

## RHAMNACEAE TREES OF ISRAEL AND PALESTINE – NUTRITION, MEDICINE AND CHEMISTRY

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### ABSTRACT

The trees of the Rhamnaceae family are part of sights of Eastern Mediterranean region and have been used by inhabitants of today's Israel and Palestine since ancient times. In this review article we will introduce the taxonomical affiliations of this family and its genera, as well as the ethnomedicinal and nutritional uses of the nine species that naturally grow in this region. The main part of this article will be briefly listing the findings of modern research of the medicinal, biological and nutritional properties of these trees, and extensive discussion will follow these findings. Structures of active phytochemicals will be presented in figures. It can be concluded here that there are significant differences in the number of published studies related to each one of the species. Finally, even though it is considered

a domesticated species, selected activities of *Ziziphus jujuba* will be introduced for comparison and completion of this presentation.

**KEYWORDS:** Chemical composition, Plant extract, Antimicrobial, Antioxidant, nutrition, Anti-inflammatory, Growth conditions, Phytoremediation, Ethnomedicine, Nanoparticles.

### 1. INTRODUCTION

Rhamnaceae (Buckthorns in English, النبقيات Arabic, אשחריים Hebrew) is one of the largest plant families and it is mainly consisted of trees, as in our reviewed region, Israel and Palestine, where nine tree species are found in the wild. The Rhamnaceae has worldwide distribution, excluding both poles and Asian and African central deserts, and include 54 genera and 900 species.<sup>[1]</sup> But these numbers are debated: 50 and 900,<sup>[2]</sup> 55 and 950,<sup>[3]</sup> and other reported estimations.

Among the nine wild Rhamnaceae species found in the reviewed region, *Ziziphus spina-christi* is the most widespread and most used by humans since antiquity. Remains of its fruits were found in two temples in Sudan, dating back to 1500-1480 BC,<sup>[4]</sup> in Kuwait from a vast era of Bronze and Hellenistic ages,<sup>[5]</sup> and wood in a boat that belonged to King Khufu of ancient Egypt (reigned 2589-2566 BC).<sup>[6]</sup> Other archeological studies revealed even greater importance of this tree for ancient Egyptians. Some scholars found that it was named *Nebes* and its first mention was on a wall writing that shows food offering in the fifth dynasty, 2494-2345 BC.<sup>[7]</sup> In this publication, O. Kadioglu *ahc* (see list of abbreviations after **Table 1**) listed five papyri that mentioned *Z. spina-christi* for its medicinal uses, dating from 1900 to 300 BC. Most of these uses were based on consumption of fruits bread and mainly treated general weaknesses. R. Elshiwly concluded that *Z. spina-christi* was named *Nbs* in ancient Egypt and lists many uses of this tree at that time, wood, food and several herbal medicines.<sup>[8]</sup>

Moving from ancient times to more recent ethnomedicine reports, *Rhamnus alaternus* is used for treatment of jaundice and liver diseases, while *Ziziphus nummularia* is used to treat diarrhea, lung inflammation, congestion<sup>[9]</sup> and astringent.<sup>[10]</sup>

Of all the nine wild Rhamnaceae species found in Israel and Palestine, *Ziziphus spina-christi* is the most reported for use in traditional medicines and beyond that. A. Dafni *ahc* list more than thirty traditional medicine uses of this tree, as well as its social and religious importance for peoples of the Middle East.<sup>[11]</sup> M. Abou Auda listed this tree as an important plant for treatment of skin disorders,<sup>[12]</sup> A. Aati *ahc* considered it “vital” and it has decorative and shade benefits, in addition to having emotional, religious importance.<sup>[13]</sup> They mentioned large number (18) of ethnomedicinal uses of this tree, mainly in the Middle East, but also in other regions of the world. N. Jaradat and A. Zaid reported that flowers decoction of *Z. spina-christi* treats male infertility.<sup>[14]</sup> D.H. Feyssa *ahc* consider this tree essential for “people’s resilience to adapt to changing environment” in Ethiopia, due to its many uses of wood, nutrition and medicine, for humans and their livestock.<sup>[15]</sup>

Finally, the Rhamnaceae trees of Israel and Palestine are *Paliurus spina-christi*, *Rhamnus alaternus*, *Rhamnus disperma*, *Rhamnus libanotica*, *Rhamnus lycioides*, *Rhamnus punctata*, *Ziziphus lotus*, *Ziziphus nummularia* and *Ziziphus spina-christi*.

## 2. Selected published activities of rhamnaceae trees of Israel and Palestine

The biological-medicinal activities of the nine Rhamnaceae trees of Israel and Palestine are notably diverse. It is also notable that some species were extensively studied and published, while for others, a few publications can be found, if any. These properties and activities are summarized in **Table 1**.

**Table 1: Selected published activities of rhamnaceae trees of Israel and Palestine.**

Testing Method, Results and Reference/s
<p><b><i>Paliurus spina-christi</i></b></p> <p>Leaves and flowers were separately extracted with ethanol, and both extracts were moderately active against MCF-7 and MDA-MB-231 breast cancer cells, tested in various time periods and concentrations (total of 12 tests). The chemical compositions of the extracts were analyzed (GC-MS) and reported, and this part of the publication includes many mistakes. See section 3, <b>Discussion</b>.<sup>[16]</sup></p> <p>Fruits methanolic and ethanolic extracts were active against STZ-induced diabetes in rats. Chemical compositions of the extracts were determined by LC-MS and the major components were: rutin, catechin, hesperidin, quinic acid and malic acid.<sup>[17]</sup></p> <p>Fruits 70% aqueous methanolic extract was prepared and fractionized with <i>n</i>-hexane, chloroform, ethyl acetate and <i>n</i>-butanol. All extracts were tested for <math>\alpha</math>-amylase and <math>\alpha</math>-glucosidase inhibition, but only the <i>n</i>-hexane fraction had significant activity. The chemical composition of this fraction was analyzed and the main components were betulin, betulinic acid, lupeol and <math>\beta</math>-sitosterol (<b>Figure 1</b>. Figures mentioned in <b>Table 1</b> appear after <b>Abbreviations</b>). All four compounds inhibited the enzymes, especially betulinic acid. Acarbose was reference in this study.<sup>[18]</sup></p> <p>Aerial parts were extracted with 70% aqueous methanol and the extract was fractionized with petroleum ether, chloroform, ethyl acetate, and methanol. All products were tested for insulin activation in HepG2 insulin-resistant cells. Both crude extract and methanolic fraction were active, indicated by several biomarkers. Metformin was reference.<sup>[19]</sup></p> <p>Fruits water extract was prepared and analyzed for phenolic compounds. It had significant <i>in vitro</i> antioxidant activity (ABTS, CUPRAC, DPPH, FRAP methods) and <i>in vivo</i> measured by several biomarkers. It also had activity in STZ-induced diabetic rats.<sup>[20]</sup></p> <p>Fruits 70% aqueous methanolic extract was prepared and fractionized with <i>n</i>-hexane, ethyl acetate and <i>n</i>-butanol. Several phenolics were isolated from the ethyl acetate and <i>n</i>-butanol fractions, and these had antigenotoxicity V79 cell lines.<sup>[21]</sup></p> <p>Fruits aqueous extracts had lipid lowering activity in high cholesterol-fed STZ-induced diabetic rats.<sup>[22]</sup></p> <p>Fruits, leaves and branches were separately extracted with ethanol, and each extract was fractionized with <i>n</i>-hexane, chloroform, and ethyl acetate extract and 60% aqueous ethanol (total 15 extracts). All extracts except the <i>n</i>-hexane fractions had significant antioxidant activity (ABTS, DPPH methods), and most of them had anti-inflammatory activity measured by 5-LOX inhibition.<sup>[23]</sup></p> <p>Bark, flowers, fruits, leaves and roots were separately extracted with 90% aqueous ethanol, and from the leaves extract, a flavonoid-rich fraction was prepared. All six extracts were active against 6 bacteria species. Oxytetracycline was reference.<sup>[24]</sup></p> <p>Stems methanolic extract was prepared and found active against <i>Trichophyton</i></p>

*mentagrophytes* fungus. It was analyzed for chemical composition, compounds\* are listed but no concentrations or percentages are provided.<sup>[25]</sup>

Leaves methanolic extract had notable antioxidant activity (CUPRAC, DPPH, FRAP methods) and its TFC and TPC were determined. It had weak to moderate activities against 11 bacteria species, but strong activity against *Candida albicans*.<sup>[26]</sup>

Fruits and leaves were separately extracted with ethanol and water, and the four extracts had notable antioxidant activity (ABTS, CUPRAC, DPPH methods). BHT and Trolox were references. The had weak to moderate activities against 8 bacteria species and *Candida albicans*, with penicillin as a reference.<sup>[27]</sup>

Aerial parts 80% aqueous methanolic extract had moderate antioxidant activity measured with DPPH method.<sup>[28]</sup>

Flowers aqueous decoction had moderate antioxidant activity (ABTS, FRAP, Fe<sup>+2</sup> chelating methods).<sup>[29]</sup>

Fruits, leaves and stems were separately extracted with *n*-hexane, ethyl acetate, dichloromethane, methanol and water (15 extracts). The phenolic compositions of all extracts were analyzed and a detailed table is provided. The antioxidant activity of the extracts was tested with six methods (ABTS, CUPRAC, DPPH, FRAP, PBD and metal chelating methods) and found notable. They also had significant enzyme inhibition activity (acetylcholinesterase, butyrylcholinesterase, tyrosinase, amylase, and glucosidase).<sup>[30]</sup>

Fruits and leaves were separately extracted with methanol and both extracts had high antioxidant activity tested by DPPH and  $\beta$ -carotene bleaching method. Both extracts had significant pancreatic lipase inhibition activity. Extracts were analyzed for phenolic compounds and molecular docking was performed for enzyme inhibition by major components: rutin, quercitrin, quercetin, kaempferol and kaempferol 3-O-glucoside-7-O-rhamnoside.<sup>[31]</sup>

Isolation of three known phenolic compounds from fruits aqueous extract.<sup>[32]</sup>

\* The listed compounds in this research are products of silylation. This process was not described in the article and was not even mentioned. In addition, the plant name is mistakenly written in the title as *PALIURUS SPINA-CHRISTII* instead of *Paliurus spina-christi*.

