

**ANCHUSA PLANTS OF ISRAEL AND PALESTINE. A CONCISE
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***Corresponding Author****Abdullatif Azab**Eastern Plants Company,
Box 868, Arara, Israel,
30025.**ABSTRACT**

Anchusa species are part of the flowering plants of Israel and Palestine, with small, very beautiful blue or white colors, and hairy stems and leaves. These plants possess very interesting chemical compositions. In traditional medicines of the reviewed region and other places over the globe, these plants were extensively used, but surprisingly, modern science is far from a situation of sufficient research of these species, generally speaking. In this mini-review article, the documented ethnobotanical-ethnomedicinal uses will be presented, along with modern research findings. The information will be provided in two separate tables, and to make it more reader-user friendly, the tested activities-properties in modern research will be highlighted. In addition, the structures of the reported natural products isolated from these plants will be presented in separate figures. An extended discussion section will follow the findings listing, followed by

conclusions. Finally, it is important to mention that each one of the six species of Israel and Palestine has a major botanical name and synonyms that will be mentioned, but we will use the major name of each species.

KEYWORDS: *Anchusa*, *Anchusa azurea*, ethnobotany, chemical composition, antimicrobial, antioxidant, antiulcer, anticancer, cardioprotective, anti-inflammatory.

Abbreviations: ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), a/c and her/his colleagues, AChE acetylcholine esterase, BuChE butyrylcholine esterase, CNS central nervous system, CUPRAC cupric reducing antioxidant capacity, DEE diethyl ether, DPPH 2,2-Diphenyl-1-picrylhydrazyl, DCM dichloromethane, EO essential oil, GCC general chemical composition, GC-MS gas chromatography mass spectrometry, HPLC high

performance liquid chromatography, LPS lipopolysaccharide, MDA malondialdehyde, ND not detected, NPs nanoparticles, PBD phosphomolybdenum (assay), PE petroleum ether, STZ streptozotocin, syn. synonym/s, TAC total antioxidant capacity, TFC total flavonoid content, TLC thin layer chromatography, TPC total phenolic content, TRPA total reducing power ability.

1) INTRODUCTION: Taxonomy, Archeology and Published Review Article

Anchusa is one of the genera of the Boraginaceae plant family, *جمحية* in Arabic *זיפנייים* in Hebrew, the two native languages of the reviewed region. This is a relatively large plant family with about 90 genera and 1600-1700 species, even though these numbers are very debated.^[1,2,3] However, 29 genera that include 82 species are native to the reviewed region.^[4] These are: *Anchusa aegyptiaca* syn. *Lycopsis aegyptiaca*, *Asperugo aegyptiaca*; *Anchusa arvensis* syn. *Lycopsis arvensis*, *Anchusa azurea* syn. *Anchusa italica*, *Anchusa milleri* syn. *Anchusa ryssosperma*, *Anchusa strigosa* syn. *Anchusa echinata*, *Buglossum echinatum*, *Buglossum syriacum* and *Anchusa undulata* syn. *Anchusa hybrida*.

In ancient times, *Anchusa* plants were used mainly for external, non-medicinal uses: textile coloration in the Middle East of the fourteenth century and in ancient Egypt^[5,6] and for perfume industry in ancient Egypt as well.^[7]

Surprisingly and strangely, no comprehensive review articles were published about the plants of *Anchusa* plants, and the only published, focused of specific species. F.H. Hussain ahc published a mini-review (despite the fact that they titled it as “comprehensive”) about *Anchusa azurea*^[8], and in even a smaller size article, A.E. Al-Snafi reviewed *Anchusa azurea* and *Anchusa strigosa*.^[9] The article of Z. Chebaro ahc reviews *Anchusa strigosa* in a notably comprehensive fashion: botany, ecology, ethnopharmacology, colored photos, selected natural products and their structures (wide blank spaces, weakness), pharmacological activities, mechanisms of action, 26 pages and 81 references.^[10]

2) Ethnobotany and Ethnomedicine of *Anchusa* Plants of Israel and Palestine

Anchusa plants were notably in ethnomedicine and ethnobotany of many peoples and cultures. The uses are of wide range and they are summarized in **Table 1**.

Table 1: Ethnobotany and Ethnomedicine of *Anchusa* Plants of Israel and Palestine.

Species	Country/Region, Uses, Reference
<i>A. aegyptiaca</i>	Iran; flowers (with flowers of <i>Myrtus</i> and <i>Cichorium</i>); decoction; skin dermatitis, herpes ^[11] Jordan; arial parts; wounds, skin infections, acne; pads are macerated applied externally ^[12]
<i>A. arvensis</i>	Pakistan; leaves; fodder (no details) ^[13,14]
<i>A. azurea</i>	<u>Albania</u> ; flowers; food (no details) ^[15] <u>Azerbaijan</u> ; unspecified; medicinal, dyes (no details) ^[16] <u>Iran</u> ; flowers (with flowers/aerial parts of <i>Thymus</i> and <i>Stachys pilifera</i>); infusion; cold, influenza. ^[11] Flowers; cold; infusion, decoction. ^[17] Flowers; analgesic; no details. ^[18] Flowers; relaxing; infusion, decoction. ^[19] Aerial parts, flowers; eaten raw, infusion, decoction, pads; fodder, burning, wounds, female hygiene, acne, sedative, antistress and antidepressant. ^[20] Aerial parts; cold, sedative; no details. ^[21] Flowers, leaves; decoction; stomach-ache ^[22] <u>Iraq</u> ; young flowers; food, tonic as tea, pulse rate relaxant. ^[23] Leaves; decoction, compress, snakebite ^[24] <u>Jordan</u> ; flowers, food sucking as juice ^[25] <u>Morocco</u> ; roots; powder, used locally for burns and wounds ^[26] <u>Spain</u> ; flowers, food sucking as juice. ^[27] Flowers, basal leaves; food, sucking for juice, stewed ^[28] <u>Turkey</u> ; basal leaves, roots; decoction; women sterility, wounds. ^[29] flowers, food sucking as juice. ^[30] Aerial parts, basal leaves, roots; unspecified; women sterility. ^[31] Aerial parts; cooked and eaten; antidote (no details). ^[32] Aerial parts; infusion; diuretic. ^[33] Aerial parts, roots; decoction; wounds, anticancer, antidote for animals (no details), food (eaten fresh). ^[34] Aerial parts, flowers, leaves; crushed and sucked; snakebite, improve mouth taste. ^[35] Leaves; food; eaten fresh or cooked. ^[36] Aerial parts; food; cooked ^[37]
<i>A. milleri</i>	<u>Saudi Arabia</u> ; whole plant; CNS stimulation; no details. ^[38] Whole plant; unspecified method; CNS stimulation, fever, cough, syphilis ^[39]
<i>A. strigosa</i>	<u>Iran</u> ; flowers; infusion; antidepressant, memory enhancement; cold. ^[40] Flowers; tea with lemon as heart tonic, sedative, exhilarating, cold catarrh (mixed with other plants). ^[41] Unspecified part(s), unspecified method(s); heart and CNS tonic, cold. ^[42] <u>Jordan</u> ; arial parts; wounds, female sterility, anthelmintic, headache; pads, vapor, decoction, respectively. ^[12] Roots; direct application; wounds, dermal ulcers, burns, tuberculosis; decoction; diuretic ^[43] <u>Turkey</u> ; aerial parts; decoction; anticancer ^[34] Leaves; infusion; analgesic. ^[35] Young leaves, flowers; food; cooked with onion, sucked for nectar. ^[44]
<i>A. undulata</i>	<u>Jordan</u> ; flowers, food sucking as juice ^[25] <u>Lebanon</u> ; leaves; food; diuretic, cough, muscular pain, stomach-ache, rheumatism; unspecified method(s). ^[45] Leaves; food, cold (medicinal); unspecified method(s). ^[46] <u>Spain</u> ; basal leaves; food, stewed ^[28] <u>Turkey</u> ; leaves; antidiabetic; cooked and eaten. ^[36] Aerial parts; food; cooked. ^[37] Flowers; food; sucked for nectar. ^[47,48,49]

3) Published Activities-Properties of *Anchusa* Plants of Israel and Palestine

Surprisingly, the number of published modern research studies of the *Anchusa* plants of Israel and Palestine is notably low. This statement is based on the fact that these species are very well-known for many uses as food and in ethnomedicine (**Table 1**). Modern science significantly investigated the chemical compositions of some of these plants, not all of them,

yet very partially studied and published their medicinal properties-activities. A summary of these findings is presented in **Table 2**.

