), and other antioxidant tests carried out in vitro [85–89] notably involving inhibition of lipid peroxidation or metal-chelation.
Natural compounds, including polyphenols, have various pharmacological activities and biological effects, such as antioxidant properties [90–95], and are known for their protective effects against many oxidative stress-related diseases [96–100].

The antioxidant properties of *R. alaternus* are associated with the presence of numerous constituents, such as flavonoids and anthraquinones [48,50]. Numerous studies focused on the antioxidant activity of natural substances extracted from *R. alaternus* [16,31,50]. Most of these studies evaluated the antioxidant properties using in vitro methods such as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [98]. Zeouk and colleagues [47] demonstrated that ethanolic extracts of *R. alaternus* obtained by maceration from leaves harvested in the Atlas Mountains of Morocco possess an interesting antioxidant capacity. Indeed, using DPPH antioxidant test, former authors isolated three fractions with a very good antioxidant activity (IC₅₀ = 58.00 ± 7.00 µg/mL), compared with Butyl hydroxytoluene BHT (positive control; IC₅₀ = 31.00 ± 3.00 µg/mL).

In 2015, Boussahel and co-workers investigated the antioxidant activity of the aqueous and methanolic extracts of *R. alaternus* barks, obtained either by maceration or by decoction [21]. They evaluated the antioxidant properties while coupling various biochemical tests: radical scavenging tests, such as the DPPH and the ABTS tests (2,2’-azino-bis-(3-ethylbenzothiazine-6-sulfonic acid), the ferric reducing ability test that uses the FRAP reagent (ferric reducing antioxidant power) and the determination of oxygen radical absorbance capacity (ORAC) assay [21]. Former authors focused first on the solubility of flavonoids in organic and aqueous solvents, and evidenced that the flavonoids of *Rhamnus alaternus* bark were more easily dissolved in methanol than in water. Concerning their antioxidant effect, the methanolic extract presented a higher content of flavonol (51.17 ± 0.41 µg QE/mg) compared to the aqueous one (24.10 ± 0.85 µg QE/mg) using quercetin as reference substance. Hence, the methanolic extract was more bioactive whatever the chemical assays used, in particular for the test of flavonoid quantification described by Tamiano et al. [101]. Also, the same extract showed the highest Trolox equivalents antioxidant capacity (TEAC = 0.75 ± 0.001 mmol TE/g) using Trolox as reference substance [21].

In another study [24], the antioxidant properties of two flavonoids isolated from *Rhamnus alaternus* leaves using Soxhlet extraction method were evaluated using the ABTS assay. The two isolated constituents—the kaempferol 3-O-β-isorhamninoside and rhamnocitrin 3-O-β-isorhamninoside (Figure S2(13),(14))—showed an interesting inhibitory activity against superoxide anion at a dose of 150 µg/assay. Former authors determined that these two compounds exhibited a potential radical scavenging activity with IC₅₀ values, ranging from 18.75 to 22.5 µg/mL. In comparison, the Trolox reference exhibited an IC₅₀ value of 0.2 µg/mL. Furthermore, Moussi et al. [48] reported the antioxidant properties of various fractions obtained from *R. alaternus* leaves by maceration and found an interesting radical scavenging capacity for all fractions separated from the methanolic extract. Besides, the antioxidant activity evaluated by DPPH assay presented a high percentage of DPPH radical inhibition (90.36 ± 0.45 %).

More recently, Ben Ammar et al. [16] isolated three new bioactive substances from various parts of *Rhamnus alaternus* (i.e., leaves, roots, and barks). They evaluated the DPPH radical scavenging activity of these new identified anthraquinones: alaternoside A, alaternoside B, and alaternoside C. These anthraquinones showed a high free radical scavenging capacity, specially alaternoside C, with an IC₅₀ value of 9.46 µg/mL and presented a higher effect than the positive reference (i.e., Vitamin E). Table 3 summarizes the antioxidant activity of *Rhamnus alaternus* collected from different geographical regions, according to the part of the plant investigated and the biological test set up.

According to the data concerning the extraction process of bioactive compounds from different parts of *R. alaternus* and the antioxidant activity of the corresponding fractions, the maceration of the leaves gave an extract, rich in bioactive substances, with the highest antioxidant activity.

Besides, the inhibition of lipid peroxidation is another indicator of antioxidant activity. Indeed, the lipid peroxidation affects unsaturated lipids, and is related to the presence...
of radical species [98]. In the long-term, lipid peroxidation process could lead to the development of various diseases such as diabetes and several cancers [99,102]. The anti-lipid peroxidation activity of various extracts from R. alaternus, produced using the Soxhlet extraction method, was estimated by calculating the values of malondialdehyde (MDA) in cultured K562 human chronic myelogenous leukemia cells [52]. Rhamnus alaternus extracts containing total oligomer flavonoids (TOF) and ethyl acetate (EA) inhibited lipid peroxidation at a concentration comprised within 200–800 µg/mL, the best activity being observed at the highest concentration (800 µg/mL). In this study, the IC₅₀ values of TOF and EA extracts were determined at 196 and 265 µg/mL, respectively. In comparison, a value of 17 µg/mL was obtained for vitamin C, used as reference substance.