### ***Rhamnus alaternus***

Leaves ethanolic extract and two of its major components, emodin and kaempferol (**Figure 2**), were active against methicillin-resistant *Staphylococcus aureus*. *R. alaternus* was more active than 14 other plants used in this research.<sup>[33]</sup>

Aerial parts were extracted with petroleum ether, chloroform, ethyl acetate, methanol and water. In addition, three flavonoid-rich fractions were prepared. All eight products were tested for activity against K562 leukemia cells and five bacteria species. Ethyl acetate and one of the fractions were most active.<sup>[34]</sup>

Aerial parts 70% aqueous ethanolic extract was prepared and fractionized with petroleum ether, ethyl acetate and *n*-butanol. Extract and fractions were tested for antioxidant activity (DPPH, FRAP, hydroxyl radical scavenging, TAC methods), antibacterial (5 bacteria species) and hemolytic activity (human blood). Positive results were observed in all tests ranging from weak to strong activities. Seven active known compounds were isolated.<sup>[35]</sup>

Bark was successively extracted with methanol and ethyl acetate and the extract had notable antioxidant (DPPH,  $\beta$ -carotene bleaching, FRAP, Fe<sup>+2</sup> chelating methods), antimicrobial (3 bacteria and 3 fungi species) activities. Extract was analyzed for anthraquinone content resulting the presence of emodin, aloe-emodin, rhein, chrysophanol and physcion (**Figure 2**). Three other *Rhamnus* species were studied in

this research.<sup>[36]</sup>

Leaves, pods and roots were combinedly extracted with three natural deep eutectic solvents (choline chloride and glycerol/ethylene glycol/urea) resulting phenolics-rich extracts. These were tested for antioxidant (ABTS, DPPH, Fe<sup>+2</sup> chelating methods) and antibacterial (5 species) activities. Moderate to high activities were observed. Chemical compositions of extracts contained many compounds including aloesin, aloesol and piperonylidene acetone (**Figure 2**).<sup>[37]</sup>

Leaves EO (hydrodistillation) had activity against *E. coli*, *S. typhimurium* and *P. aeruginosa*. The major component of this EO were (%): camphene 17.63, linalool 16.13, pulegone 15.01 and naphthalene 14.66 %.<sup>[38]</sup>

Leaves were separately extracted with petroleum ether, chloroform, ethyl acetate, methanol and 67% aqueous acetone (flavonoid-rich). All extracts had antioxidant (DPPH) and antibacterial (*S. typhimurium*) and antimutagenic (*S. typhimurium*) activities. General chemical compositions (compound families) were determined.<sup>[39]</sup>

Leaves were defatted with petroleum ether then extracted with methanol. The extract had antioxidant (DPPH,  $\beta$ -carotene bleaching methods) and antimutagenic (*Allium cepa* assay) activities. TFC and TPC were also determined.<sup>[40]</sup>

Leaves and root bark were separately extracted with methanol, and the leaves extract was used to obtain a phenolics-rich extract by using water-saturated-*n*-butanol. The original extracts were tested for antioxidant (DPPH, xanthine oxidase methods), antimutagenic (*S. typhimurium*) and antiproliferative (K562 leukemia cells) activities. General chemical composition of phenolics-rich extract was determined.<sup>[41]</sup>

Kaempferol 3-*O*- $\beta$ -isorhamninoside and rhamnocitrin 3-*O*- $\beta$ -isorhamninoside were isolated from leaves (several extraction steps), and they were found antioxidant (ABTS method) and antigenotoxic (in *E. coli*).<sup>[42]</sup>

Bark methanolic and aqueous extracts were prepared and had antioxidant (ABTS, DPPH, FRAP, ORAC methods) and anticancer (U937 leukemia cells) activities. Flavonoid content and compositions were determined.<sup>[43]</sup>

Leaves 80% aqueous acetone extract had activity against B16F10 cancer cells. The chemical composition of this extract included nine major phenolics.<sup>[44]</sup>

A follow up of previous study.<sup>[45]</sup>

Leaves methanolic extract had no significant effect in alloxan-induced diabetic rats.<sup>[46]</sup>

Leaves aqueous extract was prepared and fractionized with several solvents to determine the chemical (phenolic) compositions. The crude extract had activity against Triton-induced hyperlipidemia in rats and human HepG2 cells.<sup>[47]</sup>

Leaves aqueous extract had notable blood glucose lowering activity in STZ-induced diabetic rats. General chemical composition is provided.<sup>[48]</sup>

Bark and leaves were separately extracted with 70% aqueous methanol. Methanol was evaporated from this crude extract to obtain aqueous extract, which was fractionized with *n*-hexane, chloroform and ethyl acetate, yielding a total of ten extracts. These showed clear activity against *A. niger* and *C. albicans* fungi, with three reference drugs.<sup>[49]</sup>

Bark and leaves were separately extracted with 70% aqueous ethanol. Extracts had significant antioxidant (ABTS, DPPH, FRAP methods) and antihemolytic (AAPH method) activities. General chemical compositions are listed.<sup>[50]</sup>

Ethanol leaves extract was chromatographed to obtain 12 fractions. Crude extract and mixtures of the fractions were tested for antioxidant activity (DPPH method), and most active components were determined: emodin and kaempferol.<sup>[51]</sup>

A follow up of previously cited studies, especially,<sup>[41]</sup> and this study focused on the antioxidant (DPPH, superoxide anions and inhibition H<sub>2</sub>O<sub>2</sub>-induced lipid peroxidation



in K562 cells) activity of three known, single compounds: kaempferol 3-*O*-isorhamninoside, rhamnocitrin 3-*O*-isorhamninoside and rhamnetin-3-*O*-isorhamninoside.<sup>[52]</sup>

Leaves aqueous and methanolic extracts were prepared and had significant antioxidant activity (DPPH and  $\beta$ -carotene bleaching methods). Four known major phenolic compounds were identified in these extracts.<sup>[53]</sup>

Leaves methanolic extract was defatted and fractionized with several solvents and several steps. Crude extract and fractions were tested for antioxidant activity (DPPH method), their phenolic contents were determined and analyzed for major compounds (6).<sup>[54]</sup>

Bark and leaves were separately extracted with methanol-acetone 3:2 (v/v). Extracts were tested for antioxidant activity (DPPH, FRAP methods) with BHA as reference, showing notably higher activity of leaves extract. Hemolysis activity was measured by direct effect of extracts on human blood, showing maximum (weak) effect of 2%.<sup>[55]</sup>

One of the first reports of anthraquinones isolation from this plant. See **Figure 2**.<sup>[56]</sup>

Analysis of fruits methanolic extract afforded the six major anthocyanins shown in **Figure 2**.<sup>[57]</sup>

Leaves aqueous extract was administered (250 mg/kg/day, 9 days) to rats with CCl<sub>4</sub>-induced hepatotoxicity, resulting ameliorative effect. Silymarin was reference in this study.<sup>[58]</sup>

Leaves aqueous extract had nephroprotective (plasma electrolyte concentrations) in high salt and high fat-fed rats.<sup>[59]</sup>

Aerial parts aqueous extract had antioxidant activity (DPPH, FRAP,  $\beta$ -carotene bleaching methods), and hepatoprotective (plasma hepatic biomarkers) against AICl<sub>3</sub>-induced hepatotoxicity in rats.<sup>[60]</sup>

Case report of poisoning from Tunisia, as a result of daily drinking roots tea six months, by a person who considered this tea a herbal medicine against type II diabetes.<sup>[61]</sup>

#### ***Rhamnus disperma***

Roots ethanolic extract was prepared, fractionized with several solvents and the ethyl acetate fraction was chromatographed yielding several phenolics, four of them are methoxylated flavonoids (**Figure 3**). These compounds had significant activity against MCF7, A2780 and HT29 cancer cell lines.<sup>[62]</sup>

Same extraction, isolation and purification as in previous cited study (study<sup>[63]</sup> is 6 years earlier). Extract had amelioration effect on dinitrochlorobenzene-induced eczema in rats (1.0% w/w alcoholic extract ointment in Vaseline, 15 days). Reference was 0.1% w/w betamethazone (**Figure 3**) ointment.<sup>[63]</sup>

Roots were extracted with 70% aqueous ethanol and extract was fractionized with petroleum ether, ethyl acetate and *n*-butanol. The ethyl acetate fraction chromatography afforded four known phenolic compounds (rhamnocitrin 3-*O*- $\alpha$ -L-rhamnopyranoside, quercetin 7-*O*- $\alpha$ -L-rhamnopyranoside, kaempferol 3,7-di-*O*- $\alpha$ -L-rhamnopyranoside and quercetin 3,4'-di-*O*- $\alpha$ -L-rhamnopyranoside). These compounds had antioxidant activity measured by DPPH method.<sup>[64]</sup>

First isolation of quercetin 3,4'-di-*O*- $\alpha$ -L-rhamnopyranoside (**Figure 3**) with other known phenolics from 80% aqueous ethanol aerial parts extract. The known compounds included these mentioned in the previous citation<sup>[64],[65]</sup>

#### ***Rhamnus libanotica***

Bark methanolic extract was fractionized with several solvents and fractions were analyzed affording new derivative of physcion (**Figure 2**) along with other previously known compounds. The structure of the new compound is shown in **Figure 4**.<sup>[66]</sup>

***Rhamnus lycioides***

Leaves were extracted with several solvents, and extracts were tested for antioxidant (DPPH, FRAP,  $\text{Fe}^{+2}$  chelating methods) and acetylcholinesterase inhibition activities. Three of the extracts were analyzed for chemical composition: ethyl acetate, methanolic and anthraquinone-rich. All reported compounds are previously known.<sup>[67]</sup>

Leaves 70% aqueous methanolic extract was prepared, methanol was evaporated, and the aqueous phase was fractionized with chloroform, ethyl acetate and *n*-butanol. Crude extract and fractions were analyzed for chemical compositions where major seven compounds were obtained, including rhamnazin, rhamnocitrin and taxifolin (**Figure 5**).<sup>[68]</sup>

Aerial parts 70% aqueous methanolic extract was prepared, methanol was evaporated, and the aqueous phase was fractionized with chloroform, ethyl acetate and *n*-butanol. All materials were tested for hypotensive activity in rats.<sup>[69]</sup>

A follow up of previous study about the hypotensive activity of the plant, revealed a major active component (**Figure 5**).<sup>[70]</sup>

***Rhamnus punctata*****No published medicinal-biological activities*****Ziziphus lotus***

Fruits and leaves aqueous extracts were separately prepare and analyzed for phenolic compositions. Both extracts had significant antioxidant (ABTS, DPPH, FRAP methods, Trolox and ascorbic acid were references), antidiabetic ( $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition) and dermatoprotective (tyrosinase inhibition) activities.<sup>[71]</sup>

Fruits flavonoid-rich aqueous extract was analyzed for TFC and TPC. It had notable antioxidant (DPPH,  $\beta$ -carotene bleaching methods, BHA was the reference) and antihyperlipidemic (high fat-fed mice, blood cholesterol, triglycerides and glucose were measured) activities.<sup>[72]</sup>

Fruits were successively extracted with petroleum ether, dichloromethane and methanol, and each extract was analyzed for TFC, TPC and tannins content. The extracts were active against eight bacteria (control: amoxicillin<sup>a</sup>) and four fungi species (control: difenoconazole).<sup>[73]</sup>

Leaves, roots and seeds were separately extracted with several solvents, but only the 50% aqueous ethanolic extract<sup>b</sup> was used for antifungal activity *Aspergillus flavus-parasiticus* and *Aspergillus ochraceus*, showing notable activity. General chemical composition is presented.<sup>[74]</sup>

Honey of bees that was naturally fed by the tree flowers, was analyzed for TFC and TPC, and was active against three bacterial strains.<sup>[75]</sup>

Leaves 80% aqueous methanolic extract was prepared, analyzed for TFC and TPC, and major phenolic ingredients. It was active against 7 bacteria species and *C. albicans*. The inhibition kinetics is presented.<sup>[76]</sup>

Leaves methanolic extract was analyzed for general chemical composition and tested for antibacterial (5 species)<sup>c</sup>.<sup>[77]</sup>

Leaves methanolic extract was analyzed for general phenolic composition, had significant antioxidant (DPPH method) and antimicrobial (*E. coli*, *S. aureus*, *K. pneumoniae* and *F. culmorum*, *F. solani*, *B. cinerea*)<sup>d</sup> activities. In this research, *Ziziphus jujuba* was also studied.<sup>[78]</sup>

Seeds were separately extracted with ethanol, methanol and water. Each extract was analyzed for TPC and TFC, had significant antioxidant (DPPH method) and antibacterial (*E. coli*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *C. tropicalis*) activities.<sup>[79]</sup>

Fruits were separately extracted with ethanol, methanol and water. Each extract was analyzed for TPC and TFC and wide range of compound families. All extracts had

significant antioxidant (DPPH, TAC methods) and antibacterial (seven bacteria species and *C. tropicalis*) activities.<sup>[80]</sup>

Leaves methanolic extract was fractionized by several solvents and each fraction was tested for antimicrobial (2 bacteria and a fungi species), antileishmanial (2 species) and antioxidant (DPPH, FRAP methods). In the biological tests, the dichloromethane fraction had the highest activity, while in the antioxidant tests, the *n*-butanol fraction had the highest activity.<sup>[81]</sup>

Fruits, leaves and seeds were separately extracted with methanol and each extract had notable antioxidant (DPPH, TAC methods) and antibacterial (4 strains) activities. The extracts were analyzed for TFC and TPC, as well as single compounds. Major compounds were quinic acid, rutin, quercitrin and naringin. In this research *Ziziphus mauritiana* (nonnative of the reviewed region) was also studied.<sup>[82]</sup>