Table 2: Published Activities-Properties of *Anchusa* Plants of Israel and Palestine.

Activity-Property, Testing Method(s), Result(s), Reference
<p><i>Anchusa aegyptiaca</i> syn. <i>Lycopsis aegyptiaca</i>, <i>Asperugo aegyptiaca</i> Shoots were defatted with PE and extracted with water. Both extracts were analyzed for GCC, TFC and TPC. The aqueous extract was tested for antimicrobial (five bacterial and five fungal strains), antioxidant (DPPH method) and cytotoxicity (using chromosome abbreviation assay, seeds of <i>Vicia faba</i>) activities.^[50] Whole plant methanolic extract had moderate antiparasitic activity against <i>Schistosoma mansoni</i> adult worms.^[51] Whole plant was separately extracted with water and methanol, and both extracts were analyzed for TPC and antioxidant (DPPH method) and moderate insecticidal (against <i>Aedes aegyptii</i> larvae) activities.^[52]</p>
<p><i>Anchusa arvensis</i> syn. <i>Lycopsis arvensis</i> Whole plant was extracted with methanol and this extract was fractionized with <i>n</i>-hexane, chloroform and ethyl acetate. The crude extract and the fractions had significant anticancer activity against HepG-2 human hepatocellular carcinoma cell line. Analysis of the extract for active compounds yielded 4-methoxycatechol (Figure 1) and decane. Molecular docking was performed for these compounds.^[53] Whole plant was extracted with methanol and this extract was fractionized with <i>n</i>-hexane, chloroform and ethyl acetate. The crude extract and the fractions were tested for antioxidant (ABTS, DPPH methods, ascorbic acid was reference) and anticancer (against NCI-H460 cancer cell line) activities. GCC of crude extract was determined.^[54] Whole plant was extracted with methanol and this extract was fractionized with <i>n</i>-hexane, chloroform and ethyl acetate. The chloroform fraction was chromatographed affording a new compound, 3-hydroxyoctyl-5-<i>trans</i>-docosenoate (Figure 1). This compound was tested for antioxidant (ABTS, DPPH methods) and anticancer (against HepG-2 human hepatocellular carcinoma cell line) activities. Molecular docking was performed for anticancer activity of the new ester.^[55] Whole plant 45% aqueous ethanolic extract had potential antidiabetic activity tested by enzymes (albumin, haemoglobin and crystalline) glycation inhibition.^[56] Whole plant was separately extracted with 45% aqueous ethanol and water, and both extracts were analyzed for TFC, TPC, and tested for antioxidant (DPPH method) activity. Both extracts were chromatographed to determine the contents of cyanidin and delphinidin 3-glucosides (Figure 1).^[57] Seeds oil was extracted using PE and it was analyzed (GC-MS) for fatty acid composition. Authors highlighted the content of six acids (%) were: oleic 29, linoleic 23.8, α-linolenic 10.8, palmitic 9.2, γ-linolenic 6.7 and erucic 3.4.^[58] Whole plant was extracted for alkaloids with 0.5% HCl_(aq) and the extract was analyzed using several techniques. Seven alkaloids were detected (Figure 1).^[59]</p>
<p><i>Anchusa azurea</i> syn. <i>Italic</i> Aerial parts methanolic extract had weak activity against MCF7, HepG2 and WEHI164 cancer cell lines.^[21] Whole plant was separately extracted with water and methanol, and both extracts were analyzed for TPC and antioxidant (DPPH method) and moderate insecticidal (against <i>Aedes aegyptii</i> larvae) activities.^[52]</p>

Seeds oil was extracted using PE and it was analyzed (GC-MS) for fatty acid composition. Authors highlighted the content of six acids (%) were: oleic 31, linoleic 32, α -linolenic 0.3, palmitic 8, γ -linolenic 6.2 and erucic 10.3.^[58]

Aerial parts methanolic extract was analyzed for total monomeric anthocyanin and TFC contents, and was tested for **antioxidant** activity (methods: ABTS, DPPH, FRAP, β -carotene bleaching). It had **anticancer** activity against MCF-7, MDA-MB-231, RKO, and R2C cell lines. Analysis of this extract (HPLC) 12 previously known phenolics, where the three major compounds were (μ g/g, **Figure 2A**): astragalin 313., catechin 499.3, chlorogenic acid 699. A mechanism of action is presented.^[60]

Whole plant 95% aqueous ethanolic extract was fractionized with PE, ethyl acetate and *n*-butanol, and the *n*-butanol fraction was chromatographed yielding six compounds (**Figure 2A**): oleanazuroside 1, oleanazuroside 2, 24-hydroxytormentic acid ester glucoside, 24-epi-pinfaensin, oleanolic acid 3-O- α -l-arabinoside and (3 β ,21 β)-21-[(β -D-glucopyranosyl-(1-2)- β -D-glucopyranosyl)oxy]-3-hydroxyolean-12-en-28-oic acid (new). Oleanolic acid 3-O- α -l-arabinoside had moderate **anticancer** activity against A-549, MDA-MB-231, KB, KB-VIN and MCF-7 cell lines.^[61]

Whole plant 95% aqueous ethanolic extract was fractionized with PE, ethyl acetate and *n*-butanol, and the *n*-butanol fraction was chromatographed yielding 24 known compounds. Three of these compounds (**Figure 2B**) showed **anticomplementary** activity: medioresinol, 5-hydroxypyrrolidin-2-one and 5-hydroxyl-3', 4', 6, 7-tetramethoxy flavone.^[62]

Flowers 70% aqueous ethanolic extract and was analyzed for TPC. It had **anticonvulsant** activity against pentylenetetrazole-induced seizures in mice. It had also *in vivo* **antioxidant** activity measured by several biomarkers in serum and prefrontal cortex.^[63]

Whole plant 70% aqueous ethanolic extract had **antitussive** (ammonia-induced cough in rats), **anti-inflammatory** (carrageenan-induced paw edema in rats) and **antimicrobial** (against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans* strains) activities.^[64]

Leaves and roots were separately extracted with 70% aqueous ethanol and the extracts were qualitatively analyzed for GCC and for phenolic composition (HPLC). These extracts were tested for **burn healing** activity (rabbits), and **anti-inflammatory** (carrageenan-induced in rats), **antinociceptive** (acetic acid-induced abdominal contortions in rats) and **antidepressant** (forced swimming in rats, diazepam was positive control) activities.^[65]

Unspecified plant part(s) 80% methanolic extract had moderate **hormone-sensitive lipase inhibition** activity.^[66a]

Aerial parts methanolic extract was fractionized with DEE, chloroform, ethyl acetate and water, and all five materials were analyzed for TFC and TPC. Crude extract and the four fractions were tested for **antihemolytic** (measuring half hemolysis time) and **antioxidant** (DPPH and β -carotene bleaching methods). The **anti-inflammatory** activity of the extract was tested using phorbol 12-myristate 13-acetate, PMA- induced inflammation in mice.^[67]

An earlier study by the same group cited in the previous work using the same extraction methods. The **antioxidant** and **antihemolytic** activities tested blood serum and plasma isolated from mice using DPPH and FRAP methods. The **antibacterial** activity was tested against 11 bacterial strains.^[68]

Aerial parts 75% aqueous ethanolic extract was partitioned with PE, DCM, ethyl acetate and *n*-butanol. All five materials were tested for **cell viability** and **anti-inflammatory** activity (LPS-induced) in RAW264.7 cell lines. Anti-inflammatory effect was measured with several parameters and a mechanism of action is proposed.^[69]

Aerial parts 80% aqueous methanolic extract was supplemented to rats showing **anti-inflammatory** activity, where effect was verified by measuring pro- and anti-inflammatory biomarkers. The **antioxidant** activity was tested using DPPH method and the **burn wound healing** activity was tested on such burns in rats, using ointment containing this extract.^[70]