| Parts of Plant | Analytical Method | Values | Country | Reference |
|----------------|-------------------|--------|---------|-----------|
| Leaves         | DPPH assay        | 1.5 – 38 µg/mL equivalent vitamin C (fractions) | Tunisia | [50]      |
|                |                   | 12.60 – 90.81 %; BHT is the positive control (fraction) | Algeria | [48]      |
|                |                   | 8.22±0.01µg/mL; BHT is the positive control (extract) | Algeria | [42]      |
|                | TEAC assay        | 07.76 – 38.87 % (fractions) | Morocco | [47]      |
|                |                   | 18.75 – 22.5 µg/mL equivalent of Trolox (fractions) | Tunisia | [103]     |
|                |                   | 18.75 µg/mL |              |
|                |                   | 22.5 µg/mL equivalent of Trolox (fraction) | Tunisia | [24]      |
|                | FRAP assay        | 300 – 368 µg/mL equivalent of Trolox (fractions) | Tunisia | [31]      |
| Mixture of Leaves, Bark and Roots | DPPH assay | 2.35 – 58 µg/mL | Tunisia | [16]      |
| Leaves         | DPPH assay        | 18.84 µg/mL |          |
| Root           |                   | 7.21 µg/mL, α-tocopherol is the positive control (extract) | Tunisia | [22]      |
| Bark           | DPPH assay        | 0.39 – 0.61 mmol TE/g | Algerb | [21]      |
|                | ORAC assay        | 3.96 – 6.55 mmol TE/g |
|                | FRAP assay        | 1.24 – 1.72 mmol Fe²⁺/g |
|                | TEAC assay        | 0.65 – 0.75 mmol TE/g (extract) |
| β-carotene-linoleic acid assay | DPPH assay | 78.7±3.16 µg/mL | Croatia | [8]       |
|                |                   | 250±6.84 µg/mL |          |
| Reducing power assay SRP | TEAC assay | Ascorbic acid, quercetin and BHT are used as positive controls (extract) |
| Chelating activity | DPPH assay | 0.91±0.01 mg⁻¹ |
|                |                   | 1760±60.7 µg/mL |
| Atrial part    | DPPH assay        | 52.32 – 87.34 % α-tocopherol is the positive control (extract) | Tunisia | [51]      |

Table 3. Antioxidant activity of Rhamnus alaternus collected from various countries.

5.2.3. Antiproliferative Activity

Every year in developed countries, many people die because of cancer [104,105]. Many effective anticancer drugs contain biomolecules such as flavonoids, an important class of natural substances found in numerous plants, among them R. alaternus. These molecules are characterized by their antiproliferative activities, which are related to their properties to prevent or delay the growth and spread of cells, especially malignant cells,
into surrounding tissues. Hence, these molecules could be either used as traditional drugs, or pills once encapsulated [106–110].

The antiproliferative test determines the effect of an investigated biomolecule or extract to prevent the growth of a given cell type. The objective of this assay is to determine the inhibitory concentration (IC50) of the investigated biomolecule, i.e., the concentration that inhibits half's proliferation of the overall cell defined [111–114].

The antiproliferative effect of roots and leaves extracts obtained from R. alaternus maceration was investigated against K562 human cell line and L1210 mouse lymphoma cells, at various concentrations comprised between 100 and 800 µg/mL [22]. The proliferation of these leukemia cells was followed by the MTT assay, which uses the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) as reagent [115]. The leaves and roots extracts from R. alaternus showed interesting antiproliferative, and dose-dependent effects. The root extract was more effective than the leaves one, on both types of leukemia cells. Indeed, concerning the K562 human cell, the IC50 values of roots and leaves extracts were determined at 165 and 260.69 µg/mL, respectively. Concerning the L1210 cells, the IC50 values of roots and leaves extracts were determined at 210.73 and 343.10 µg/mL, respectively, in the presence of α-tocopherol as positive control. According to these former data, the K562 cell line showed a higher sensitivity to the inhibitory effect of the tested extracts.

5.2.4. Antimicrobial Activity

The antimicrobial activity concerns all active substances or agents that kill or inhibit the growth of bacteria [116]. A large number of plants have always been used to treat prevalent infectious diseases in humans [117–120], these ones being caused by various micro-organisms, including bacteria. Hence, plants endowed with antimicrobial activity are often used as a part of the usual treatment of various diseases [121–123].

Many methods are reported in the literature for the evaluation or detection of antimicrobial effect. The disc diffusion method is a qualitative method since it only gives a trend about the presence of antimicrobial activity of the investigated substances [124–126]. The principle of this method is based on the surface culturing of inoculated microbes, which are exposed to small disks containing a quantity of extracts. If the extract has the capacity to inhibit the bacterial growth, it results in a zone of inhibition around the disk after incubation. On the contrary, the dilution method is a quantitative test: it can determine the level of microbial resistance to an extract or an antimicrobial agent by making serial dilutions of the tested agent, in order to define the minimal inhibitory concentration (MIC).

Ben Ammar and colleagues (2007) investigated the antimicrobial activity of aerial parts of various R. alaternus extracts obtained by Soxhlet extraction. Such extracts were tested against two Gram-positive bacteria, both from the American type culture collection (ATCC): Staphylococcus aureus and Enterococcus faecalis. In addition, their antimicrobial activities were tested on three Gram-negative bacteria as well: Escherichia coli ATCC, Salmonella enteritidis ATCC and Salmonella typhimurium. The antimicrobial activity of R. alaternus was determined using the micro-dilution method, the range of concentration varying between 50 µg/mL and 6 mg/mL. According to the investigated bacterial species, the MIC values varied from 62.5 µg/mL up to 6 mg/mL, while the minimal bactericidal concentration (MBC) values varied from 1.75 µg/mL to more than 6 mg/mL. The authors observed an important antimicrobial activity for the ethyl acetate (EA) and the total oligomer flavonoids (TOF) extracts, in addition to the most concentrated fraction in flavonoids obtained, the so-called A2 fraction [20].

In 2013, Kosalec and colleagues [8] investigated the antimicrobial activity of R. alaternus in addition to three other Rhamnus species. The methanolic bark extracts obtained by ultrasonic assisted extraction was tested against various bacterial strains: Escherichia coli ATCC 10535, Staphylococcus aureus ATCC 6538, Microsporum gypseum MFBF 3, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 10231, and Aspergillus niger ATCC
The MIC values were determined using the micro-dilution method, with ethanol 96% as negative control; all extracts from *Rhamnus species*, notably the *R. alaternus* one, exhibited an interesting antimicrobial activity against the previous microorganisms.

More recently, Zeouk et al. (2019) investigated the antimicrobial activity of *Rhamnus alaternus* [127] against various staphylococcal strains. After the ethanolic maceration, leaves’ extracts were investigated in a range of concentration varying between 0.5 and 16 mg/mL; with other antibiotics in the strain’s antibiogram, the ampicillin (100 µg/mL) and distillated water were respectively used as positive and negative control. With various resistance intensity, this former extract showed anti-staphylococcal activity notably against *Staphylococcus epidermidis* strains, *Staphylococcus aureus*, and its clinical isolate. Whatever the strain investigated, the MIC values varied between 0.5 and 2 mg/mL.