Methanolic extracts of fruits and leaves were prepared. The fatty acids composition of fruit extract included 13 compounds, including the isomers C18:1 *cis* (oleic acid, 88.1%) and C18:1 *trans* (elaidic acid, 7.9%). Leaves extract had higher phenolic, stronger antioxidant (ABTS, DPPH methods) and stronger antimicrobial (seven bacteria and three fungi species) activities.<sup>[83]</sup>

Fruits from 11 different locations in Morocco were analyzed for mineral content, extracted with *n*-hexane for EO, and this EO was analyzed for fatty acids composition. Separately, fruits were successively extracted with 70% aqueous acetone, ethanol, methanol and with water. The general composition of the combined extracts was determined and their antioxidant activity was tested with DPPH, FRAP and TEAC methods, showing significant results.<sup>[84]</sup>

Follow up of previous study, by the same group, focusing on nutritional properties.<sup>[85]</sup>

Methanolic extract was analyzed for phenolics and fatty acids compositions. It had notable antioxidant (DPPH, FRAP methods) activity, and protective effect (against oxidation) of corn oil during frying. *Moringa oleifera* was also studied in this research.<sup>[86]</sup>

Fruits leaves and roots were separately extracted with five solvents (15 extracts). They had notable antioxidant (ABTS, DPPH, FRAP methods) and cytotoxic (against SH-SY5Y cell line) activities. Extracts were separately analyzed for TFC, TPC and tannins content, and combinedly for chemical composition, revealing many major compounds where 13-epimanol (**Figure 6**) had one of the highest concentrations. Drying process of plant materials effect on the tested properties was also studied.<sup>[87]</sup>

Fruits without seeds were dried, analyzed for phenolic content and tested for antioxidant activity (DPPH method). The powder was added to cakes, resulting improvement of their taste, nutritional value and sensory quality.<sup>[88]</sup>

Honey of bees that was naturally fed by the tree flowers, had healing effect on hard palate ulcers (mechanical cut) in rats.<sup>[89]</sup>

Fruits aqueous extract had antiurolithiatic effect in ethylene glycol-induced lithiasis in rats (150 mg/kg/day, 14 days), indicated by several biomarkers, mainly  $\text{Ca}^{+2}$  and  $\text{C}_2\text{O}_4^{-2}$  concentrations.<sup>[90]</sup>

Leaves were successively extracted with petroleum ether, dichloromethane, ethyl acetate, *n*-butanol and water distilled. The combined extracts were tested *in vitro* for prevention of the formation of calcium oxalate after the mixing of  $\text{CaCl}_2$  (aq) and  $\text{Na}_2\text{C}_2\text{O}_4$  (aq).<sup>[91]</sup>

Aerial parts were specially extracted for alkaloids ( $\text{NH}_4\text{OH}-\text{CH}_3\text{OH}-\text{C}_6\text{H}_6$  1:1:98), resulting the isolation of five 14-membered frangulanine-type cyclopeptide alkaloids. Two of these alkaloids were new (**Figure 6**).<sup>[92]</sup>

Leaves were defatted with *n*-hexane and chloroform, then extracted with methanol.



Analysis of the extract afforded four new dammarane saponins (**Figure 6**).<sup>[93]</sup>

Fruits were defatted with *n*-hexane and separately extracted with 80% aqueous ethyl acetate and 80% aqueous methanol. Extracts were analyzed for chemical compositions and detailed results are presented. TFC, TPC, total anthocyanins and tannins contents were also determined. Testing antioxidant activity with DPPH showed significant results.<sup>[94]</sup>

EOs of aerial parts (not including fruits) and fruits were prepared by hydrodistillation and analyzed for chemical compositions: aerial parts EO contained much larger number of compounds.<sup>[95]</sup>

Fruits powder had excellent activity of Cr(VI) removal from aqueous solution. Kinetics, thermodynamics and the effect of various factors (pH, temperature, particle size) were studied.<sup>[96]</sup>

Leaves ethanolic extract had inhibition effect on corrosion of carbon steel under strong acidic conditions (1 M HCl).<sup>[97]</sup>

Activated carbon prepared from leaves had high capacity of methylene blue (decolorization) from textile wastewater. Kinetics, thermodynamics and the effect of various factors were studied.<sup>[98]</sup>

Cellulose was extracted from trunks with two methods (alkaline extraction then bleaching or vice versa), then it was carboxymethylated with  $\alpha$ -chloroacetic acid. The product was used to prepare membranes that had significant activity of pollutants removal (decolorization) from aqueous solutions.<sup>[99]</sup>

Honey of bees that was naturally fed by the tree flowers was tested for color, pH, moisture, electrical conductivity, and various nutritional contents such water, protein, total and single carbohydrates, phenolics and minerals. It had high antioxidant activity measured by DPPH method.<sup>[100]</sup>

Honey of bees that was naturally fed by the tree flowers was tested for external properties resulting clear dependence of these on the source of pollen.<sup>[101]</sup>

Aqueous extract of fruits pulp had modulatory effect on the humoral immune response in mice, antibacterial activity against *S. aureus* and antioxidant activity tested with DPPH method. It had hemolytic activity in low concentrations and antihemolytic activity in high concentrations.<sup>[102]</sup>

Fruits powder had high capacity of removing  $Pb^{+2}$  and  $Cd^{+2}$  from aqueous solution. The kinetics of the phytoremediation process was studied, as well as pH effect.<sup>[103]</sup>

a) Amoxicillin mistakenly written as amoxillin and difenoconazole mistakenly written as difenocazole.

b) This publication includes several mistakes. For example, under the title of "Extraction of flavonoids" it is written: "About 50 g powder of *Z. lotus* (seeds, roots, and leaves) were heated separately to 900°C under reflux in a mixture (distilled/ethanol water) (250 ml/250 ml) for 4 hrs".

c) Despite the fact that the title of this article is "Evaluation of antibacterial and antioxidant activities of *Artemisia campestris* (Astraceae) and *Ziziphus lotus* (Rhamnaceae)", no tests of antioxidant activity were presented. In addition, plant family names should not be written in *italics*, and in this case, both names are misspelled: Astraceae should be Asteraceae and Rhamnacea should be Rhamnaceae.

d) The antimicrobial activity is presented in this article for "ethanolic" and "aqueous" extracts, but practically, these are solutions of the original methanolic extract.

e) This article has a mistake in the title, "Immunosuppressive" instead of "Immunosuppressive". We did not correct it in the references list.

#### ***Ziziphus nummularia***

Methanolic roots extract was fractionated with water, of *n*-hexane, chloroform, ethyl

acetate, and ethanol. The crude extract and fractions tested for four activities in mice. Sedative, by treatment with 50 or 100 mg/kg; hypnotic effect against phenobarbitone-induced sleep, with positive control of bromazepam; antipyretic effect in (yeast) *S. cerevisiae*-induced hyperthermia, with paracetamol as control; and acetic acid-induced inflammation, with diclofenac as control. In all tests positive effects were observed.<sup>[104]</sup>

Leaves ethanolic extract was administered (three doses) to rats with formaldehyde-induced arthritis, showing positive effect measured by several biomarkers, especially oxidative/antioxidative. Diclofenac as reference in this study.<sup>[105]</sup>

Leaves 70% aqueous ethanolic extract had activity against Capan-2 human pancreatic cancer cells. Various mechanistic pathways were tested and it was found that for example, activity has clear relation to downregulation of ERK1/2 and NF- $\kappa$ B signaling pathways, among other actions.<sup>[106]</sup>

Leaves 80% aqueous ethanolic extract had activity against MDA-MB-231 human breast cancer cells. Authors linked this activity to the high antioxidant capacity of this extract that was proved with DPPH method. Qualitative chemical composition analysis revealed that extract contains 81 compounds.<sup>[107]</sup>

Fruits and leaves were separately extracted with 70% aqueous ethanol, and both extracts had glucose diffusion inhibition activity. TPC of both extracts is reported.<sup>[108]</sup>

Leaves 90% aqueous ethanolic extract was used to prepare a topical gel (20 and 30%), that had anti-inflammatory (carrageenan-induced paw edema) and wound healing (mechanical cut) activities in rats.<sup>[109]</sup>

Root bark ethanolic extract had anti-inflammatory activity *in vitro* (RAW 264.7 cells) and *in vivo* (rats). Inflammations were induced by several agents: carrageenan, arachidonic acid, xylene and LPS. Authors report the active compound shown in **Figure 7**.<sup>[110]</sup>

Leaves 70% aqueous ethanolic extract attenuated TNF- $\alpha$ -induced phenotypic inflammation in human aortic smooth muscle cells.<sup>[111]</sup>

Leaves were successively extracted with petroleum ether, chloroform, ethyl acetate, ethanol and water. Each extract was tested for anti-inflammatory (inhibition of protein denaturation test) and antioxidant (DPPH, hydroxyl radical scavenging methods) activities.<sup>[112]</sup>

Root bark 70% aqueous ethanolic extract was chromatographed affording a novel cyclic peptide-alkaloid (**Figure 7**) with anti-inflammatory activity in LPS/IFN- $\gamma$  stimulated RAW 264.7 cells (INF, interferons). Molecular docking study is reported.<sup>[113]</sup>

Fruits and leaves were separately extracted with water, methanol, ethanol and chloroform (8 extracts). Each extract was tested against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes*, *C. albicans*. Both alcoholic extracts had high activity and aqueous extract was weakest. TFC, TPC and alkaloid content were determined.<sup>[114]</sup>

Roots aqueous and ethanolic extracts had activity against several bacteria species. For some strains, ethanolic extract was more active, and vice versa. General chemical compositions of both extracts are presented.<sup>[115]</sup>

Leaves aqueous extract had antibacterial (against 6 species) and antioxidant (ABTS, DPPH,  $\beta$ -carotene bleaching methods) activities. TPC and general chemical compositions are reported.<sup>[116]</sup>

Fruits were extracted with chloroform-methanol (1:1, v/v) and this extract was active against four bacteria and two fungi species. It had antioxidant activity measured with DPPH method. Its general and detailed chemical compositions are presented with pyrogallol (**Figure 7**) as major component.<sup>[117]</sup>

Aerial parts methanolic extract antioxidant activity was tested with FRAP method.<sup>[118]</sup>

Fruits aqueous extracts was analyzed for nutritional and general compositions, and its

antioxidant activity was tested with DPPH, FRAP and  $\text{Fe}^{+2}$  chelating power.<sup>[119]</sup>

Fruits methanolic extract (100, 200, 400 mg/kg) had antiulcer (mechanical cut, pyloric ligation method) activity in rats, with omeprazole as a reference.<sup>[120]</sup>

Fruits ethanolic extract had cardioprotective activity in isoproterenol induced myocardial infarcted rats, indicated by activity of several cardiac enzymes.<sup>[121]</sup>

Similar isolation methods to ref. 92 and ref. 113 afforded 11 new cyclic peptide-alkaloid with structures like those shown in **Figure 6** and **Figure 7**.<sup>[122,123,124]</sup>

General chemical compositions of fruits and leaves 70% aqueous ethanolic extracts.<sup>[125]</sup>

Leaves 70% aqueous ethanolic extract was analyzed for general and detailed chemical compositions, resulting N-[3-methyl-2-butenylidene]methanamine as second major component, where the first is not specified ethyl glucose ether.<sup>[126]</sup>

Comparative chemical compositions (whole plant) analysis with *Z. jujuba* revealed many similarities and many differences. For example, jujuboside A and jujuboside B (**Figure 7**) are present in both species, but their concentrations in *Z. jujuba* are higher.<sup>[127]</sup>

Sieves were obtained by acidic hydrolysis of the tree wood and had high adsorption capacity of bromocresol green. The kinetics of the adsorption process was studied, including effects of pH, dye concentration, amount of adsorbent, size of adsorbent particles, and contact time on removal (decolorization).<sup>[128]</sup>