Aerial parts and roots were separately extracted with methanol and water. The methanolic extract was partitioned with *n*-hexane and *n*-butanol, and the *n*-butanol fraction was chromatographed affording rosmarinic acid. All four materials had **anti-inflammatory** activities in carrageenan-induced inflammation in rats. The **antiulcer** activity was tested against indomethacin-induced ulcer. Rosmarinic acid showed anti-inflammatory activity using the same model, with ibuprofen as a reference drug.^[71]

Flowers 70% aqueous ethanolic extract was analyzed for TFC and TPC and tested for **anti-ischemia** activity. Effect was tested by measuring **antioxidant** activity (DPPH method) and *in vivo* **anti-inflammatory** (rats, NO and MDA production inhibition). A mechanism of action is proposed.^[72]

Whole plant 60% aqueous ethanolic extract was analyzed for very detailed qualitative chemical composition. It had **ischemia** activity, and a mechanism of action is proposed.^[73]

Leaves and roots were separately extracted with 70% aqueous ethanol and both extracts were analyzed for TFC and TPC. Both extracts had significant **antimicrobial** (seven strains) and **antioxidant** (DPPH, FRAP, TAC methods) activities.^[74]

Whole plant 80% aqueous ethanolic extract was partitioned with ethyl acetate, *n*-butanol and pentanol (unspecified isomer). Extract and fractions were analyzed for TPC and tested for **antimicrobial** (four bacteria and two fungi species). The extract was chromatographed yielding Kaempferol 3-*O*-Rutinoside (**Figure 2B**).^[75]

Whole plant methanolic extract analysis afforded 17 known compounds but some of them were isolated from this plant and/or genus and/or family for the first time (**Figure 2B**): 4-Hydroxy-*N*-(4-(3-(4-hydroxyphenyl)-*E*-acryloylamino)-butyl)-benzamide (1), 1-*O*- β -D-Glucopyranosyl-1,4-dihydroxy-2-(3',3'-dimethylallyl)-benzene (2), Oresbusin A (3), Benzyl-*O*- β -D-glucopyranoside (4), Methyl-3,4-dihydroxycinnamate (5), Lolilide (both enantiomers, 6,7) and Syringaresinol (both enantiomers, 8,9). Two of the 17 compounds, previously isolated from this plant, had weak to moderate antibacterial activity.^[76]

Aerial parts aqueous extract was analyzed for TFC and TPC, and tested for **antioxidant** activity using ABTS, CUPRAC, DPPH, FRAP and TRPA methods.^[77]

Roots aqueous, methanolic and ethyl acetate extracts were analyzed for TFC, TPC, and tested for **antioxidant** activity using ABTS, CUPRAC, DPPH, FRAP, PBD and ferrous-chelating methods. They also had notable **enzyme inhibition** activity: AChE, BuChE, α -amylase, α -glucosidase and tyrosinase.^[78]

Areal parts 75% aqueous ethanolic extract was chromatographed affording 27 compounds, which have the general structure shown in **Figure 2C**. Some compounds with similar general structures are shown in **Figure 2A**. Two of these compounds, 2 α ,3 β ,19 α ,23-tetrahydroxyurs-12-en-28,21 β -olide and laevigin C (**Figure 2C**, compounds 6,14 in the cited article, respectively), have slightly different structures. All 27 compounds were tested for protective effects on hypoxia/reoxygenation induced cardiomyocytes injury and this compound was most active. A mechanism of action is proposed.^[79]

Whole plant 60% aqueous ethanolic extract was purified with 75% aqueous ethanol yielding phenolics-rich extract, which was chromatographed resulting the presence of major well-known compounds: hesperidin, kaempferol, naringenin, quercetin and rutin. The final extract improved cardiac function and alleviated cardiac remodelling post myocardial infarction in mice.^[80]

Aerial parts 75% aqueous ethanolic extract was purified with five aqueous ethanolic solutions (0, 30, 50, 70 and 95%), and the fourth fraction was chromatographed. Fourteen compounds were isolated, three of them were new (**Figure 2C**): 3-oxo-21 β -{[β -D-glucopyranosyl(1-2)- β -D-glucopyranosyl]oxy}-23-hydroxyolean-12-en-28-oic acid (1), 2 α ,3 β -dihydroxy-19-oxo-1819-secours-1113(18)-dien-24-formyl-28-*O*- β -D glucopyranoside (2), 21-oxo-2 α ,3 β ,23-trihydroxyolean-12-en-28-oic acid β -D-glucopyranoside(3). All 14 compounds were tested for protective effect on cardiomyocytes injured by hypoxia/reoxygenation in cultured neonatal rat cells, but only six of them showed significant activity.^[81]

Roots methanolic extract was chromatographed resulting isolation of five previously known

phenolics: astragalin, isoquercetin, rutin, kaempferol rutinoside and rosmarinic acid.^[82]

Analysis of aerial parts EO (hydrodistillation) afforded 33 previously known compounds, where the major two were: diisobutyl- 14.6 and dibutyl phthalate 9%.^[83]

Whole plant methanolic extract had significant **antioxidant** activity tested with FRAP method. EO of the plant (dry heating, 60-70 °C) was analyzed (GC-MS) affording 42 known compounds, where the major (%) three were: *trans*-caryophyllene 13.26, spathulenol 11.27 and germacrene D 10.47, (**Figure 2D**).^[84]

Flowers were investigated for microstructure and qualitatively analyzed for chemical composition. Ninety compounds were identified.^[85]

Whole plant 70% aqueous ethanolic extract was fractionized with PE, DCM, ethyl acetate and *n*-butanol. The ethyl acetate and *n*-butanol fractions were chromatographed yielding 31 compounds of which six are new (**Figure 2D**): 2 α ,3 β ,19 α -trihydroxy-23-formyl-urs-12-en-28,21 β -olide (1), (2R,6R,9S)-9-hydroxy-4-megastigmen-3-one-2-O- β -D-glucopyranoside (2), (2R,6S,9S)-9-hydroxymegastigman-4,7-dien-3-one-2-O- β -D-glucopyranoside (3), (+)-isololiolide β -D-glucopyranoside (4), (2S,8R)-loliolide β -D-glucopyranoside (5) and (2R,8S)-loliolide β -D-glucopyranoside (6). Twenty of the isolated compounds were tested *in vitro* in experimental model of hypoxia/reoxygenation (H/R) injury in neonatal rat cardiomyocytes (**cardioprotective**), showing moderate to weak activity.^[86]

Whole plant methanolic extract was partitioned with chloroform, ethyl acetate and water. All for materials for TFC and TPC. They were tested for **xanthine oxidase inhibition**, *in vitro* and *in vivo* (mice): in the first case none to weak effect was observed, while in the second case, the effect was moderate. They were also tested for **hypouricemic** activity *in vivo* showing significant effect.^[87]

Flowers 70% aqueous ethanolic extract was analyzed for TFC and TPC and tested for **antioxidant** activity: *in vitro* using DPPH method and *in vivo* using rats brain serum. It was also tested for **neuroprotective** effect on global cerebral ischemia and reperfusion in rats, showing significant results.^[88]

Roots and shoots were subjected to separate ultrasonically assisted separately extraction using 100, 80 and 60% aqueous methanol, and the three extracts of each plant part were combined. Shoots (combined) extract was analyzed for allantoin (ND), 4-hydroxybenzoic acid (ND), rutin (ND), hydrocaffeic acid (ND), rosmarinic acid (28 mg/g) and chlorogenic acid (ND). Roots (combined) extract was analyzed for allantoin (0.3 mg/g), hydrocaffeic acid (ND), shikonin (ND) and rosmarinic acid (3.8 mg/g).^[89]

a) In "Plant extraction" section of this article, it is stated that "sample of each ground plant material of the used parts (Table 1)". In "Table 1", the only table in this article, plant parts are not indicated.