From Table 1, we can conclude that the aerial parts extracts (i.e., leaves, bark, and berries), obtained by Soxhlet extraction, showed an interesting number of bioactive compounds with the highest antimicrobial activity against various bacterial strains.

5.2.5. Toxicity

Cytotoxicity is defined as the property of a chemical or a biological agent to be toxic towards cells [128]. Hence, cytotoxicity tests are very important in the biomedical field [129]. The cytotoxicity of various extracts and fractions from *Rhamnus alaternus* plant was investigated on the human chronic myelogenous K562 cells and the murine lymphocytic L1210 leukemia cells, using the 3(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay [22]. In the presence of *R. alaternus* leaves and roots extract, former authors observed variations in the measured absorption of blue formazan produced from the MTT reduction by the mitochondrial dehydrogenase. Thus, these extracts have not a cytotoxic response. On the contrary, the cytotoxicity of leaves extract was lower than the one of roots extract on K562 and L1210 cells line.

In 2015, Boussahel and colleagues evaluated the cytotoxicity of bark extracts of *Rhamnus alaternus* on the human leukemia U937 cells and peripheral blood mononuclear cells (PBMCs), using the trypan blue assay. The bark methanolic extract was very toxic for U937 cells compared to the bark aqueous extract obtained by maceration and the infusion bark extract, the IC₅₀ values measured being of 6.39, 76.74, and 84.65 µg/mL, respectively. In comparison, the value of Taxol, a reference cytotoxic compound, was 2.47 µg/mL. Besides, the extracts of *R. alaternus* were not toxic for normal PBMC cells line, when the methanolic, aqueous and traditional extracts had an IC₅₀ value of 220.35, 38.81, and 29.35 µg/mL, respectively [21].

In 2015, a Tunisian man diagnosed with renal failure and rhabdomyolysis was intoxicated by the daily drink of *Rhamnus alaternus* roots solution for six months, which was used as a traditional treatment of diabetes [57]. Hence, Elyebderi and colleagues (2017) classified *Rhamnus alaternus* as a toxic plant at certain conditions of use in folk medicine, such as its daily consumption of leaves infusion [130].

However, the cytotoxic effect of crude methanolic extract (CME) of *Rhamnus alaternus*’ leaves was investigated on hepatic cells (HepG2) by the lactate dehydrogenase (LDH) release assay [23]. In this latter study, the CME, used up to 500 µg/mL, did not exhibit any toxicity effect.

5.2.6. Antigenotoxic Activity

The genotoxicity describes the damages of the genetic information inside cells, caused by chemical agents, able to induce cancer. The genotoxicity assays, either carried out in vitro and/or in vivo, aim to discover biomolecules, which can reduce damages on cells [131,132]. Thus, the antigenotoxic activity represents the potential of a bioactive compound to reduce or inhibit the DNA damage generated by various genotoxic agents such as alkylating and exogenous agents [133].
The genotoxicity was measured by the Son of Sevenless (SOS) chromotest microplate assay using the *E. coli* strain [134–136]. This quantitative bacterial colorimetric assay enables to determine the antigenotoxicity effect of the plant extracts against the toxic effect induced by the aflatoxin B1 [134,137,138].

In 2008, Ben Ammar and colleagues investigated the antigenotoxic activity of *R. alaternus*’ leaves extracts obtained by Soxhlet extraction on *Escherichia coli* PQ37 strain [53]. First, they injected the mutagens dose of aflatoxin B1 (AFB1) and nifuroxazide at 10 and 20 µg/assay, respectively. The aflatoxin was used as positive control at 10 µg/assay while the negative control did not contain neither AFB1 nor extracts. No toxicity was observed on *Escherichia coli* PQ37 strain since these concentrations were just below the ultimate genotoxic effect. Besides, the nifuroxazide induction factor (IF) decreased down to 46.6% in the presence of *R. alaternus* extracts with SOS chromotest [53]. Thus, *R. alaternus* leaves extracts using Soxhlet technique contain bioactive compounds with antigenotoxic properties, which contribute to the inhibitory effect of mutagens.

In another study, Ben Ammar and colleagues observed that *R. alaternus* aerial part extracts reduced the genotoxicity induced by AFB1 and nifuroxazide mutagens used at 10 and 20 µg/assay, respectively. The high genotoxic-reducing percentage was comprised between 79% and 90% for the three extract concentrations investigated (10, 50, and 250 µg/assay). The AFB1 mutagen was used as positive control, while the negative control did not contain neither extracts nor mutagen AFB1 [58]. In 2011, Bhouri and coworkers evaluated the antigenotoxic activity of two flavonoids isolated from *R. alaternus* leaves using Soxhlet extraction (i.e., kaempferol 3-O-β-isorhamninoside and rhamnocitrin 3-O-β-isorhamninoside), on *E. coli* PQ37 [24]. Former authors led the SOS chromotest with two positive controls—i.e., nifuroxazide and aflatoxin B1—used at 10 µg/assay and 5 µg/assay, respectively. The assay carried out in absence of both aflatoxin B1 and extracts constituted the negative control. For the three flavonoid concentrations studied (1, 5, and 10 µg/assay), the antigenotoxic activity of rhamnocitrin 3-O-β-isorhamninoside (Figure S2(14)) was higher than the one determined for kaempferol 3-O-β-isorhamninoside (Figure S2(13)).

According to the studies, which focused on the antigenotoxic activity of various parts from *R. alaternus*, a good antigenotoxic activity was present in leaves extracts from *Rhamnus alaternus* using Soxhlet extraction.

### 5.2.7. Antimutagenic Activity

The antimutagen agents reduce the genotoxic activity of mutagens such as intercalating and deaminating agents, which can increase the rate of mutation into cells [139–141]. The antimutagenic activity of roots and leaves extracts from *Rhamnus alaternus* was determined by the biological Ames assay in order to determine the mutagenic potential of its compounds with sodium azide mutagen [142,143].