Activated carbon was prepared from fruit kernel and it had high erythrosine removal capacity. The kinetics and thermodynamics of the decolorization process are presented, with detailed effects of various factors as in the previous study.<sup>[129]</sup>

Adsorbent (pyrene) prepared from the plant waste had high erythrosine removal capacity. This study is a follow up of the study cited in reference 129, and it compares this adsorbent with another prepared from Sorghum.<sup>[130]</sup>

Seven months old infants and lambs were fed with the same amount and for the same time period, with fruits and a mineral food additive. Infants gained more nutritional benefits than lambs.<sup>[131]</sup>

Leaves polyphenol-rich extract was studied as part of feed block with *Vigna sinensis* and *Acacia nilotica*. The study tested *in vitro* rumen fermentation kinetics, methane production and digestibility.<sup>[132]</sup>

Leaves 80% aqueous ethanolic extract was tested for four activities: castor oil-induced diarrhea in mice, antisecretory activity in castor oil-induced fluid accumulation in mice, against potassium chloride-induced contractions in isolated rabbit jejunum tissues, and against ethanol-induced gastrointestinal ulcer in rats. In all tests positive effect was observed.<sup>[133]</sup>

Fruits, leaves and stems were separately powdered and these powders had significant metal ions\* ( $\text{Pb}^{+2}$ ,  $\text{Cd}^{+2}$ ,  $\text{As}^{+5}$ ,  $\text{Hg}^{+2}$ ) accumulation from aqueous solutions.<sup>[134]</sup>

Similar isolation methods to ref. 92 and ref. 113 afforded two new cyclic peptide-alkaloid with structures like those shown in **Figure 6** and **Figure 7**, along with five previously known. In this research *Z. spina-christi* was studied.<sup>[135]</sup>

Fruits EO was obtained by headspace solid-phase micro extraction and it was analyzed, revealing 39 known compounds. In this research *Z. spina-christi* was studied.<sup>[136]</sup>

\* In section 2.2 of this article it is written: "Prepare the standard solution [Lead, cadmium, Arsenic and Mercury] or as per the requirement containing the element of interest by the following method". Solutions can not contain these elements. They contain their ions.

#### *Ziziphus spina-christi*

Similar isolation methods to ref. 92 and ref. 113 afforded two new cyclic peptide-alkaloid with structures like those shown in **Figure 6** and **Figure 7**, along with five

previously known. In this research *Z. nummularia* was studied.<sup>[135]</sup>

Fruits EO was obtained by headspace solid-phase micro extraction and it was analyzed, revealing 41 known compounds. In this research *Z. nummularia* was studied.<sup>[136]</sup>

Leaves 70% aqueous ethanolic extract was used to prepare a topical solution with clindamycin to treat patients with mild to moderate acne, for six weeks. The results showed significant reduction.<sup>[137]</sup>

Molecular docking of anti-acne of christinins (A, B, C, D), natural products from the fruits of this tree. Their molecular structures are shown in **Figure 6** and **Figure 7**, taken from references 93 and 127.<sup>[138]</sup>

Leaves aqueous extract had allelopathic activity against *Triticum durum* and *Raphanus sativus* seeds.<sup>[139]</sup>

Leaves methanolic extract was analyzed for TPC and tannins content. It had antioxidant (DPPH method) and allelopathic (against *Trigonella foenum-graecum* and *Lens culinaris* seeds) activities.<sup>[140]</sup>

Root bark was extracted with *n*-hexane, chloroform, ethyl acetate and methanol. The methanolic extract was eluted with chloroform-methanol (7:3, v/v), and this fraction was the most active against pain (induced by acetic acid, formalin or hot plate) in rats, and had anti-inflammatory (egg-albumin-induced paw edema) in mice.<sup>[141]</sup>

Leaves were extracted with 80% aqueous ethanol yielding crude extract, which was eluted with ethanol-water gradient, resulting three more extracts: ethanolic, 50% aqueous-ethanolic and aqueous. The ethanolic extract had the highest activity against MCF-7 cancer cells.<sup>[142]</sup>

Leaves methanolic extract had strong anticancer (human rhabdomyosarcoma cell line) and antioxidant (DPPH method) activities.<sup>[143]</sup>

Leaves 70% aqueous ethanolic extract was fractionated with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. The last fraction was analyzed affording christinin E and its two new derivatives (**Figure 8a**). These compounds had activity against lung cancer (A549), glioblastoma (U87), breast cancer (MDA-MB-231), and colorectal carcinoma (CT-26) cell lines.<sup>[144]</sup>

Leaves 70% methanolic extract had anticancer activity: *in vivo* against diethyl nitrosamine-induced hepatocarcinogenesis in rats, and *in vitro* against HepG2 cancer cell line. It had antioxidant activity measured by DPPH and hydroxyl radical scavenging methods. The chemical composition of this extract is presented.<sup>[145]</sup>

Leaves ethanolic extract was used to prepare five shampoos (5%) that had anti-dandruff (caused by *Malassezia furfur*) activity in human volunteers.<sup>[146]</sup>

Leaves 75% aqueous ethanolic extract had significant activity of blood glucose lowering and insulin upregulation in STZ-induced diabetic rats.<sup>[147]</sup>

Fruits were separately extracted with 80% aqueous ethanol and water, and both extracts had positive effect *in vivo* (alloxan-induced diabetic rats, measured by several biochemical parameters), and *in vitro* (inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase).<sup>[148]</sup>

Leaves 50% aqueous ethanolic extract had notable ameliorating effect in alloxan-induced diabetic rats, indicated by several liver and kidney biomarkers.<sup>[149]</sup>

Fruits, leaves and seeds were separately extracted with ethanol, and these extract had positive effect (blood glucose lowering and insulin upregulation) in hyperglycemia induced by hyperlipidemic diet (no details) in albino rats.<sup>[150]</sup>

Leaves 80% aqueous methanolic extract was prepared and fractionated with ethyl acetate, *n*-butanol and water. All four materials had blood glucose lowering activity (and other biochemical indicators) in STZ-induced diabetic rats. It also had high antioxidant activity measured by ABTS and DPPH methods.<sup>[151]</sup>

A follow up of the previous study. Same extract was fractionized with *n*-hexane,



chloroform, ethyl acetate, *n*-butanol and water. These materials had hepatoprotective and nephroprotective effects in CCl<sub>4</sub>-induced hepatotoxicity in rats, where both activities were measured by several biomarkers. These materials had significant  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition activity.<sup>[152]</sup>

Six extracts were prepared: seeds were separately extracted with dichloromethane, ethyl acetate and 80% aqueous methanol; leaves, roots and stems were separately extracted with 50% (v/v) methanolic dichloromethane. All materials were tested in two anti-inflammatory activity tests, and molecular docking was performed for epigallocatechin, gallic acid, spinosin, 6''-feruloylspinosin and 6''-sinapoylspinosin (**Figure 8a**).<sup>[17]</sup>

Leaves hydroalcoholic extract (procedure is not specified) attenuated pentylenetetrazole-induced kindling in mice brain tissue, measured by several biochemical parameters.<sup>[153]</sup>

Seedless fruits and seeds were separately extracted with 70% aqueous ethanol. Antioxidant activity was determined with DPPH method and anti-inflammatory activity was determined with denaturation method.<sup>[154]</sup>

Leaves 80% aqueous methanolic extract reduced the concentration of Hg<sup>+2</sup> (HgCl<sub>2</sub>) in rats liver hepatotoxicity, and activated antioxidant enzymes: superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.<sup>[155]</sup>

Aerial parts were separately extracted with water, 95% aqueous ethanol, petroleum ether, and ethyl acetate, but only aqueous and aqueous-ethanolic extracts were tested against microbial strains of *S. aureus*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *K. pneumoniae* and *C. albicans*, showing significant activities.<sup>[156]</sup>

Leaves 80% aqueous ethanolic extract was active against bacterial strains of *S. typhi*, *P. mirabilis*, *S. dysenteriae*, *E. coli*, *K. pneumoniae*, *B. melitensis*, *B. bronchiseptica* and *P. aeruginosa*.<sup>[157]</sup>

Aerial parts ethanolic extract was prepared and fractionated with petroleum ether, ethyl acetate and methanol. All materials were active against bacterial strains: *S. aureus*, *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, *S. para typhi* B, and *K. pneumoniae*.<sup>[158]</sup>

Fruits, leaves, seeds and stems were separately and successively extracted with petroleum ether, chloroform, methanol and water. All 16 extracts were tested against six bacteria and two fungi species. The aqueous extract was not active, and all extracts had no activity against fungi, while against bacteria, organic extracts had moderate activities.<sup>[159]</sup>

Seeds EO was obtained by extraction with *n*-hexane and it was active against *S. aureus*, *E. coli*, *Shigella* ssp. and *P. aeruginosa*.<sup>[160]</sup>

Fruits 80% aqueous methanolic extract was active against six bacteria species.<sup>[161]</sup>

Bark, fruits, leaves, roots and seeds were separately extracted with ethanol and methanol (10 extracts), and these extracts were tested against *P. aeruginosa*, *E. cloacae*, *E. aerogenes*, *E. coli*, *K. pneumoniae*, *E. faecalis* and *S. aureus*. Notable activity was observed in most cases.<sup>[162]</sup>

Leaves aqueous and ethanolic extracts were mildly active against five bacterial strains. In this research *Ziziphus abyssinica* and *Ziziphus mauritiana* (nonnative to the reviewed region) were also studied.<sup>[163]</sup>

Leaves aqueous and ethanolic extracts were mildly active against three bacterial strains. In this research *Ziziphus jujuba* was also studied.<sup>[164]</sup>

Stem bark was separately extracted with petroleum ether, ethyl acetate, ethanol, water and methanol, in microwave-assisted method. Extracts were tested against four bacteria and a fungi species, showing moderate activities, except the petroleum ether extract that was inactive. General chemical compositions were presented.<sup>[165]</sup>

Leaves and stem bark aqueous extracts were separately and combinedly tested against



12 bacterial strains, resulting high activities.<sup>[166]</sup>

Leaves aqueous, 60% aqueous ethanolic and 60% aqueous methanolic extracts were prepared and found active against four bacterial isolates.<sup>[167]</sup>

Leaves 96% aqueous ethanolic extract tested against ten bacterial species and found active. This extract was analyzed and 30 compounds were identified, where the major three were (%): *cis*-9-hexadecenal 31.6, *n*-hexadecanoic acid 20.1, squalene 5.2. Theoretical analysis of antibacterial activity of all 30 compounds was performed.<sup>[168]</sup>

Leaves aqueous and ethanolic extracts were active against six bacteria and two fungi species.<sup>[169]</sup>

Leaves 70% aqueous ethanolic extract was active against *Streptococcus mitis*.<sup>[170]</sup>

Leaves 70% aqueous ethanolic extract was active against *Streptococcus oralis*.<sup>[171]</sup>

Leaves aqueous extract was active against *Streptococcus warneri*.<sup>[172]</sup>

Leaves aqueous and 96% aqueous ethanolic extracts were active against four bacterial strains.<sup>[173]</sup>

Leaves 70% aqueous ethanolic extracts was active against five bacteria species.<sup>[174]</sup>

Whole plants ethyl acetate extract had mild activity against four bacteria species, and moderate antioxidant activity, tested with DPPH method.<sup>[175]</sup>

Aerial parts EO (hydrodistillation) had high activity against *A. niger*, *P. digitatum* and *K. pneumonia*, and high antioxidant activity (DPPH method). The chemical composition of this EO was analyzed and the major components were (%): carotol 42.2, hexadecanoic acid 13.75%, linoleic acid 11.76, vetivenic (zizanoic) acid 9.56% and valeranone 7.06. The structures of carotol vetivenic acid, and valeranone are shown in **Figure 8a**.<sup>[176]</sup>