Anchusa milleri* syn. *Ryssosperma

Whole plant was extracted for alkaloids with 0.5% HCl (aq) and the extract was analyzed using several techniques. Nine alkaloids were detected (**Figure 3**), including supinine which is shown in **Figure 1**.^[59]

Aerial parts aqueous extract was analyzed for GCC, TFC and TPC, and had significant **Antioxidant** activity tested with DPPH method, with ascorbic acid as a reference. It had notable **antibacterial** activity (against *E. faecalis*, *S. aureus*, *P. aeruginosa*, and *K. pneumonia* strains), **antiviral** moderate activity against two viruses (CoxB4, HSV-1) and **anticancer** activity against Vero and HepG2 cell lines.^[90]

Anchusa strigosa* syn. *echinata*, *Buglossum echinatum*, *B. syriacum

Whole plant aqueous and methanolic extracts were analyzed for GCCs. Both extracts had ameliorating **antiarthritic** effect (complete Freund's adjuvant-induced arthritis) in rats, with betamethasone as reference drug. Effect was measured with four biomarkers.^[91]

Leaves and roots were separately extracted with PE, *n*-hexane, chloroform, methanol and ethanol, using two methods: cold maceration and Soxhlet hot extraction. Extracts were for **anticancer** activity against MCF-7, MDA-MB-231, T-47D and Caco-2 cell lines. Authors reported that extracts

were analyzed with TLC, presented photos of two TLC plates, indicated that 11 “compounds” were identified, but these “compounds” were not listed.^[92]

Leaves aqueous extracts were prepared using maceration and ultrasound assisted extractions and both extracts were analyzed for TFC and TPC. Extracts were tested for **anticancer** and **wound healing** (against Capan-2 pancreatic cancer cells) and **antioxidant** (DPPH method) activities. Extracts were qualitatively analyzed using LC-MS yielding mainly phenolic and other acids along with other well known phenolic compounds.^[93]

Whole plant (?) was separately/successively (?) extracted with *n*-hexane, ethyl acetate, ethanol and methanol. Extract/s (?) was/were (?) analyzed with TLC and HPLC resulting five well known phenolics. Extract/s (?) was/were (?) tested for **anticancer** (against A357 and RSAR001 cancer cells) and **antioxidant** (DPPH method) activities.^[94]

Flowers aqueous extract had **antidiabetic** activity in STZ-induced diabetic rats. Effect was mainly detected by blood glucose lowering.^[95]

Flowers, leaves and roots were separately defatted with *n*-hexane and analyzed for alkaloids content, where the highest was in leaves. The extracts and their mixtures were tested for **antifeedant** activity against to *Spodoptera exigua* and *Pieris brassicae* larvae where leaves extract was most potent. This extract was chromatographed for alkaloids yielding eight compounds (**Figure 4A**), two of them (7 and 8) were new.^[96]

Flowers *n*-hexane extract was analyzed for fatty acid composition resulting octadecanoic (stearic) acid as major component, 4.6 µg/100 g dry extract. Extract had **antibacterial** activity against ten bacterial strains showing weak to moderate effect.^[97]

Roots aqueous extract had **antiulcer** activity against ethanol-induces ulcer in rats.^[98]

Roots were separately extracted with ethanol, *n*-butanol, chloroform and PE. Ethanolic extract for alkaloids presence resulting significant result. The other threes extracts were tested for **antiulcer** activity against ethanol-induced ulcer in rats, resulting good to high activities (PE > chloroform > *n*-butanol). PE extract was analyzed for chemical composition where four compounds with steroidal structures were major components: oleanolic acid, β-amyrin, crataegolic acid and β-sitosteryl glucoside.^[99]

An earlier study of the research cited by reference 96. Roots were successively extracted with chloroform, 90% chloroform-methanol and methanol. The chloroform-methanol extract was chromatographed affording nine new compounds (**Figure 4A** and **Figure 4B**).^{b[100]}

Roots aqueous extract had **pepsin inhibition** activity.^[101]

Leaves 70% aqueous ethanolic extract had **no toxicity** in rats.^[102]

Flowers and leaves 70% aqueous ethanolic extract ^c was qualitatively analyzed for chemical composition yielding nine compounds or compound families (such as alkaloids). The extract and its identified compounds had **wound healing** (in human dermal fibroblasts cell lines) and **antibacterial** (against *E. coli* and *S. enteritidis*) activities. Isovaleraldehyde and cubebene (**Figure 4B**) were among the volatile compounds found in this extract.^[103]

b) Compound 7 in this research (5 in the article) is identical to compound 5 in our figure 4, so it is not new, unless the stereochemistry (*S,S,S,S*) which is not indicated in reference 100, is different.

c) Authors direct readers to three of their previous publications for extraction method(s). In these past publications, several extracting solvents were used, while in present study, there is no clue which of the solvents was used.

Anchusa undulata* syn. *hybrida

Seeds oil was extracted using PE and it was analyzed (GC-MS) for fatty acid composition. Authors highlighted the content of six acids (%) were: oleic 42.7, linoleic 12.5, α-linolenic 1.2, palmitic 8, γ-linolenic 1.1 and erucic 5.7.^[58]

Roots and shoots were subjected to separate ultrasonically assisted separately extraction using 100, 80 and 60% aqueous methanol, and the three extracts of each plant part were combined. Shoots (combined) extract was analyzed for allantoin (2.9 mg/g), 4-hydroxybenzoic acid (0.2), rutin (0.5),

hydrocaffeic acid (ND), rosmarinic acid (29) and chlorogenic acid (ND). Roots (combined) extract was analyzed for allantoin (0.09), hydrocaffeic acid (ND), shikonin (ND) and rosmarinic acid (30).^[89]

Aerial parts and roots were combinedly extracted with 80% aqueous methanol and the resulting extract was analyzed for TFC and TPC. It was tested for **antidiabetic** (inhibition of α -amylase and α -glucosidase), **antioxidant** (ABTS and DPPH methods) and **enzyme inhibition** (AChE and BuChE) activities.^[104]

Aerial parts methanolic extract had significant **antimicrobial** activity against nine bacteria and fungi species. The extract was chromatographed yielding a new compound, undulatoside (**Figure 5**): 3-O-(β -D-glucopyranosyl)-29-O-(β -D-glucopyranosyl)-2 α ,23-dihydroxyolean-12-en-28-oic acid. In addition to seven known triterpene glycosides (with structures close to oleanazuroside 1, reference 61, **Figure 2A**) and quercetin 3-O-Rutinoside (**Figure 5**) were isolated for the first time from this species.^[105]

Aerial parts methanolic extract was analyzed for phenolic composition affording 13 known compounds, where rosmarinic acid had highest concentration, 5.53 mg/g dry extract.^[106]

Aerial parts methanolic extract was analyzed for TFC, TPC, total condensed tannins, fatty acids and total saponins contents. Single fatty acids were also identified. Extract was tested for **neuroprotective** (inhibition of AChE), **skin care** (inhibition of tyrosinase), **antidiabetic** (inhibition of α -amylase and α -glucosidase) and **antioxidant** (β -carotene-linoleic acid, PBD, DPPH, ABTS, hydroxyl radical scavenging, superoxide anion scavenging, nitric oxide scavenging, reduction of ferric to ferrous ions, CUPRAC, FRAP and ferrous ion chelating, methods) activities.^[107]

* Unless indicated otherwise, solvent mixtures are volume/volume, v/v.