The antimutagenicity test of roots and leaves’ extracts obtained from *R. alaternus* maceration was carried out in the presence of two strains of *Salmonella Typhimurium* (i.e., TA1535 and TA100) and with a sodium azide concentration of 1.5 µg/plate as mutagen inductor. Sodium azide and spontaneous revertants were used as controls [22]. These *R. alaternus* extracts indicated a result of 434 ± 5 and 51 ± 3 revertants/plate for roots, and 362 ±6 and 442 ±7 revertants/plate for leaves extracts with TA1535 and TA100 strains, respectively. The incubation of various doses of *R. alaternus* extracts (i.e., roots, leaves) with *S. typhimurium* TA100 strain evidenced that the leaves’ extract was more efficient to reduce the sodium azide-induced mutagenicity than the root’s one. The incubation of the *Rhamnus alaternus* roots and leaves extracts at the concentration of 5 µg/plate was also investigated in the presence of the *Salmonella Typhimurium* TA1535 strain. For this latter bacterial strain, the roots extract reduced more significantly the sodium azide-induced mutagenicity than the leave’s extract used at the same concentration.

In another study [51], the authors investigated the antimutagenic activity of leaves extracts by the Ames assay, using the mutagen Aflatoxin B1 (AFBI) at a concentration of
10 μg/plate. This former experiment was carried out with two strains of *Salmonella Typhimurium* (i.e., TA98 and TA100) in the presence of various extracts, and spontaneous revertant was used as control. Petroleum ether, chloroform, methanol, aqueous, and total oligomers flavonoids (TOF) extracts obtained by *R. alaternus* maceration were investigated at various doses (10, 50, and 250 μg/plate) and remarkably reduced the AFB1-induced mutagenicity. In this study, the ethyl acetate fraction obtained from the *R. alaternus* aqueous extract was the most effective at a dose of 250 μg/plate. At such dose, the inhibition percentage of mutagenicity was determined by the Ames assay up to 78% for the TA98 strain.

The studies carried out by Ben Ammar and colleagues [22,51] showed that leaves, bark, and roots extracts obtained by maceration or Soxhlet extraction present a good antimutagenic activity.

6. Conclusions

For the first time, to the author’s knowledge, the various extraction processes applied to *Rhamnus alaternus* in order to obtain bioactive compounds were reviewed here, and related to their biological activities. In this present work, we discuss relevant information concerning this plant, its pharmacological effects, phytochemical profiles, and cytotoxicity of different parts of *R. alaternus* when extracted using different processes (i.e., maceration, decoction, Soxhlet, ultrasonic assisted extraction, and hydrodistillation). Furthermore, the antioxidant, antigenotoxic, and antimicrobial activities of *R. alaternus* were reported. Besides, the natural substances isolated and identified from this plant were presented. *R. alaternus* contains many phytochemical compounds (i.e., flavonoids, tannins, and anthocyanins), which are endowed with important medicinal potentials. These aforementioned compounds render the *Rhamnus alaternus* a suitable source to be used for its natural therapeutic substances. In addition, a large number of pharmacological studies are still insufficient to determine the effects and beneficial therapeutic properties of *R. alaternus*. Yet, this plant may help in the discovery of new bioactive substances for treatment of various digestive diseases and health problems.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s2, Figure S1: Extraction processes commonly applied on *R. alaternus*. Figure S2: Biomolecules found in *Rhamnus alaternus*. Table S1: name of compounds of figure S2.

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**References**

1. Stocker, P.; Yousfi, M.; Djerridane, O.; Perrier, J.; Amziani, R. Effect of flavonoids from various Mediterranean plants on enzymatic activity of intestinal carboxylesterase. *Biochimie* 2004, 86, 919–925, doi:10.1016/j.biochi.2004.09.005.
2. Lu, C.M.; Yang, J.J.; Wang, P.Y.; Lin, C.C. A new acylated flavonol glycoside and antioxidant effects of Hedyotis diffusa. *Planta Med.* 2000, 66, 374–377, doi:10.1055/s-2000-8544.
3. Mai, L.P.; Dumontet, V.; Van Tri, M.; Hill, B.; Thoison, O.; Se, T. Cytotoxicity of Rhamnosylantheraquinones and Rhamnosylanthrone from Rhamnus nepalensis. *J. Nat. Prod.* 2001, 64, 1162–1168, doi:10.1021/np010030v.
4. Locatelli, M.; Epifano, F.; Genovese, S.; Carlucci, G.; Končić, M.Z.; Kosalec, I.; Kremer, D. Anthraquinone profile, antioxidant and antimicrobial properties of bark extracts of Rhamnus catharticus and R. orbiculatus. *Nat. Prod. Commun.* 2011, 6, 1275–1280, doi:10.1179/1934578x1100600917.

5. Coskun, M.; Satake, T.; Horii, K.; Saiki, Y.; Tanker, M. Anthraquinone glycosides from Rhamnus Libanoticus. *Phytochemistry* 1990, 29, 2018–2020, doi:10.1016/0031-9422(80)90650-S.

6. Chermat, S.; Ghazrouli, R. Ethnobotanical Study of Medicinal Flora in the North East of Algeria - An Empirical Knowledge in Djebel Zdimm (Setif). *J. Mater. Sci. En. A* 2015, 5, 50–59, doi:10.17265/2161-6213/2015.1.2.007.

7. Marzouk, M.S.; El-Toumy, S.A.A.; Merfort, I.; Nawwar, M.A.M. Polyphenolic metabolites of Rhamnus disperma. *Phytochemistry* 1999, 52, 943–946, doi:10.1016/S0031-9422(99)00262-9.

8. Kosalec, I.; Kremer, D.; Locatelli, M.; Epifano, F.; Genovese, S.; Carlucci, G.; Randić, M.; Zovko Končić, M. Anthraquinone profile, antioxidant and antimicrobial activity of bark extracts of Rhamnus alaternus, R. fallax, R. intermedia and R. pumila. *Food Chem.* 2013, 136, 335–341, doi:10.1016/j.foodchem.2012.08.026.