Leaves 70% aqueous ethanolic extract was active against two bacteria species and a fungus, and notable antioxidant (hydroxyl radical scavenging) activity.<sup>[177]</sup>

Fruits *n*-hexane extract had significant antibacterial (against *P. Carotovorum*, *D. solani*, *R. solanacearum*, *E. cloacae*, *B. pumilus*), and antioxidant (DPPH,  $\beta$ -carotene bleaching methods) activities. The chemical composition (GC-MS) of this extract included mainly phenyl long chain alkane derivatives.<sup>[178]</sup>

Leaves 70% aqueous methanolic extract was prepared from three different locations in Tunisia. It was active against three bacteria and three fungi species, had significant antioxidant (DPPH method) and insecticidal (against *Tribolium castaneum*) activities.<sup>[179]</sup>

Leaves ethanolic extract was active against three fungi species and had significant antioxidant (DPPH method) activity. The major volatile ingredient of this extract was  $\text{CH}_3(\text{CH}_2)_{16}\text{CO}_2\text{CH}(\text{CH}_2\text{OH})_2$  octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (18.8%).<sup>[180]</sup>

Leaves ethanolic and methanolic extracts had activity against COVID-19 virus. Analysis of extracts yielded christinin E (**Figure 8a**) as major active component.<sup>[181]</sup>

Stem bark ethanolic extract was fractionized with chloroform, diethyl ether and *n*-butanol, and all these four materials were tested against two fungal and three bacterial species. The *n*-butanol fraction was most active. The ethanolic was analyzed and authors link the antimicrobial activity to betulin and betulinic acid (**Figure 1**), and molecular docking was performed for these compounds. In total, 36 compounds were identified in the ethanolic extract and diethyl ether and *n*-butanol fractions, most were shown in **Figures 1-8a**.<sup>[182]</sup>

Fruits and leaves were separately extracted with methanol, and both extracts were found active against three fungi species: *A. alternata*, *A. citri*, and *A. radicina*. Fruits extract was more active. The chemical compositions of both extracts is presented, and the major compound in each extract is shown in **Figure 8a**.<sup>[183]</sup>

Aerial parts 90% aqueous ethanolic extract (crude) was prepared, dissolved in 50% aqueous ethanol, and this solution was fractionized with petroleum ether, chloroform, ethyl acetate and *n*-butanol. All fractions were tested for pancreatic lipase inhibition, where chloroform and *n*-butanol fractions were most active. The crude extract was analyzed for chemical composition, and the major components were tested for the same activity. The three most active compounds were christinin A, daphnoretin and daphnodorin (**Figure 8b**).<sup>[184]</sup>

Leaves ethanolic extract had significant antioxidant activity, tested by DPPH and hydroxyl radical scavenging methods. In this research *Ziziphus mauritiana* was also studied.<sup>[185]</sup>

Fruits 80% aqueous ethanolic extract had notable antioxidant activity, measured by DPPH, hydroxyl radical scavenging, FRAP, TRPA and Fe(II) chelating.<sup>[186]</sup>

Leaves methanolic extract had notable antioxidant activity tested by DPPH method.<sup>[187]</sup>

Fruits powder or its 80% ethanolic extract, were separately, combinedly or in combination with zinc, fed to rats that were poisoned with sodium fluoride. The poisoning increased the total oxidant status in the kidneys and liver, and the intervention ameliorated that. Analysis of the extract revealed that its major component is nimbolide (**Figure 8b**).<sup>[188]</sup>

Fruits and leaves were separately extracted with 80% aqueous ethanol, and the extracts had significant antioxidant activity tested by DPPH and FRAP methods.<sup>[189]</sup>

Acetic acid-induced ulcerative colitis in rats was treated with 80% aqueous methanolic fruits extract, by suppressing oxidative stress biomarkers.<sup>[190]</sup>

Aqueous, ethanolic and methanolic leaves extracts had significant antioxidant activity tested with DPPH (methanolic was highest) and FRAP (ethanolic) methods.<sup>[191]</sup>

The effect of natural fermentation on general chemical compositions, TFC and TPC, was separately studied for whole fruits. This effect was also studied on the positive antioxidant activities using DPPH, FRAP, TRPA and hydroxyl scavenging methods.<sup>[192]</sup>

Mice were infected with *Schistosoma mansoni* and were supplemented with 70% aqueous ethanolic root bark extract. This resulted in amelioration of oxidative stress, measured by five oxidative/antioxidative biomarkers, and activities of seven hepatic enzymes.<sup>[193]</sup>

Mice were infected with *Bilharzia* (schistosomiasis) and were supplemented with ethanolic root bark extract. This intervention reduced hepatic worm burden and glaucoma, and increased ova count.<sup>[194]</sup>

Follow up of the study cited in reference 193 with three differences: leaves extract not roots, the major tested biomarkers were liver granuloma and fibrosis, and a mechanism of action is proposed in this study: downregulation of fibrinogenic signaling.<sup>[195]</sup>

Ethanolic leaves extract was fractionized to achieve saponin-rich extract, which was tested against B16F10 (melanoma) and L929 (fibroblasts) cell lines, resulting high activity against the first.<sup>[196]</sup>

Seedless fruits were defatted with petroleum ether and analyzed for fatty acids, protein, sugars and unsaponifiable fractions. The petroleum ether, fatty acids and unsaponifiable fractions were tested against three bacteria and three fungi species, showing significant activity against bacteria and no activity against fungi.<sup>[197]</sup>

Seeds methanolic extract had genotoxicity against *Biomphalaria alexandrina* (snails), with control of *Daphnia magna* (plankton), where the extract had very low toxicity.<sup>[198]</sup>

Leaves aqueous extract used for treatment of *Enterobiasis vermicularis*-infected children in Egypt, separately or in combination with mebendazole, a standard antiparasitic drug.<sup>[199]</sup>

Molecular docking work of pentacyclic peptides contained in this plant (see **Figures 6 and 7**) against SARS-COVID-2 main protease enzyme.<sup>[200]</sup>

Leaves 70% aqueous ethanolic extract had activity against scopolamine-induced anxiety in rats, measured by rotarod task method, with diazepam as a reference. The extract antioxidant activity was determined with DPPH method.<sup>[201]</sup>

Leaves 70% methanolic extract had ameliorative effect on mice sepsis induced by cecal ligation and puncture (CLP). This effect was measured by the activity of antioxidant and anti-inflammatory biomarkers in heart and kidneys. The extract was analyzed by GC-MS where squalene and piperidine were the major components.<sup>[202]</sup>

Aerial parts general chemical composition is presented.<sup>[203]</sup>

Leaves were extracted with 35% ethyl acetate in ethanol, and extract was fractionized and analyzed for chemical composition. Twenty-two compounds were isolated and characterized, including christinin-like (10) and phenolics (12).<sup>[204]</sup>

Isolation of betulinic acid\* from stem bark 95% aqueous ethanolic extract.<sup>[205]</sup>

Ethanolic stem bark extract was fractionized with diethyl ether, chloroform, ethyl acetate and *n*-butanol. GC-MS analysis revealed that the major components were (%): ethyl palmitate 18.94 in diethyl ether, betulin 55.23 in chloroform,  $\alpha$ -sitosterol 43.89 in ethyl acetate and 23.51 in *n*-butanol. In addition, compositions comparison is presented.<sup>[206]</sup>

Fruit pulp nutritional composition is presented.<sup>[207]</sup>

Leaves 95% aqueous ethanolic extract was supplemented to bulls in three dosages, 1, 3, and 5%, resulting increase of quantity and quality of the animals semen.<sup>[208]</sup>

Branches were added to lambs diet (10, 20, 30%) resulting higher digestibility and greater weigh gain compared with control group. *Z. mauritiana* was also studied.<sup>[209]</sup>

Fruits, pulp and aqueous extract (hot or cold) were used to produce jam.<sup>[210]</sup>

Leaves 70% aqueous methanolic extract attenuated liver and spleen *Plasmodium berghei*-induced injury in mice, by modulating antioxidant enzymes.<sup>[211]</sup>

Follow up of previous study with two differences. First, malaria was induced by *Plasmodium chabaudi*, and second, more biomarkers were tested in this research.<sup>[212]</sup>

Dynamics of nectar secretion from flowers, its sugar content and its potential for honey production.<sup>[213]</sup>

Honey of bees that naturally fed from the tree flowers was active against *S. aureus*, *L. monocytogenes*, *S. typhimurium* and *E. coli*.<sup>[214]</sup>

Honey of bees that naturally fed from the tree flowers was active against *B. cereus*, *S. aureus*, *E. coli*, *S. enteritidis* and *T. mentagrophytes*. The activity was compared with two standard antibiotics, flucoral and mycosat.<sup>[215]</sup>

Honey of bees that naturally fed from the tree flowers was active against *S. aureus*, *S. aureus*, *E. coli*. Molecular docking found that some of the active compounds were isopropyl 2-furoate, (S)-5-(2-methylpropyl)-2,4-imidazolidinedione, methyl- $\beta$ -D-thiogalactoside and  $\gamma$ -sitosterol (**Figure 8b**).<sup>[216]</sup>

Roots were separately extracted with acetone, ethanol, methanol and water, and these extract were analyzed TPC, and tested for antioxidant activity (ABTS, DPPH,  $\text{Fe}^{+2}$  chelating methods). Extracts tests for insecticidal activity against cigarette beetle (*Lasioderma serricorne*). In all these tests, ethanolic extract was most active.<sup>[217]</sup>

Roots aqueous extract had positive effect on central nervous system of mice, tested with exploratory behaviour pattern, spontaneous motor activity, motor coordination (rotarod) and pentobarbital-induced hypnosis.<sup>[218]</sup>

Leaves 80% aqueous methanolic extract attenuated  $\text{HgCl}_2$ -induced hepatotoxicity in rats, measured by decreased Hg concentration, lipid peroxidation, and nitric oxide, increased glutathione, superoxide dismutase, catalase, peroxidase and reductase, and

upregulated nuclear factor-erythroid 2-related factor 2.<sup>[219]</sup>

Same extract was used by the same group of previous research to ameliorate HgCl<sub>2</sub>-induced brain toxicity in rats, mainly by lowering Hg(II) concentration in the cortex and regulation of oxidant/antioxidant biomarkers.<sup>[220]</sup>

Biosorbent prepared from seeds (stones) was efficient for removal of Zn(II) and Cd(II) from their aqueous solutions.<sup>[221]</sup>

Follow up of the previous study with two differences. First, Pb(II) was added, and second, the metal ions were in wastewater, not prepared aqueous solutions.<sup>[222]</sup>

Young trees grown in Cu(II) contaminated soil showed accumulative activity.<sup>[223]</sup>

Leaves 70% aqueous ethanolic extract was used to prepare shampoos with 5, 10 or 20%. These were tested for physical appearance, pH value, surface tension, wetting time, rheological property, dirt dispersion, foam volume and stability, detergency, eye irritation, skin sensitization and overall stability.<sup>[224]</sup>

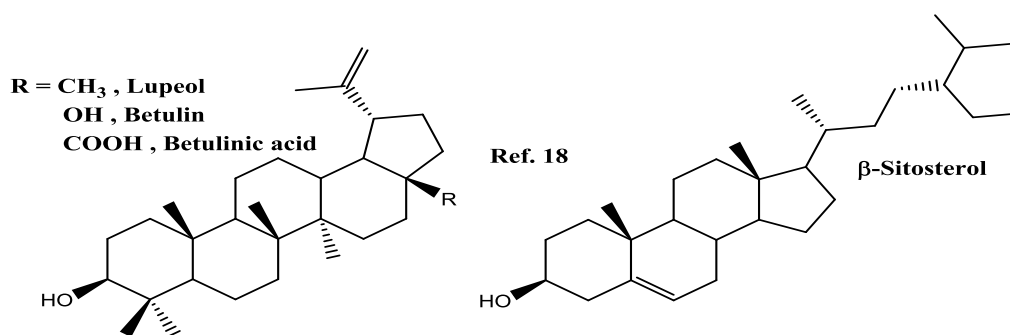
Leaves aqueous extract with extracts of another four plants were used, in various proportions, to prepare shampoos, and these were compared for different properties with two commercial shampoos.<sup>[225]</sup>