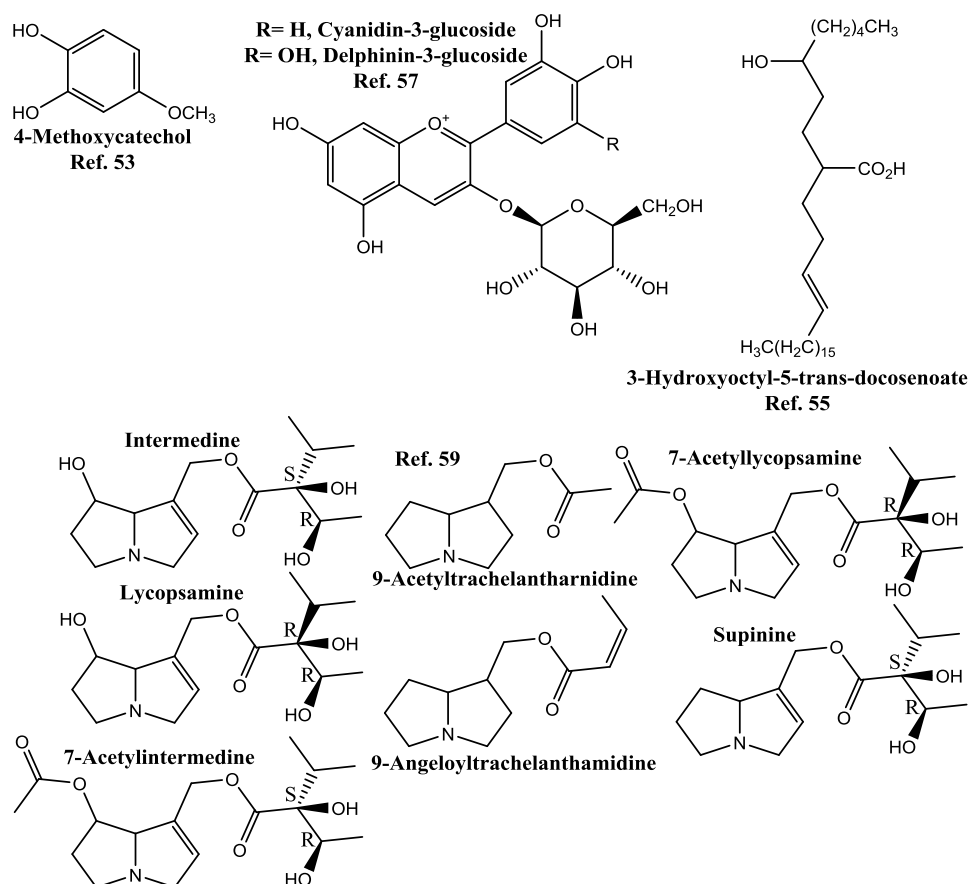


Figure 1: Natural products isolated from *Anchusa arvensis*.

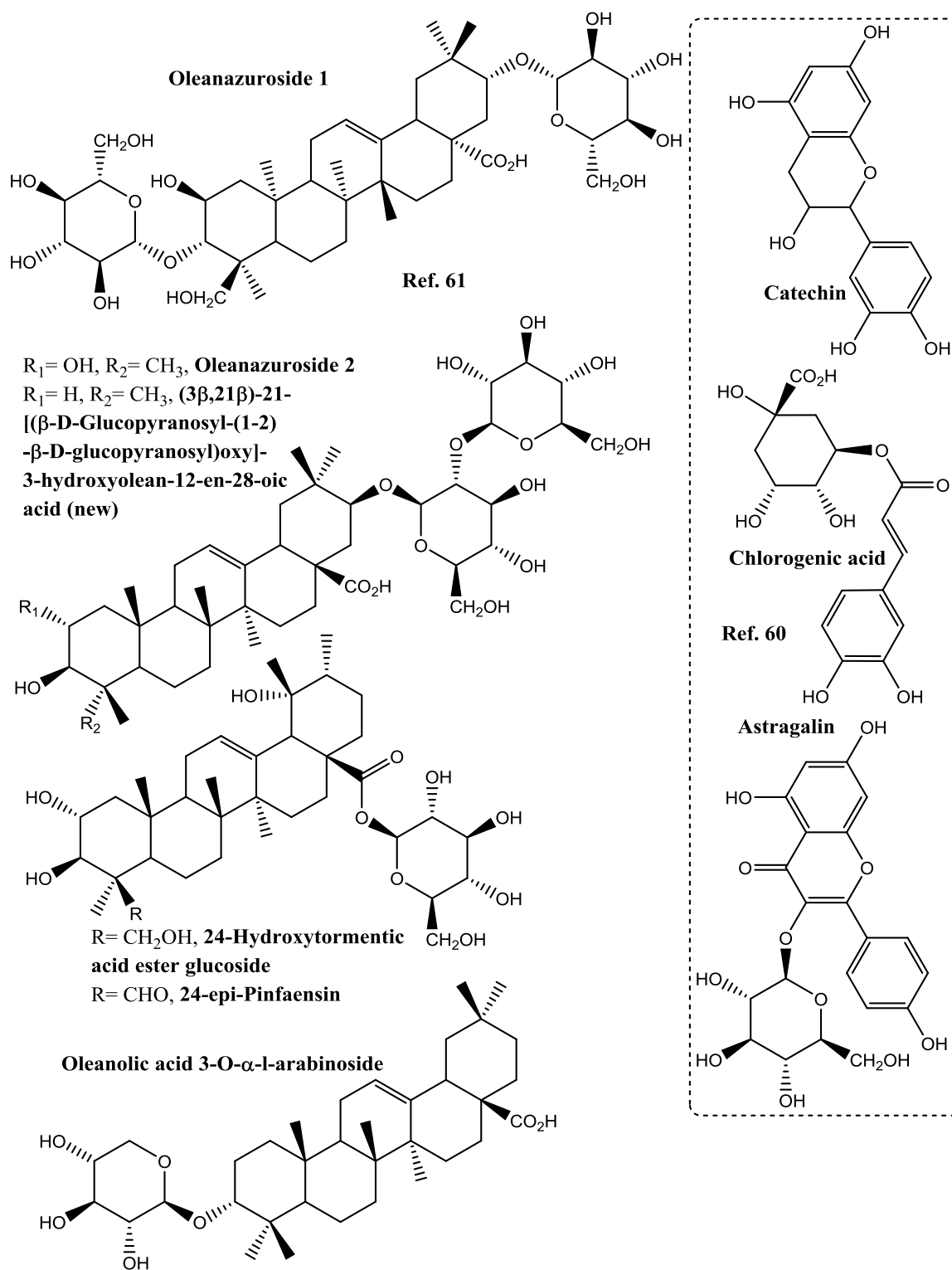


Figure 2A: Natural products isolated from *Anchusa azurea*.