9. Locatelli, M.; Genovese, S.; Carlucci, G.; Kremer, D.; Randić, M.; Epifano, F. Development and application of high-performance liquid chromatography for the study of two new oxyprrenylated anthraquinones produced by Rhamnus species. *J. Chromatogr. A* 2012, 1225, 113–120, doi:10.1016/j.chroma.2011.12.085.

10. Lu, T.M.; Ko, H.H. A new anthraquinone glycoside from Rhamnus nakaharai and anti-tyrosinase effect of 6-methoxysorigenin. *Nat. Prod. Res.* 2016, 30, 2655–2661, doi:10.1080/14786419.2016.1138300.

11. Gonçalves, R.S.; Silva, E.L.; Hioka, N.; Nakamura, C.V.; Bruschi, M.L.; Caetano, W. An optimized protocol for anthraquinone isolation from Rhamnus frangula L. *Nat. Prod. Res.* 2018, 32, 366–369, doi:10.1080/14786419.2017.1356836.

12. Genovese, S.; Epifano, F.; Curini, M.; Kremer, D.; Carlucci, G.; Locatelli, M. Screening for oxyprrenylated anthraquinones in Mediterranean Rhamnus species. *Biochem. Syst. Ecol.* 2012, 43, 125–127, doi:10.1016/j.bse.2012.03.001.

13. Bas, J.M.; Oliveras, J.; Gómez, C. Myrmecochory and short-term seed fate in Rhamnus alaternus: Ant species and seed characteristics. *Acta Oecologica* 2009, 35, 380–384, doi:10.1016/j.actao.2009.02.003.

14. Tshabar, E.; Friedman, J.; Izhaki, I. Impact on Fruit Removal and Seed Predation of a Secondary Metabolite, Emodin, in Rhamnus alaternus. *Food Chem.* 2002, 79, 290–299, doi:10.1016/S0308-8146(02)00290-x.

15. Arroyo, J.M.; Rodríguez, R.; Rigueiro, C.; Hampe, A.; Jordano, P. Isolation and characterization of 12 microsatellite loci for Rhamnus alaternus (Rhamnaceae). *Mol. Ecol. Resour.* 2009, 9, 1214–1216, doi:10.1111/j.1755-0998.2009.02616.x.

16. Ben Ammar, R.; Kilani, S.; Bouhlel, I.; Skandrani, I.; Naffeti, A.; Boubaker, J.; Ben Sghaier, M.; Bhouri, W.; Mahmoud, A.; Chekir-Ghedira, L.; et al. Antibacterial and cytotoxic activities of extracts from (Tunisian) Rhamnus alaternus L.: Combination with the phytochemical composition. *Drug Chem. Toxicol.* 2008, 31, 61–80.

17. Canale, A.; Benvenuti, S.; Raspi, A.; Benelli, G. Insect pollinators of the late winter flowering Rhamnus alaternus L., a candidate for honeybee-friendly scrubland spots in intensively managed agricultural areas. *Plant Biosyst.* 2014, 128, 37–41, doi:10.1080/11263504.2014.993742.

18. Longo, L.; Vasapollo, G.; Rescio, L. Identification of anthocyanins in Rhamnus alaternus L. berries. *J. Agric. Food Chem.* 2005, 53, 1723–1727, doi:10.1021/jf048253p.

19. Miralles, J.; Martínez-Sánchez, J.J.; Franco, J.A.; Bañón, S. Rhamnus alaternus growth under four simulated shade environments: Morphological, anatomical and physiological responses. *Sci. Hortic. (Amst.)* 2011, 127, 562–570, doi:10.1016/j.scienta.2010.12.005.

20. Ben Ammar, R.; Kilani, S.; Bouhlel, I.; Skandrani, I.; Naffeti, A.; Boubaker, J.; Ben Sghaier, M.; Bhouri, W.; Mahmoud, A.; Chekir-Ghedira, L.; et al. Antibacterial and cytotoxic activities of extracts from (Tunisian) Rhamnus alaternus (Rhamnaceae). *Ann. Microbiol.* 2007, 57, 453–460, doi:10.1080/07345910701370598.

21. Boussahel, S.; Speciale, A.; Dahamaha, S.; Amar, Y.; Bonaccorsi, I.; Cacciola, F.; Cimino, F.; Donato, P.; Ferlazzo, G.; Harzallah, D.; et al. Flavonoid profile, antioxidant and cytotoxic activity of different extracts from Algerian Rhamnus alaternus L. bark. *Pharmacogn. Mag.* 2015, 11, S102–S109, doi:10.4103/0973-1296/2015.1255707.

22. Ammar, B.; Kilani, S.; Bouhlel, I.; Ezzi, L.; Skandrani, I.; Boubaker, J.; Ben Sghaier, M.; Naffeti, A.; Mahmoud, A.; Chekir-Ghedira, L.; et al. Antiproliferative, antioxidant, and antimutagenic activities of flavonoid-enriched extracts from (Tunisian) Rhamnus alaternus L.: Combination with the phytochemical composition. *Drug Chem. Toxicol.* 2008, 31, 61–80.

23. Tcherassiout, M.; Petrov, P.D.; Mattonai, M.; Ribeichini, E.; Ribot, J.; Bonet, M.L.; Khettal, B. Antihyperlipidemic effect of a Rhamnus alaternus leaf extract in Triton-induced hyperlipidemic rats and human HepG2 cells. *Biomed. Pharmacother.* 2018, 101, 501–509, doi:10.1016/j.biopha.2018.02.106.

24. Bhouri, W.; Ben Sghaier, M.; Kilani, S.; Bouhlel, I.; Dijoux-Franca, M.G.; Ghedira, K.; Chekir Ghedira, L. Evaluation of antioxidant and antigenotoxic activity of two flavonoids from Rhamnus alaternus L. (Rhamnaceae): Kaempferol 3-O-β-isorhamminoside and rhamnocitrin 3-O-β-isorhamminoside. *Food Chem. Toxicol.* 2011, 49, 1167–1173, doi:10.1016/j.fct.2011.02.011.

25. Martínez-Sánchez, A.; Gil-Izquierdo, A.; Gil, M.I.; Ferreres, F. A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. *J. Agric. Food Chem.* 2008, 56, 2330–2340, doi:10.1021/jf072975z.