Leaves 70% methanolic extract was used to prepare a shampoo with similar extracts of *Salix babylonica* leaves and *Glycyrrhiza glabra* rhizomes. In addition to some listed above shampoo properties, its antioxidant (DPPH method) and antimicrobial (against *S. aureus*, *B. cereus*, *Proteus* spp., *E. coli* and *C. albicans*) activities were tested.<sup>[226]</sup>

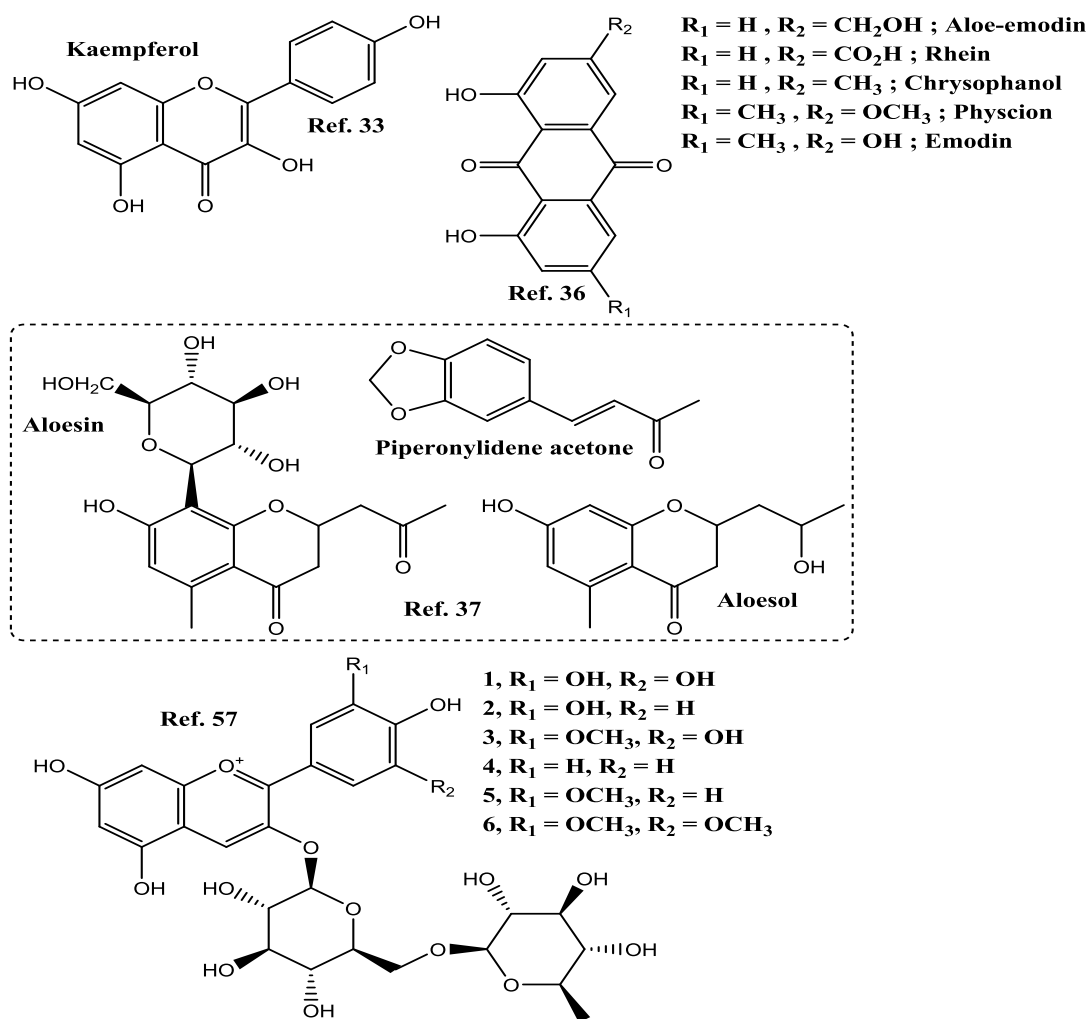
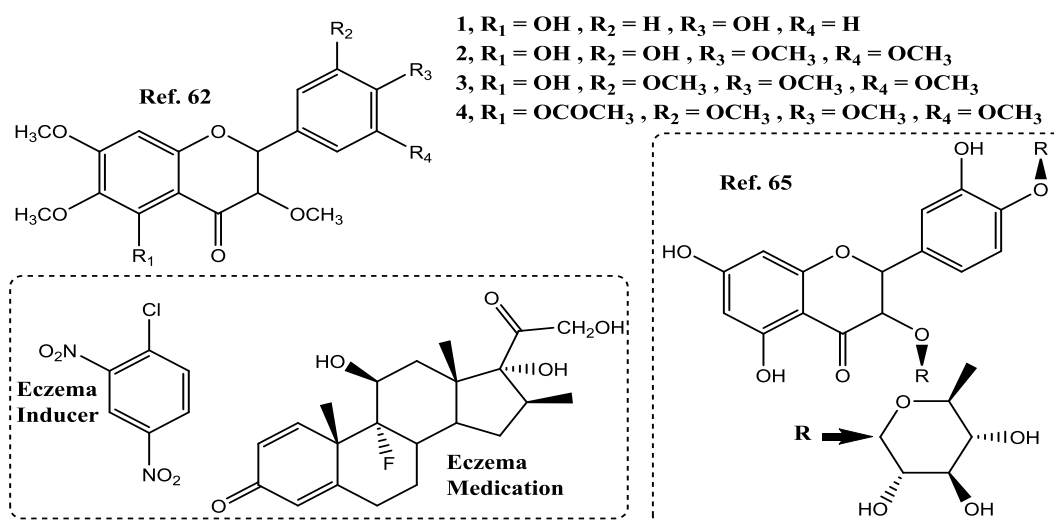
Wet leaves powder was very efficient in disappearance of severe rash that was caused by erlotinib cancer treatment.<sup>[227]</sup>

\* Mistakenly written in the title and 6 other mentions as “betunilic acid”.

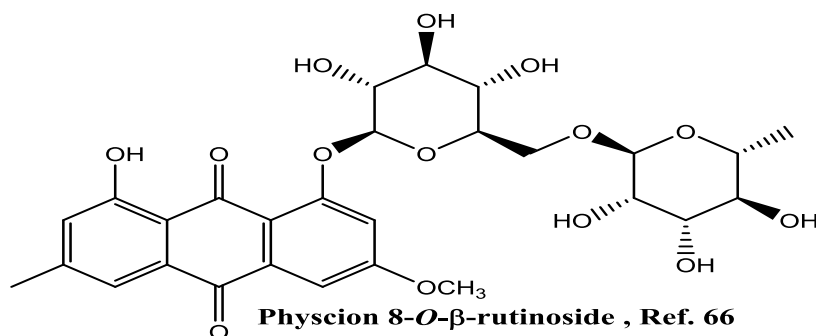
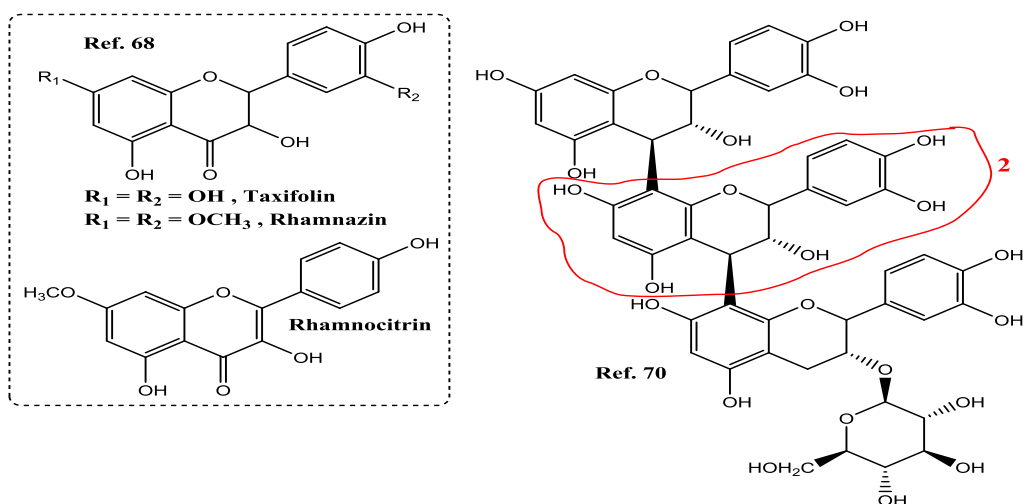
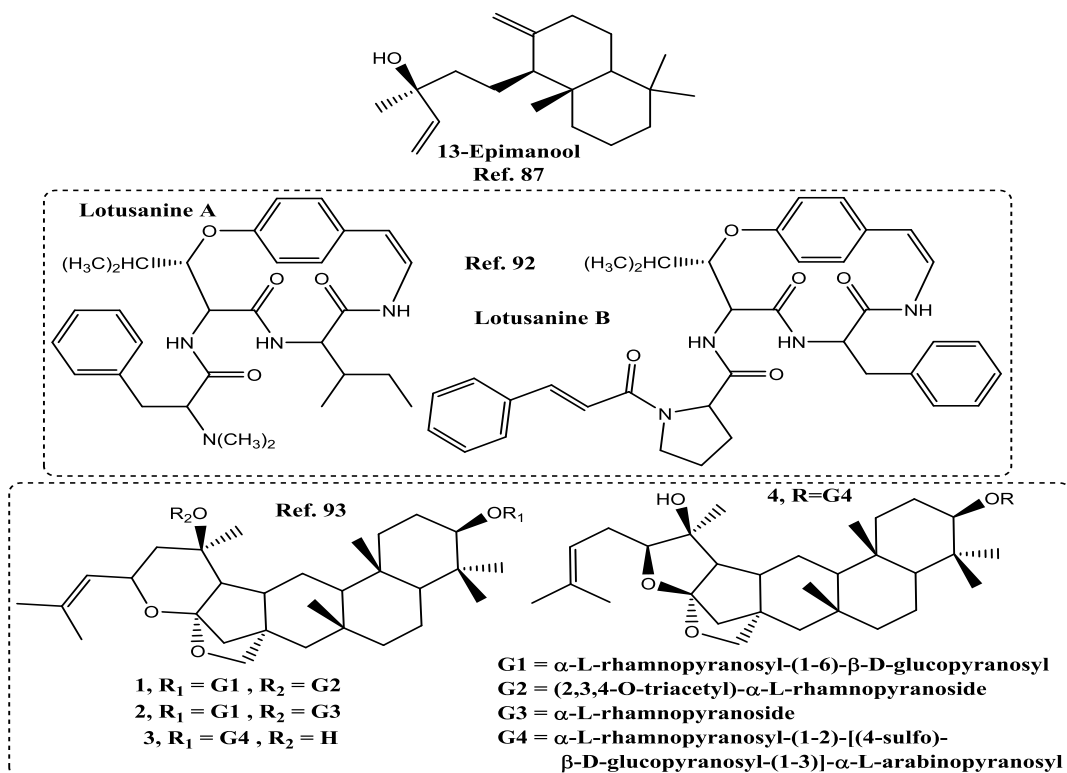
**Abbreviations:** AAPH 2,2'-azobis(amidinopropane) dihydrochloride, ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ahc and her/his colleagues, BHA butylated hydroxyanisole, BHT butylhydroxytoluene, CUPRAC cupric reducing antioxidant capacity, DPPH 2,2-Diphenyl-1-picrylhydrazyl, EO essential oil, FRAP ferric reducing activity power, GC-MS gas chromatography mass spectrometry, LC-MS liquid chromatography mass spectrometry, LPS lipopolysaccharide, LOX lipoxygenase, ORAC oxygen radical absorbance capacity, PBD phosphomolybdenum (assay), STZ streptozotocin, TAC total antioxidant capacity, TEAC Trolox equivalent antioxidant capacity, TFC total flavonoid content, TPC total phenolic content, TRPA total reducing power ability.