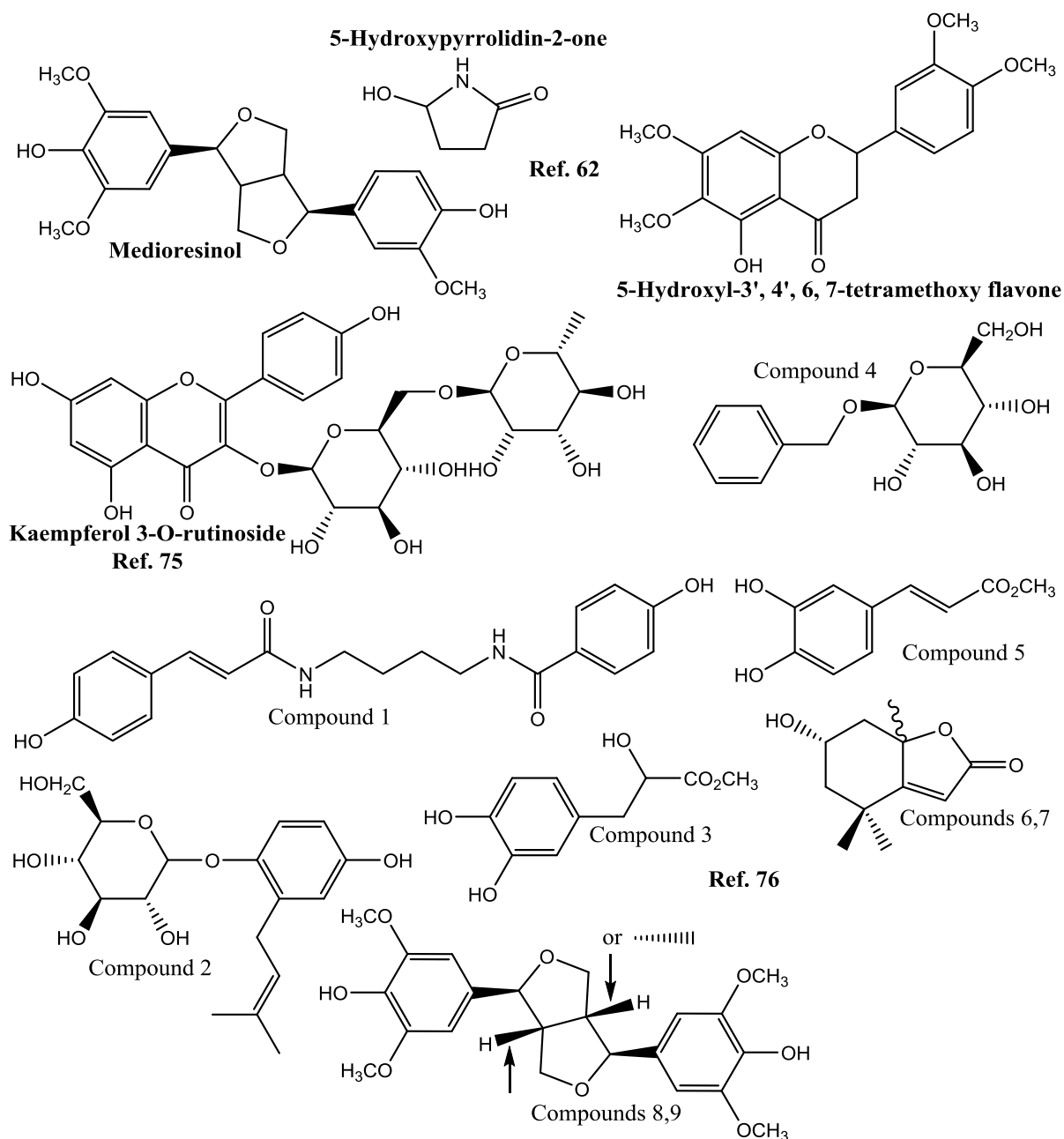


Figure 2B: Natural products isolated from *Anchusa azurea*.

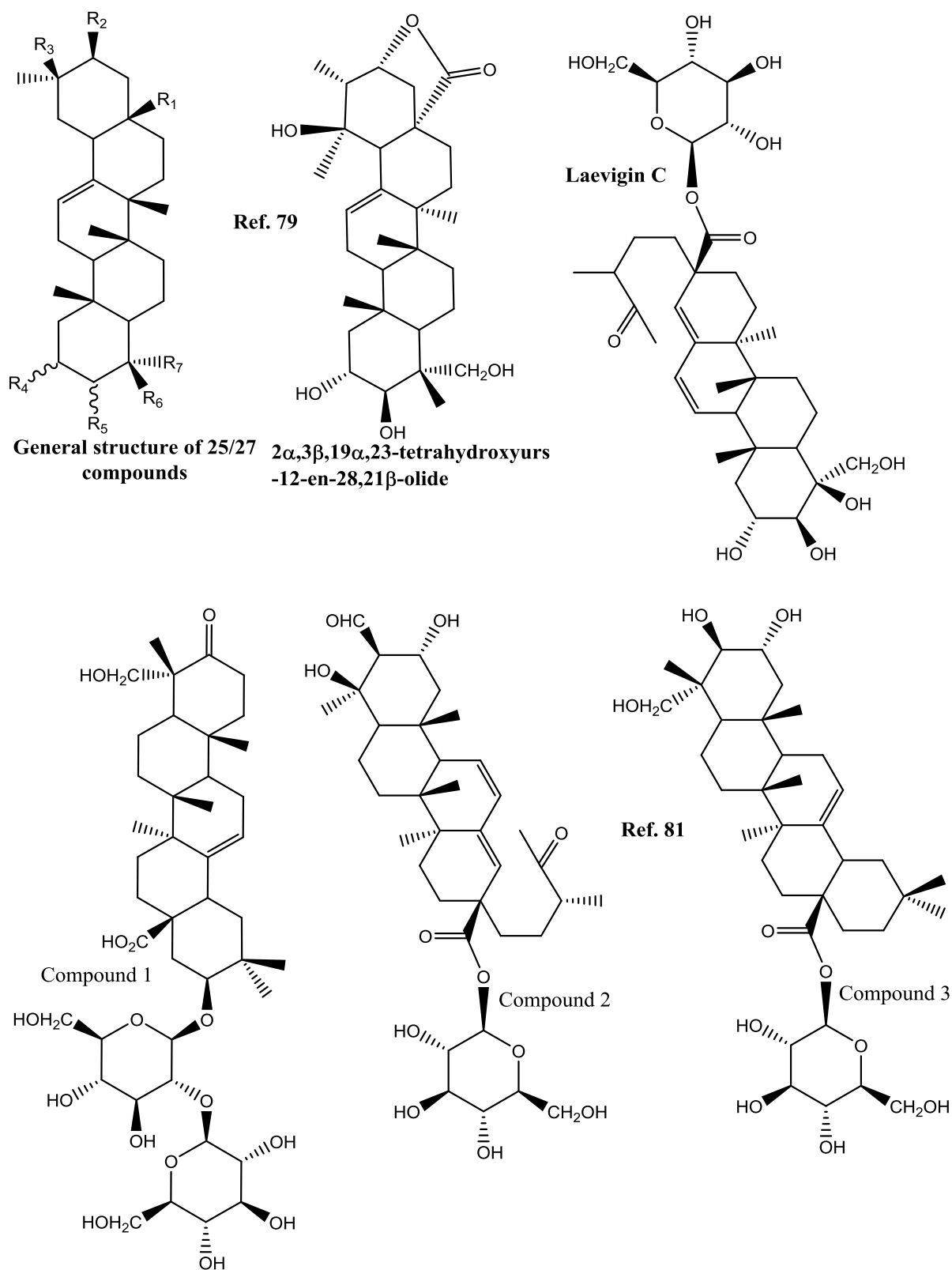
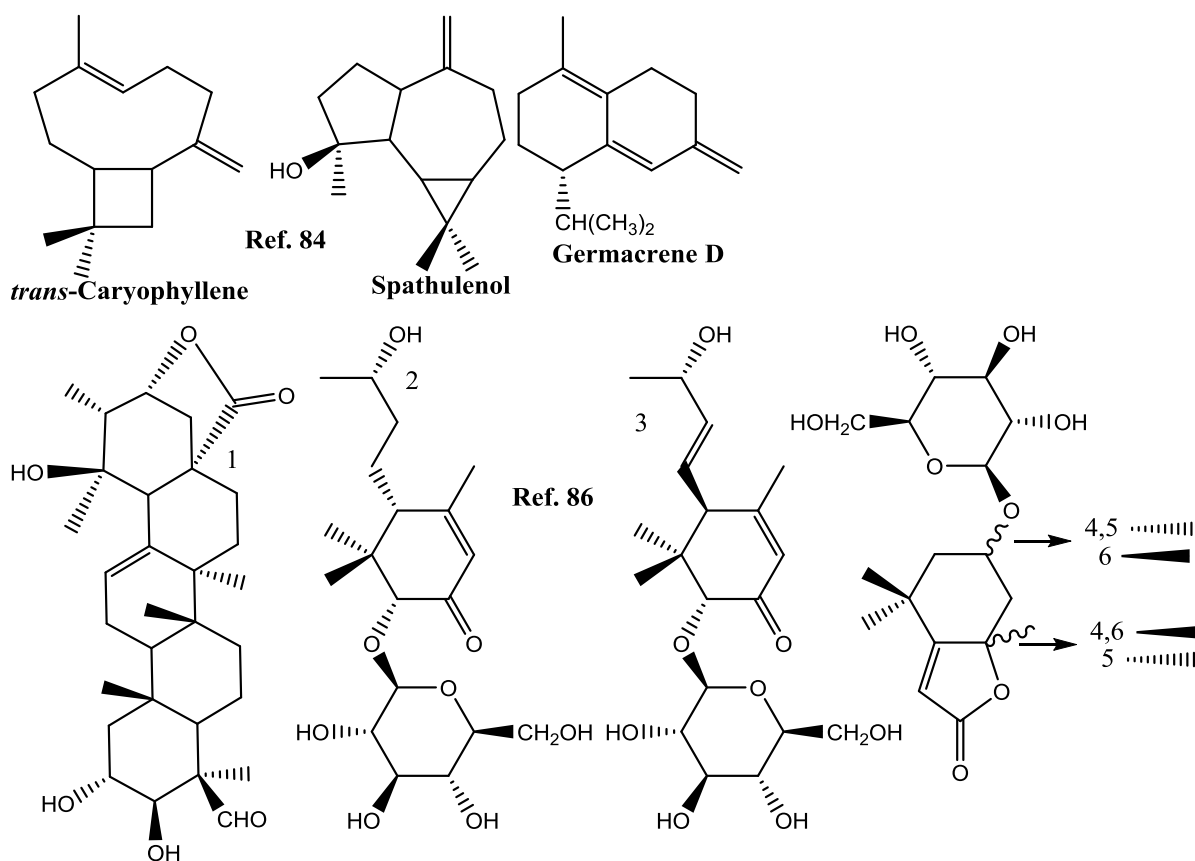
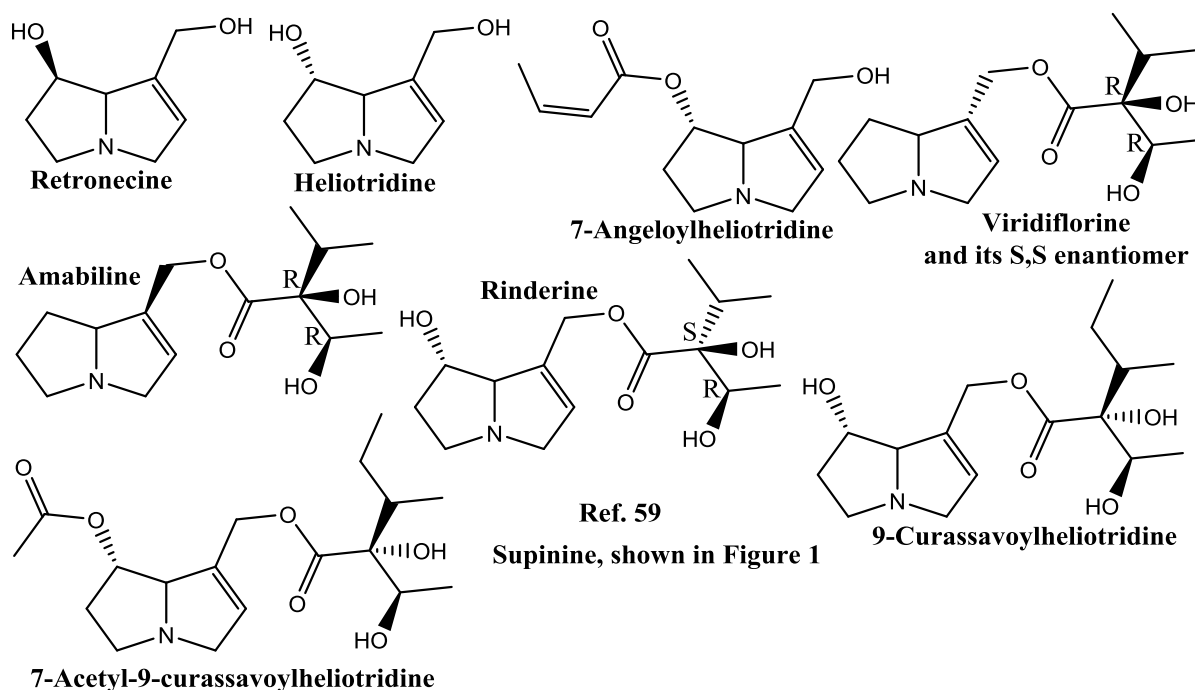