26. Ferriol, M.; Llorens, L.; Gil, L.; Boira, I. Influence of phenological barriers and habitat differentiation on the population genetic structure of the balearic endemic Rhamnus ludovici-salvatoris Chodat and R. alaternus L. *Plant Syst. Evol.* 2009, 277, 105–116, doi:10.1007/s00606-008-0110-3.

27. Penzig, O. *Flore Coloriée de Poche du Littoral Méditerranéen de Gênes à Barcelone y Compris la Corse*; KlinkPRESS, P., Ed.; Librairie Paris: Paris, France, 1902;
Antioxidants 2021, 10, 300
90. Inoue, T.; Hayashi, M.; Takayanagi, K.; Morooka, S. Lipid-lowering therapy with fluvastatin inhibits oxidative modification of low density lipoprotein and improves vascular endothelial function in hypercholesterolemic patients. *Atherosclerosis* **2002**, *160*, 369–376, doi:10.1016/S0021-9150(00)588-8.

91. Ma, Y.; Jiang, C.; Yao, N.; Li, Y.; Wang, Q.; Fang, S.; Shang, X.; Zhao, M.; Che, C.; Ni, Y.; et al. Antihyperlipidemic effect of *Cylocarya paliurus* (Batal.) Iljinskaia extract and inhibition of apolipoprotein B48 overproduction in hyperlipidemic mice. *J. Ethnopharmacol.* **2015**, *166*, 285–296, doi:10.1016/j.jep.2015.03.030.

92. Shattat, G.F. A review article on hyperlipidemia: Types, treatments and new drug targets. *Biomed. Pharmacol. J.* **2014**, *7*, 399–409, doi:10.13055/bpj/504.

93. Rodès, J.; Benhamou, J.-P.; Blei, A.T.; Reichen, J.; Rizzetto, M.; Dufour, J.-F.; Friedman, S.L.; Ginès, P.; Valla, D.-C.; Zoulim, F.; et al. *Textbook of Hepatology: From Basic Science to Clinical Practice*, 3rd ed; Oxford University Press; Blackwell Publishing Ltd: Oxford, UK, 2007; ISBN 9788578110796.

94. Asztalos, B.F.; Schaefer, E.J. HDL in atherosclerosis: Actor or bystander? *Atheroscler. Suppl.* **2003**, *4*, 21–29, doi:10.1016/S1567-56800006-0.

95. National Cholesterol Education Program NCEP, *Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report.* "Circulation," *106*, p. 3143; Volume 106, American Medical Association., Ed., Bethesda, USA, 2002.

96. Carlos, L.C.; Diego, A.S.; David, S.S.; Mahendra, R. *Natural Antioxidants and Biocides from Wild Medicinal Plants*; CAB: London, UK, 2013; ISBN 9781780642338.

97. Sharma, O.P.; Bhat, T.K. DPPH antioxidant assay revisited. *Food Chem.* **2009**, *113*, 1202–1205, doi:10.1016/j.foodchem.2008.08.008.

98. Gupta, D.K.; Palma, J.M.; Corpas, F.J. *Antioxidants and Antioxidant Enzymes in Higher Plants*; Springer Nature Switzerland AG, Ed., Gewerbestrasse: Cham, Switzerland, 2018; ISBN 9783319750880.

99. Atta, E.M.; Mohamed, N.H.; Abdelgawad, A.A.M. Antioxidants: An Overview on the Natural and Synthetic Types. *Eur. Chem. Bull.* **2017**, *6*, 365, doi:10.17628/ecb.2017.6.365-375.

100. Marc, F.; Davin, A.; Deglène-benbrahim, L.; Ferrand, C.; Bacaunaud, M.; Fritsch, P.; Marc, F.; Davin, A.; Deglène-benbrahim, L.; Ferrand, C.; et al. Studies of several analytical methods for antioxidant potential evaluation in food. *M / S Médecine Sci.* **2004**, *20*, 458–463, doi:10.1051/medsci/2004204158.

101. Krishnaiah, D.; Sarbatly, R.; Nithyanandam, R. A review of the antioxidant potential of medicinal plant species. *Food Bioprod. Process.* **2010**, *9*, 217–233, doi:10.1016/j.fbp.2010.04.008.

102. Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* **2006**, *97*, 654–660, doi:10.1016/j.foodchem.2005.04.028.

103. Montoro, P.; Braca, A.; Pizza, C.; De Tommasi, N. Structure-antioxidant activity relationships of flavonoids isolated from different plant species. *Food Chem.* **2005**, *92*, 349–355, doi:10.1016/j.foodchem.2004.07.028.

104. Nenadis, N.; Wang, L.F.; Tsimidou, M.Z.; Zhang, H.Y. Radical scavenging potential of phenolic compounds encountered in *O. europaea* products as indicated by calculation of bond dissociation enthalpy and ionization potential values. *J. Agric. Food Chem.* **2005**, *53*, 295–299, doi:10.1021/jf049776x.

105. Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774–2779, doi:10.1021/jf0101413m.

106. Susanti, D.; Sirat, H.M.; Ahmad, F.; Ali, R.M.; Aimi, N.; Kitajima, M. Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L. *Food Chem.* **2006**, *97*, 710–716, doi:10.1016/j.foodchem.2006.09.011.

107. Spector, A. Review: Oxidative Stress and Disease. *J. Ocul. Pharmacol. Ther.* **2000**, *16*, 193–201, doi:10.1089/jop.2000.16.193.

108. Dziedzic, S.Z.; Hudson, B.J.F. Phenolic acids and related compounds as antioxidants for edible oils. *Food Chem.* **1984**, *14*, 45–51, doi:10.1016/0308-814690017-7.

109. Zheng, W.; Wang, S.Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **2001**, *49*, 5165–5170, doi:10.1021/jf0100413m.

110. Lisbeth, A.; Noratto, G.; Hingorani, L.; Talcott, S.T.; Mertens-Talcott, S.U. Protective effects of standardized pomegranate (Punica granatum L.) polyphenolic extract in ultraviolet-irradiated human skin fibroblasts. *J. Agric. Food Chem.* **2008**, *56*, 8434–8441, doi:10.1021/jf0705307.