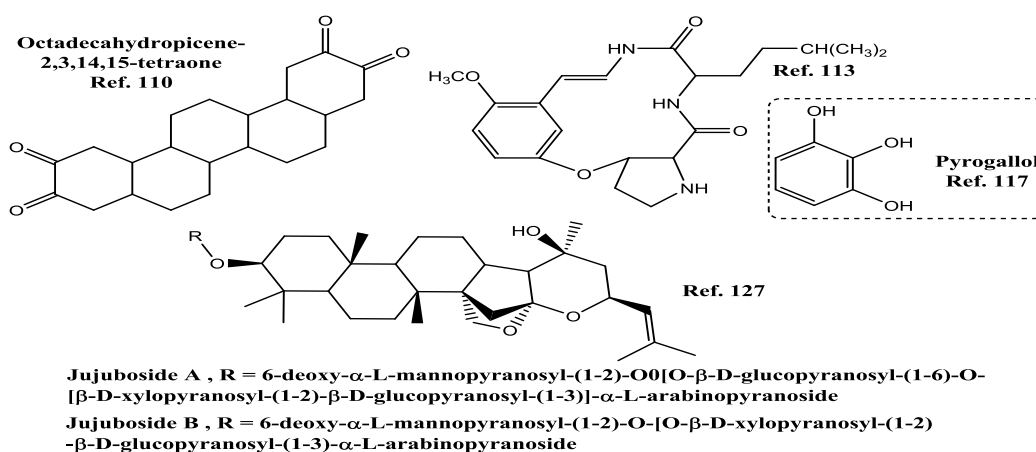


**Figure 1: Natural products isolated from *Paliurus spina-christi*.**

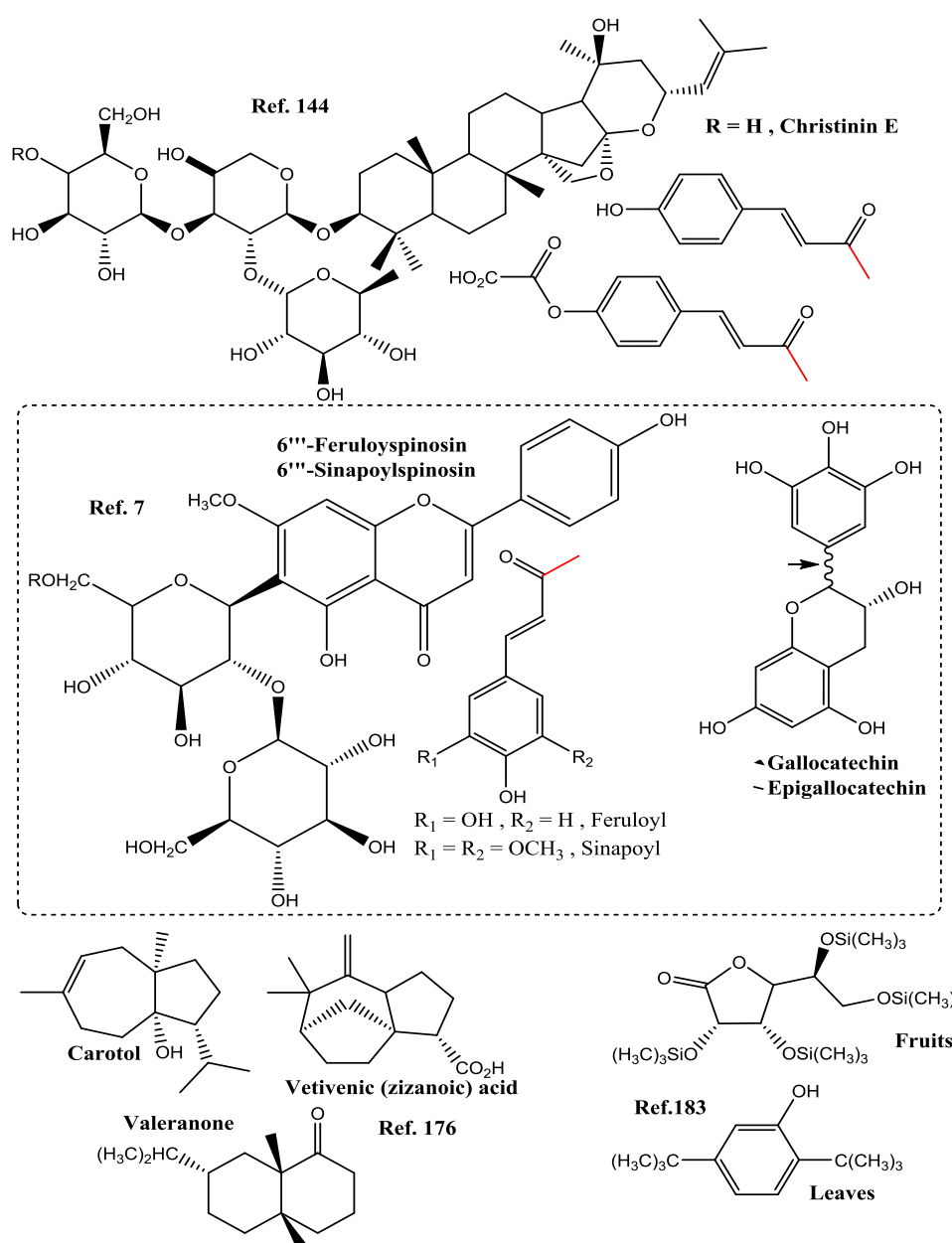
Figure 2: Natural products isolated from *Rhamnus alaternus*.Figure 3: Natural products isolated from *Rhamnus disperma*.



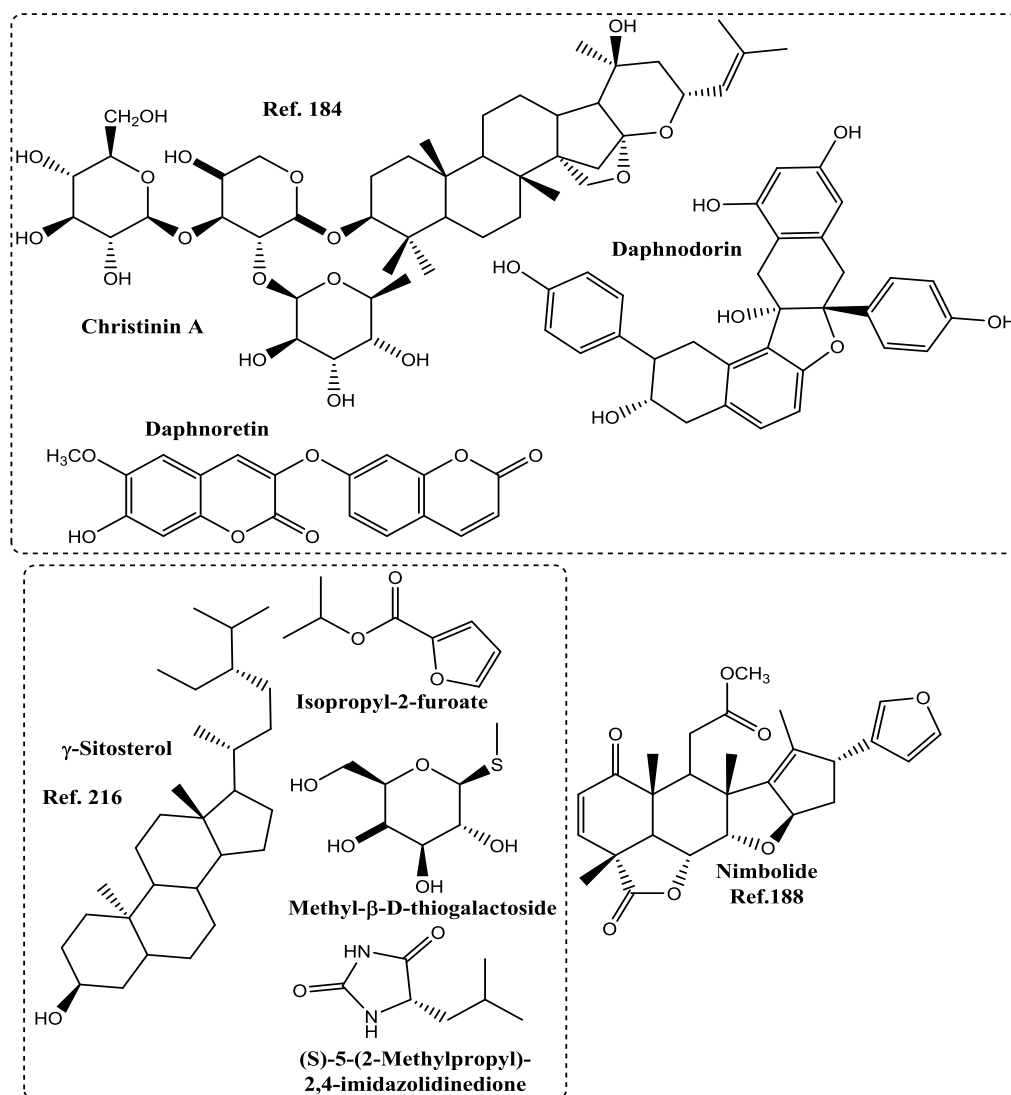
Figure 4: Natural products isolated from *Rhamnus libanotica*.Figure 5: Natural products isolated from *Rhamnus lycioides*.Figure 6: Natural products isolated from *Ziziphus lotus*.



**Figure 7: Natural products isolated from *Ziziphus nummularia*.**



**Figure 8a: Natural products isolated from *Ziziphus spina-christi*.**



**Figure 8b:** Natural products isolated from *Ziziphus spina-christi*.

### 3. DISCUSSION

The trees of the Rhamnaceae family growing between the Jordan river and the Mediterranean sea are very important part of the flora of this region. Traditionally, they were used for a variety of purposes, such as shade, medicine, wood and above all, nutrition. In fact, in more arid regions in Western Asia and North Africa, they are major source of income.<sup>[228]</sup>

As we mentioned several times in previous publications, accurate reporting is vital for the progress of science. With this obvious foundation of science, we found the publication of F., Oguz,<sup>[16]</sup> very inaccurate and misleading. Our statement refers to the chemical compositions listed in the article (table 1 and table 2, there). There are many examples but we will present only two. First, “2-decanal”. This compound cannot exist since aldehyde group cannot be second carbon. Same mistake applies for “2-undecenal”. In addition, for both compounds, the

presented molecular formulas are wrong: decanal should be  $C_{10}H_{20}O$ , not  $C_{10}H_{18}O$ , and undecanal should be  $C_{11}H_{20}O$ , not  $C_{13}H_{18}O_2$ . Even the formulas of simpler compounds such as cyclohexanone are mistakenly reported:  $C_{10}H_{14}O$  instead of  $C_6H_{10}O$ . Second, authors reported the presence of “squalane” in the text (page 359 there) but in table 1 there, the compound is “squalene”, with molecular formula of  $C_{30}H_{50}$ , which is correct. These mistakes and many others, severely limit the reliability of this publication.

Interesting findings were reported by S. Aichour ahc about hemolytic activity of *Rhamnus alaternus* extracts,<sup>[35]</sup> while S. Kherbachi ahc reported antihemolytic activity.<sup>[50]</sup> These seemingly contradicting results are practically not. Plant parts are different (aerial parts, bark and leaves, respectively), extraction solvent (petroleum ether, 70% aqueous ethanol) and method of testing (direct effect on human blood, against AAPH-induced hemolysis). These differences can also explain the great gap in the results of S. Aichour ahc that reported 90% hemolysis, and T.M. Chaouche ahc,<sup>[55]</sup> who used the same method and same concentration, but different plant part (bark and leaves) and different extraction solvent (methanol-acetone 3:2, v/v). They reported 2% hemolysis. It can be concluded that more polar solvents, yield higher phenolic content, lower hemolytic and higher antihemolytic activities.