Figure 2C: Natural products isolated from *Anchusa azurea*.

Figure 2D: Natural products isolated from *Anchusa azurea*.Figure 3: Natural products isolated from *Anchusa milleri*.

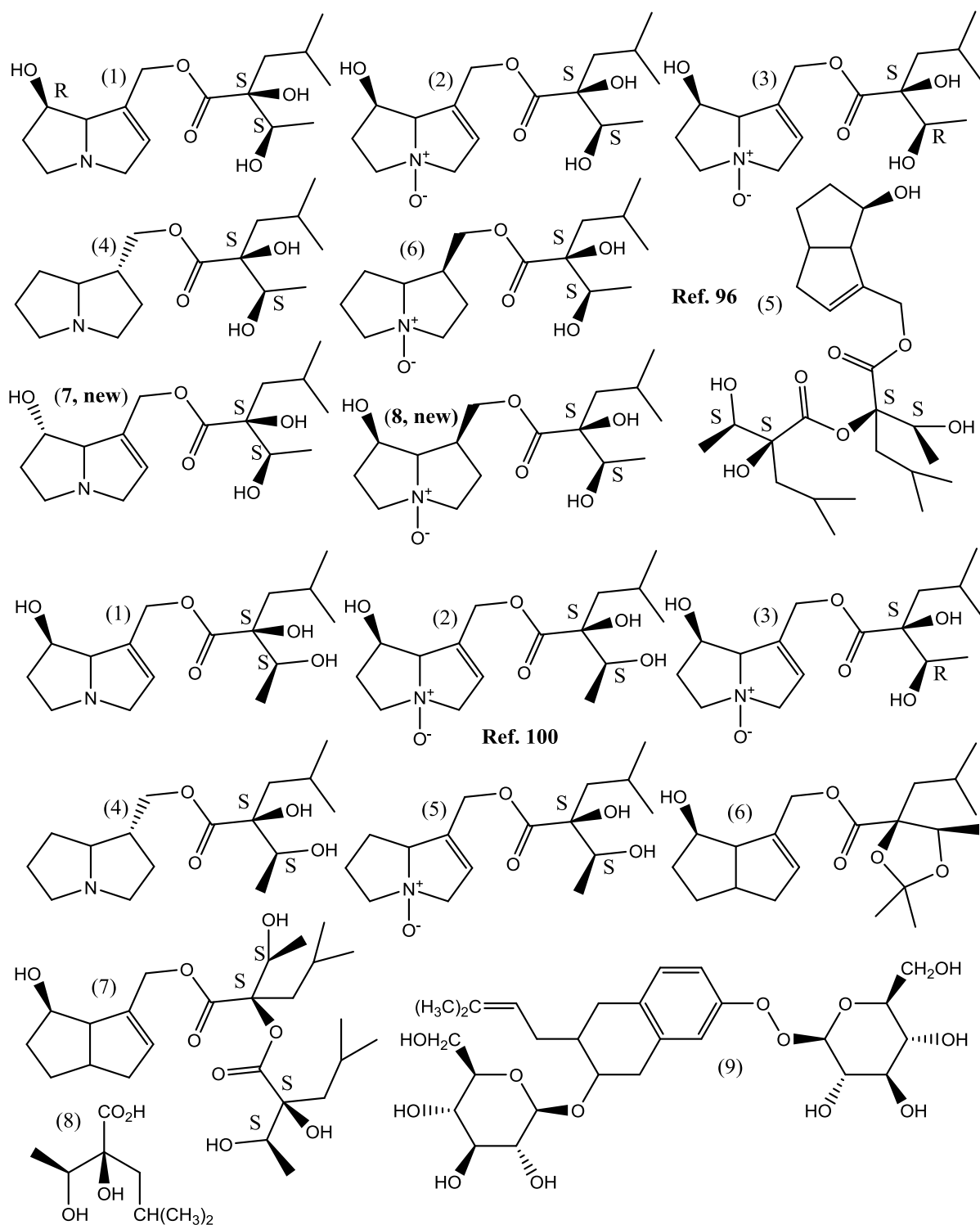


Figure 4A: Natural products isolated from *Anchusa strigosa*.