111. Chen, L.B.; Hu, J.Y.; Wang, S.Q. The role of antioxidants in photoprotection: A critical review. *J. Am. Acad. Dermatol.* **2012**, *67*, 1013–1024, doi:10.1016/j.jaad.2012.02.009.

112. Tomaino, A.; Martorana, M.; Arcoraci, T.; Monteleone, D.; Giovinazzo, C.; Saija, A. Biochimie Antioxidant activity and phenolic pro fi le of pistachio (Pistacia vera L., variety Bronte) seeds and skins. *Biochimie* **2010**, *92*, 1115–1122, doi:10.1016/j.biochi.2010.03.027.

113. Plaa, G.L.; Witshi, H. Chemicals, drugs, and lipid peroxidation. *Annu. Rev. Pharmacol. Toxicol.* **1976**, *16*, 125–142, doi:10.1146/annurev.pa.16.040176.001013.

114. Longo, L.; Vasapollo, G.; Rescio, L.; Cuoco, G.; Mathe, C.; Vieillescazhes, C.; Bhouri, W.; Ben Sghairer, M.; Kilani, S.; Bouhlel, I.; et al. Critical stages in the recruitment process of Rhamnus alaternus L. *Acta Oecologica* **2011**, *37*, 1167–1173, doi:10.1016/j.actao.2010.04.004.

115. World Health Organization. *World Health Statistics Annual 1996*; World Health Organization, Geneva, Switzerland, 1998.
105. Cianciosi, D.; Forbes-Hernández, T.Y.; Ansary, J.; Gil, E.; Amici, A.; Bompadre, S.; Simal-Gandara, J.; Giampieri, F.; Battino, M. Phenolic compounds from Mediterranean foods as nutraceutical tools for the prevention of cancer: The effect of honey polyphenols on colorectal cancer stem-like cells from spheroids. *Food Chem.* 2020, doi:10.1016/j.foodchem.2020.126881.

106. Ghellab, S.E.; Han, X. Lipid tubes formation induced by electroosmotic flow. *Chem. Phys. Lett.* 2018, 706, 515–519, doi:10.1016/j.cplett.2018.06.058.

107. Peterson, J.; Dwyer, J.; Stern, F.; England, N.; Mayer, J. Flavonoids: Dietary occurrence and biochemical activity. *Nutr. Res.* 1998, 18, 1995–2018, doi:10.1016/S0271-5370(98)00094-3.

108. Kim, J.H.; Kim, D.; Kim, J.; Hwang, J.K. Eucheversia horstfeldii Benn. activates peroxisome proliferator-activated receptor α and regulates expression of genes involved in fatty acid metabolism in human HepG2 cells. *J. Ethnopharmacol.* 2011, 133, 244–247, doi:10.1016/j.jep.2010.09.029.

109. Ghellab, S.E.; Li, Q.; Fuhs, T.; Bi, H.; Han, X. Electroformation of double vesicles using an amplitude modulated electric field. *Colloids Surf. B Biointerfaces* 2016, 160, 697–703, doi:10.1016/j.colsurfb.2017.10.025.

110. Chen, G.; Wu, J.; Li, N.; Guo, M. Screening for anti-proliferative and anti-inflammatory components from Rhamnus davurica Pall. using bio-affinity ultrafiltration with multiple drug targets. *Avail. Bioanal. Chem.* 2018, 410, 3587–3595, doi:10.1007/s00216-018-0953-6.

111. He, X.; Rui, H.L. Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple’s anticancer activity. *J. Agric. Food Chem.* 2007, 55, 4366–4370, doi:10.1021/jf063563o.

112. Ueda, J.Y.; Tezuka, Y.; Banskota, A.H.; Le Tran, Q.; Tran, Q.K.; Harimaya, Y.; Saiki, I.; Kadota, S. Antiproliferative activity of Vietnamese medicinal plants. *Biol. Pharm. Bull.* 2002, 25, 753–760, doi:10.1248/bpb.25.753.

113. Chen, G.; Li, X.; Saleri, F.; Guo, M. Analysis of flavonoids in Rhamnus davurica and its antiproliferative activities. *Molecules* 2016, 21, 1275, doi:10.3390/molecules2112175.

114. Dantas-Santos, N.; Almeida-Lima, J.; Vidal, A.A.J.; Gomes, D.L.; Oliveira, R.M.; Pedrosa, S.S.; Pereira, P.; Gama, F.M.; Rocha, H.A.O. Antiproliferative activity of fucan nanogel. *Mar. Drugs* 2012, 10, 2002–2022, doi:10.3390/md10092002.

115. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 1983, 65, 55–63, doi:10.1016/0022-1759(83)90022-4.

116. Michael, T.M.; John, M.M.; David, A.S.; David, P.C. *Brock Biology of Microorganisms*; 13th ed; Pearson Education., Ed., San Francisco, CA, USA, 2012; ISBN 9780321649638.

117. Heinrich, M.; Barnes, J.; Gibbons, S.; Williamson, E.M. *Fundamentals of Pharmacognosy and Phytotherapy*; Chirchil, L., Ed.; Elsevier: Edinburgh, United Kingdom, 2004.

118. Gibbons, A. Exploring New Strategies to Fight Drug-Resistant Microbes. *Science* 1992, 257, 1036–1038.

119. Martini, N.D.; Katerere, D.R.; Eloff, J.N. Biological activity of five antibacterial flavonoids from Combretum erythrophyllum (Combretaceae). *J. Ethnopharmacol.* 2004, 93, 207–212, doi:10.1016/j.jep.2004.02.030.

120. Al-Qura’n, S. Ethnopharmacological survey of wild medicinal plants in Showbak, Jordan. *J. Ethnopharmacol.* 2009, 123, 45–50, doi:10.1016/j.jep.2009.02.031.

121. Arora, D.S.; Ohlan, D. In vitro studies on antifungal activity of tea (Camellia sinensis) and coffee (Coffea arabica) against wood-rotting fungi. *Basic Microbiol.* 1997, 37, 159–165, doi:10.1002/jobm.3620370302.