Following the reports cited in the two paragraphs above, the work of A.H. Brantner and Z. Males has special importance: they presented a method of quality assessment and assurance for *Paliurus spina-christi* extracts.<sup>[229]</sup> Their work focused on phenolic glycosides, their extraction from different plant parts, chromatography and use of standards. And S. Guesmi ahc extracted these active polyphenolic compounds from the leaves of *Rhamnus lycioides* and studied their capacity as radiation ( $^{60}Co$ , 0-30 kGy) bio-dosimeter.<sup>[230]</sup>

Several works were published about the influence of extraction conditions of *Ziziphus lotus* on the results of the extraction, especially on the chemical compositions of the extracts. E. El Maaiden ahc used a multi-step extraction process, that was presented with a comprehensive flowchart, where they extracted four plant parts, with several solvent, *Ziziphus lotus* and *Ziziphus spina-christi*.<sup>[231]</sup> They determined the general chemical composition of each species, and the effect of the different extraction path is clearly evident. T. Letaief ahc studied the results and the kinetics of enzymatic extraction of *Z. lotus* fruit and syrup compared with traditional extraction process.<sup>[232]</sup> They used pectinase and cellulase and report that this method has higher yield and efficiency. M. Ouhammou ahc dried leaves of *Z. lotus* with temperatures ranging from room conditions to 90 °C.<sup>[233]</sup> Expectedly, they found out notable

differences in the chemical compositions of the resulting extracts (acetone) and their antioxidant activity, tested by DPPH method. F. Dahlia ahc studied the influence of locations with different environments, on the chemical composition of several extracts (leaves, pulp, seeds) of this tree.<sup>[234]</sup> They found clear differences in compound families content, as well as  $\beta$ -carotene, lycopene, chlorophyll a and chlorophyll b.

Green nanoparticles have high importance in medicine, catalysis, environmental protection and many other applications.<sup>[235]</sup> Consequently, several publications reported uses of two *Ziziphus* species aqueous extracts for synthesis of green nanoparticles. A summary of these publications is shown in **Table 2**.

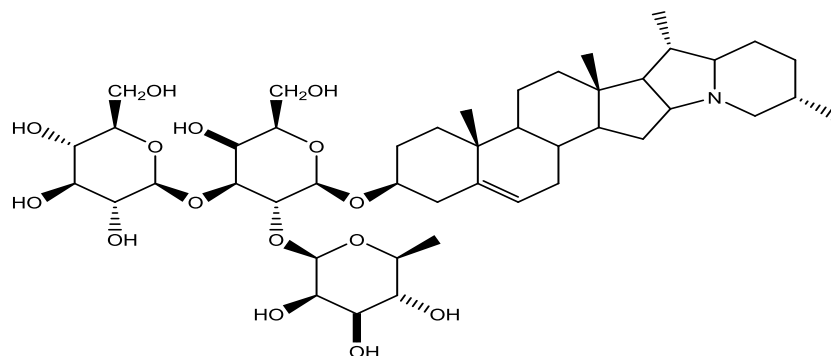
**Table 2: Green Nanoparticles (NPs) Prepared with *Ziziphus* species and Their Activities.**

Species	NPs	Activities/Reference
<i>Ziziphus nummularia</i>	AgNPs	Antimicrobial, antioxidant <sup>[236]</sup>
<i>Ziziphus nummularia</i>	AgNPs	Antimicrobial <sup>[237]</sup>
<i>Ziziphus nummularia</i>	AgNPs	Antimicrobial, antioxidant, cytotoxic, genotoxic <sup>[238]</sup>
<i>Ziziphus nummularia</i>	AgNPs	Antimicrobial, antioxidant (encapsulated) <sup>[239]</sup>
<i>Ziziphus nummularia</i>	AgNPs	Antimicrobial, hair growth promoter <sup>[240]</sup>
<i>Ziziphus nummularia</i>	AgNPs	Antimicrobial <sup>[241]</sup>
<i>Ziziphus nummularia</i>	AgNPs AuNPs	Antimicrobial, antioxidant <sup>[242]</sup>
<i>Ziziphus spina-christi</i>	AgNPs	Antimicrobial <sup>[243]</sup>
<i>Ziziphus spina-christi</i>	AgNPs	Antimicrobial, neurotoxic <sup>[244]</sup>
<i>Ziziphus spina-christi</i>	AgNPs	Antimicrobial (Extract loaded) <sup>[245]</sup>
<i>Ziziphus spina-christi</i>	AgNPs	Antimicrobial (Antifungal) <sup>[246]</sup>
<i>Ziziphus spina-christi</i>	ZnONPs	Antimicrobial, Antioxidant <sup>[247]</sup>

In addition to nanoparticles, *Ziziphus spina-christi* products were published for use in preparations of other modern applications. M. Safian-Boldaji used leaves EO (hydrodistillation) as a natural surfactant based on its hydrophobic nature and lipophilic components (%): geranyl acetone 14, methyl hexadecanoate 10, methyl octadecanoate 9.9, farnesyl acetone 9.9, hexadecanol 9.7 and ethyl octadecanoate 8.<sup>[248]</sup> S. Emadi also used leaves EO that was prepared with same method to prepare a surfactant, exploiting this property of saponins contained in this EO.<sup>[249]</sup> They explain the surfactant property of the saponin by the hydrophobic nature of the steroid part and the hydrophilic nature of the glycoside moiety. E.M. Abdel Bary ahc used cellulose that was obtained from the stem bark fibers, to produce a superabsorbent membranes after modifying with polyvinyl alcohol.<sup>[250]</sup>



Leaves aqueous extracts of various concentrations were used by A. Mahmoud *et al.* to reduce graphene oxide.<sup>[251]</sup> The reduced form had decolorization (aqueous methylene blue), antioxidant (DPPH method) and antibacterial (*E. coli*, *K. pneumonia*, *B. subtilis*, *S. aureus*) activities. M.A. El Hamd *et al.* used hydrothermal method to prepare carbon dots from the powdered leaves and concentrated phosphoric acid.<sup>[252]</sup> These were used as a green fluorometric biosensor for the assessment of rifaximin (antibiotic). To conclude this part, it is highly important to re-indicate the critical effect of the extraction method on the outcome of the extraction. In this context, M. Mohaddes-Kamranshahi *et al.* developed saponin green extraction method from the leaves of this tree, using hydrothermal, microwave and Bain-Marie water bath heating.<sup>[253]</sup> By this, they managed to isolate a structurally interesting saponin-alkaloid shown in **Figure 9**, which they mistakenly state that it contains amide group, where it is actually amine group.



**Figure 9:** Saponin-alkaloid isolated from *Ziziphus spina-christi*.<sup>[253]</sup>

*Ziziphus spina-christi* can tolerate almost all types of climate and other conditions in Western Asia and North Africa. M. Sohail *et al.* studied the salinity tolerance of this tree by using sodium chloride solution and discovered that it can tolerate low to medium salinity.<sup>[254]</sup> They concluded that based on the mineral composition of the fruits that was not affected, and they suggest using *Ziziphus spina-christi* for re-vegetation of lands with these degrees of salinity. S. Naghmouchi and M. Alsubeie reported that this species is highly tolerant towards drought, and they concluded that after testing growth, biomass and proline content, compared with control trees.<sup>[255]</sup> Y. Zait *et al.* conducted experiments that included an exposure of young trees to salinity, drought and high temperatures, and they discovered that the trees tolerated all these stressful conditions.<sup>[256]</sup>

So, although *Ziziphus spina-christi* is very tolerant to external harsh conditions, its content and the consequential properties are clearly dependent on maturity stage, as in all other

plants. A.R. Aldhanhani *ahc* tested several properties of the fruits in three maturity stages: unripe, half-ripe and fully ripe.<sup>[257]</sup> In addition to morphological characteristics and general compositions, they found out clear differences: TFC, TPC, antioxidant activity (ABTS, DPPH methods) and antibacterial activity (four species). Very significant differences in the compounds content of the fruits in these stages were reported. First example is gallic acid ( $\mu\text{g/g}$  of fruit, unripe, half-ripe, fully-ripe): 5.8, 2.22, 7.27. And in the second example of catechol, the amounts are 0.76, 3.36 and 5.95, which means that the amount of this compound in the fully-ripe fruits is around 783% of that in unripe fruits. That is a very large difference. In this research *Z. mauritiana* was also studied. And as in numerous published works, storage conditions have a key role in the quality and the contents of plant material, M.S. AL-Saikhan reports that fruits storage with calcium chloride increased the preservation of the fruits and their contents.<sup>[258]</sup> These findings are consistent with many other results such as those of D.R. Katuwal *ahc* that studied the effect of calcium chloride on tomato fruits shelf life.<sup>[259]</sup>

Finally, these trees can have a key role in environmental protection for the benefit of humans. N. Al-Naimi *ahc* compared the contents of the fruits of *Ziziphus spina-christi* on roadsides and rural areas in Qatar.<sup>[260]</sup> They found clear differences in fruit contents, such as ascorbic acid. These differences are indicative for air quality, and this authors report, is consistent with findings in other countries like Saudi Arabia and Iraq.

#### 4. Selected activities of *ziziphus jujuba*

*Ziziphus jujuba* is a very widespread domestic trees in the reviewed region between the Jordan river and the Mediterranean sea. Most scholars agree that it was domesticated in China, although its domestication date is debated. Recent scientific estimates this time about 6200 years before present.<sup>[261]</sup> It is mainly used for food consumption but its medicinal activities and chemical composition were extensively studied and published.<sup>[262]</sup> Since it is closely related and sometimes confused with other *Ziziphus* trees in this region, we will cite some selected articles about its medicinal activities. This summary is shown in **Table 3**.

**Table 3: Selected published activities of *ziziphus jujube*.**

Activity, Testing Method, Results and Reference
<b>Alzheimer's disease</b> Fruits 70% aqueous ethanolic extract had significant ameliorating effect in amyloid $\beta_{25-35}$ -induced Alzheimer's disease. Activity was observed by behavioral three tests and measured with four biomarkers. <sup>[263]</sup>
<b>Anti-inflammatory</b>

Fruits 95% aqueous ethanolic extract had notable activity against *E. coli*-induced diarrhea and against carrageenan-induced paw edema in rats.<sup>[264]</sup>

#### **Antioxidant**

Fruits from eight locations in five maturation stages, were extracted with water. The obtained extracts were analyzed for TFC, TPC, ascorbic acid, and tested for antioxidant activity by ABTS and DPPH, showing high results.<sup>[265]</sup>

#### **Cardioprotective**

Fruits 80% aqueous ethanolic extract had attenuative effect in isoprenaline-induced myocardial infarction in rats, with or without aerobic training. In both groups positive effect was observed and in the trained group it was higher.<sup>[266]</sup>

#### **Gastroprotective**

Dried fruit juice was supplemented to ethanol-induced ulcerative rats, positive effect measured by three biomarkers. Phenolic composition was determined and antioxidant activity was measured with ABTS and CUPRAC methods.<sup>[267]</sup>

#### **Hepatoprotective**

Fruits 70% aqueous ethanolic extract had ameliorative effect against acetaminophen-induced hepatic toxicity in rats. Effect was measured by concentrations of alanine aminotransferase and aspartate aminotransferase.<sup>[268]</sup>

## **5. CONCLUSIONS**

- 1) Trees of Rhamnaceae family growing in the reviewed region have great nutrition, economic and environmental potentials.
- 2) The published data of these trees has vast range of medicinal and nutritional activities.
- 3) These species are highly tolerant towards stressful conditions so they can be used for vegetation of landscapes with such conditions.
- 4) These plants contain natural products with unique structures and substructures that can be very useful in drug development and discovery.
- 5) Some of the species were not or very limitedly published, and this can open future research horizons and opportunities.

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