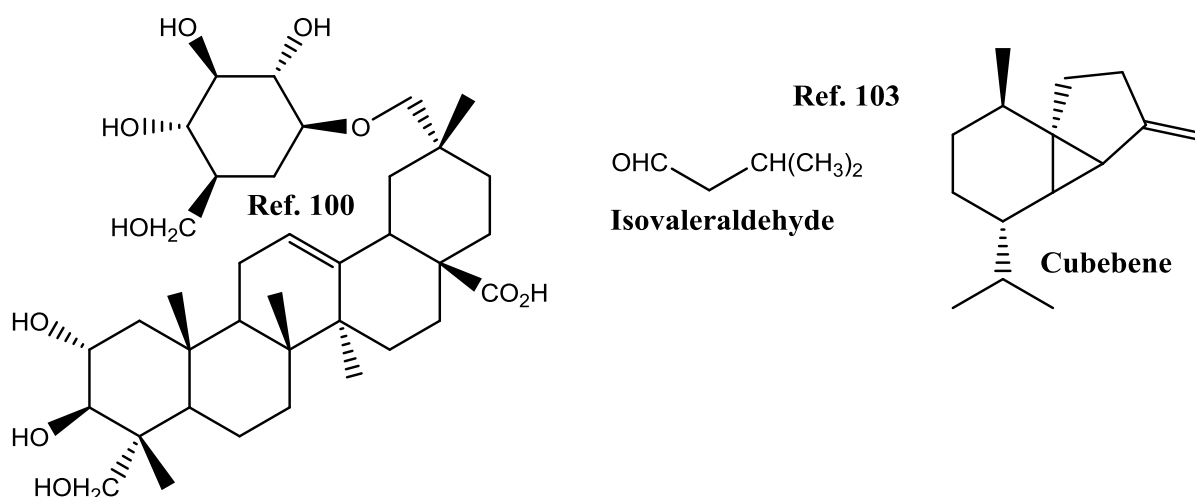


Figure 4B: Natural products isolated from *Anchusa strigosa*.

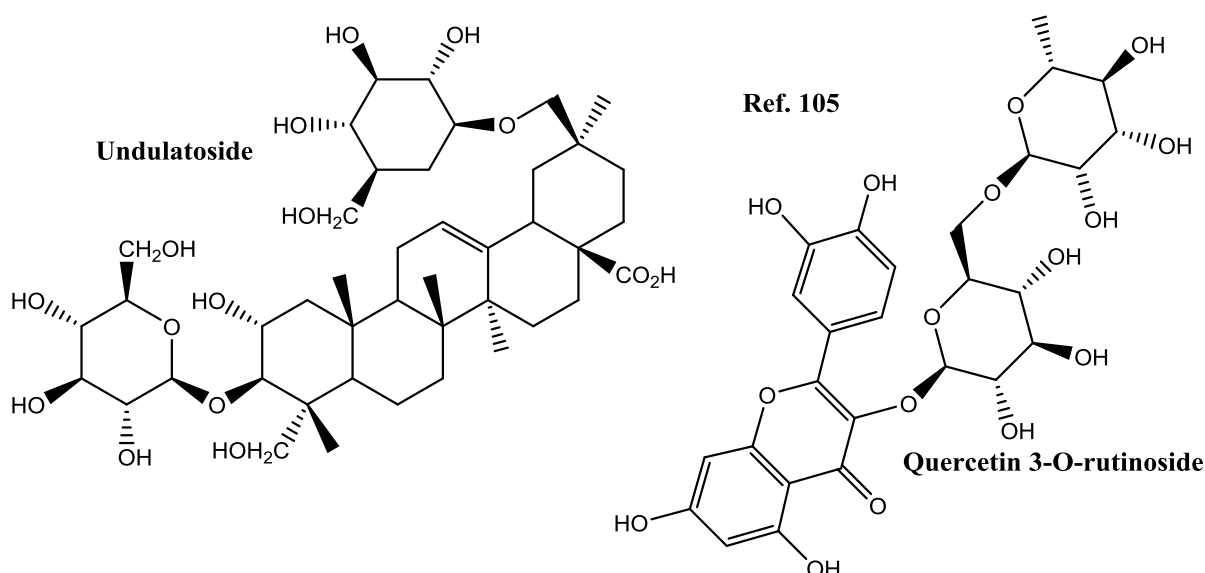


Figure 5: Natural products isolated from *Anchusa undulate*.

4) DISCUSSION

Relying on personal experience, *Anchusa* plants of Israel and Palestine are relatively wide spread and known. In Arab societies we used to suck the nectar of the flowers. Due to the rough touch of other aerial parts except the flowers, these plants are generally named “ox tongue”, even though some other species belonging to other families are named so.

The differences between the number of published articles about each one of *Anchusa* species are very large. While very few articles were published about *Anchusa aegyptiaca*, many articles were published about *Anchusa azurea*, especially about the antimicrobial activity of the second species, see **Table 2**. It is not clear why there is no single publication about the chemical composition of *Anchusa aegyptiaca*.

Contrary to the scarcity of the published data about *Anchusa aegyptiaca*, *Anchusa azurea* was extensively studied and published, see **Table 2**. One of the notable studies was published by A. Kuruuzum-Uz ahc^[71], who contributed several publications about this plant. In this article, they reported anti-inflammatory activity of the extracts of this plant, and authors relate this activity with rosmarinic acid [**Figure 6**].

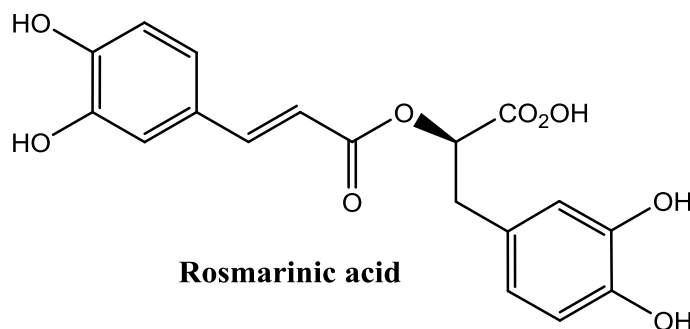


Figure 6: Natural products isolated from.

This compound was isolated from other *Anchusa* genus plants, and it is well known for its anti-inflammatory activity.^[108,109]

In addition to the 33 publications that were cited in **Table 2** about *Anchusa azurea*, several other studies about this plant were published, and in our humble opinion they are not directly linked with the activities-properties in that table. T. Aysu ahc reported the production of bio-oil from this plant and optimized the conditions of this production.^[110] V. Barbakadze ahc prepared water-soluble, high-molecular weight polymer from the crude polysaccharides of the roots, and its structure was elucidated as poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene].^[111] The same group with M. Merlani ahc followed up the previous research and they prepared poly[3-(3,4-dihydroxyphenyl)glyceric acid], which was tested for antioxidant activity using DPPH method.^[112] T.A. Alaridhee ahc reported the production of pentacene thin-film doped with flowers extracted dye.^[113] This thin film notable optical-electronic properties.

Finally, several nanoparticles (NPs) preparations were reported using *Anchusa* species, including some that are not native to the reviewed region (for example, silver nanoparticles Ag-NPs were prepared using leaves aqueous extract of *Anchusa officinalis*,)^[114] but we will present only those. These NPs and their activities-properties are presented in **Table 3**.

Table 3: Nanoparticles prepared using *Anchusa* plants of Israel and Palestine and their activities-properties.

Species	Author(s) [Reference]	Nanoparticles, Activities
<i>A. arvensis</i>	J. Ali ahc [115] M. Mohany ahc [116]	Ag, anticancer, antiparasitic, toxicity Ag, protein kinase inhibition, antioxidant, antimicrobial
<i>A. azurea</i>	O. Odemis ahc [117] O. Odemis ahc [118] S. Azizi ahc [119]	CuO, detection of Hg ⁺² ions CuO, effect on photovoltaic activity ZnO, antimicrobial
<i>A. strigosa</i>	S.A. Khit ahc [120] S.A. Khit ahc [121]	CuO, pollutants adsorption CuO, pollutants adsorption

5) CONCLUSIONS

- 1) *Anchusa* species of Israel and Palestine were very differently studied: some were extensively investigated while others just hardly.
- 2) *Anchusa* species of Israel and Palestine contain very special natural products, especially alkaloids, that should be thoroughly studied for medicinal activities.
- 3) It is important to determine for some species that are not included in this review article whether they are species of the reviewed region or not.
- 4) It is important to use as less synonyms of the scientific names as possible.
- 5) There are many medicinal activities of *Anchusa* species of Israel and Palestine that were not published so far.

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