122. Cushnie, T.P.T.; Lamb, A.J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 2005, 26, 343–356, doi:10.1016/j.ijantimicag.2005.09.002.

123. Rios, J.L.; Recio, M.C. Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.* 2005, 100, 80–84, doi:10.1016/j.jep.2005.04.025.

124. Valgas, C.; De Souza, S.M.; Smânia, E.F.A.; Smânia, A. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.* 2007, 38, 369–380, doi:10.1590/S1517-83822007000200034.

125. Michael, T.M.; Kelly, S.B.; Daniel, H.B.; W., M.S.; David, A.S. *Brock Biology of Microorganisms*; Global Edition; Pearson: London, UK, 2019; ISBN 978129235103.

126. Djenane, D.; Yangüela, J.; Derriche, F.; Bouarab, L. Utilisation des composés de feuilles d’ olivier comme agents antimicrobiens ; application pour la conservation de la viande fraîche de dinde. *Nat. Technol.* 2012, 7, 53–61.

127. Zeouk, I.; El Oouali Lalami, A.; Bekhti, K. In Vitro Antibacterial Activity of Medicinal Plants in the Central North of Morocco: A Possible Source of Alternative Drugs Against Methicillin-Resistant Staphylococcus Aureus. *Asian J. Pharm. Clin. Res.* 2019, 12, 285–292, doi:10.22159/ajpcr.2019.v12i3.30395.

128. Li, L.; Mak, K.Y.; Shi, J.; Koon, H.K.; Leung, C.H.; Wong, C.M.; Leung, C.W.; Mak, C.S.K.; Chan, N.M.M.; Zhong, W.; et al. Comparative in vitro cytotoxicity study on uncoated magnetic nanoparticles: Effects on cell viability, cell morphology, and cellular uptake. *J. Nanosci. Nanotechnol.* 2012, 12, 9010–9017, doi:10.1166/jnn.2012.6755.

129. Mukherjee, P.K. *Quality Control and Evaluation of Herbal Drugs: Evaluating Natural Products and Traditional Medicine*; Elsevier: Amsterdam, The Netherlands, 2019; ISBN 9780128133989.

130.ELYEBDRI, N.; BOUAREB, A.; ITOUZ, S. Ethnobotanical Study on the Usage of Toxic Plants in Traditional Medicine in the City of Tlemcen ,. *Int. Sch. Sci. Res. Innov.* 2017, 11, 642–646, doi:10.5281/zenodo.1132779.

131. Dhand, A.; Baijayee, M. *Genotoxicity Assessment Methods and Protocols*; Springer: London, UK, 2013; ISBN 9781627035286.

132. Jena, G.B.; Kautil, C.L.; Ramarao, P. Genotoxicity testing, a regulatory requirement for drug discovery and development: Impact of ICH guidelines. *Indian J. Pharmacol.* 2002, 34, 86–99.
133. Swift, L.H.; Golsteyn, R.M. Genotoxic Anti-Cancer Agents and Their Relationship to DNA Damage, Mitosis, and Checkpoint Adaptation in Proliferating Cancer Cells. *Int. J. Mol. Sci.* 2014, 15, 3403–3431, doi:10.3390/ijms15033403.

134. Quillardet, P.; Hofnung, M. The SOS Chromotest, a colorimetric bacterial assay for genotoxins: Procedures. *Mutat. Res. Mutagen. Relat. Subj.* 1985, 147, 65–78, doi:10.1016/0165-1161(90)90020-2.

135. Quillardet, P.; Huisman, O.; D’Ari, R.; Hofnung, M. SOS chromotest, a direct assay of induction of an SOS function in Escherichia coli K-12 to measure genotoxicity. *Proc. Natl. Acad. Sci. USA* 1982, 79, 5971–5975, doi:10.1073/pnas.79.19.5971.

136. Kocak, E. Investigation of potential genotoxic activity using the SOS Chromotest for real paracetamol wastewater and the wastewater treated by the Fenton process. *J. Environ. Heal. Sci. Eng.* 2015, 13, 1–5, doi:10.1186/s40201-015-0220-0.

137. Mokdad-Bzeouich, I.; Kilani-Jaziri, S.; Mustapha, N.; Bedoui, A.; Ghedira, K.; Chekir-Ghedira, L. Evaluation of the antimutagenic, antigenotoxic, and antioxidant activities of Eriobotrya japonica leaves. *Pharm. Biol.* 2015, 53, 1786–1794, doi:10.3109/13880209.2015.1008145.

138. Chabchoub, F.; Messaâd, M.; Ben Mansour, H.; Chekir-Ghedira, L.; Salem, M. Synthesis and antigenotoxic activity of some naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine derivatives. *Eur. J. Med. Chem.* 2007, 42, 715–718, doi:10.1016/j.ejmech.2006.12.002.

139. Berhow, M.A.; Wagner, E.D.; Vaughn, S.F.; Plewa, M.J. Characterization and antimutagenic activity of soybean saponins. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* 2000, 448, 11–22, doi:10.1016/S0027-5107(00)00225-0.

140. Yen, G.C.; Chen, H.Y. Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. *J. Agric. Food Chem.* 1995, 43, 27–32, doi:10.1021/jf00049a007.

141. Waters, M.D.; Stack, H.F.; Jackson, M.A.; Brockman, H.E.; De Flora, S. Activity profiles of antimutagens: In vitro and in vivo data. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* 1996, 350, 109–129, doi:10.1016/0027-5107(96)00097-6.

142. Maron, D.M.; Ames, B.N. Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* 1983, 113, 173–215, doi:10.1016/0165-1161(90)90010-9.

143. Zani, F.; Cuzzoni, M.T.; Daglia, M.; Benvenuti, S.; Vampa, G.; Mazza, P. Inhibition of Mutagenicity in *Salmonella typhimurium* by Glycyrrhiza glabra: Extract, glycyrrhizinic acid, 18α- and 18β-glycyrrhetinic acids. *Planta Med.* 1993, 59, 502–507, doi:10.1055/s-2006-959748.