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URTICA (NETTLE) SPECIES OF ISRAEL AND PALESTINE – A MINIREVIEW

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ABSTRACT

Urtica (Nettle) species are well known in the "Old World" for their painful stings. This property attracted human attention since antiquity and humans used these plants for various medicinal purposes, and for limited nutrition. Modern research approved the ethnomedicinal uses and expanded them, especially anti-inflammatory and antimicrobial. Four species of *Urtica* grow wild in the reviewed region (RR) of Israel and Palestine. Two of them were reasonably studied and published while the other two were hardly published. In this review article ethnopharmacological knowledge will be presented in summary, along with modern research findings. Selected natural products that were isolated from these species will be shown in clear figures.

KEYWORDS: *Urtica*, *Urtica pilulifera*, *Urtica urens*, anticancer, antimicrobial, antioxidant, anti-inflammatory, nutrition, ethnomedicine, chemical composition.

Abbreviations: ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ahc and her/his colleagues, βCBM β-Carotene bleaching method, CAT catalase, DCM dichloromethane, DPPH 2,2-Diphenyl-1-picrylhydrazyl, ED erectile dysfunction, EO essential oil, EtOAc ethyl acetate, FSH follicle stimulating hormone, FRAP ferric reducing activity power, GC-MS gas chromatography mass spectrometry, GPx glutathione peroxidase, GSH glutathione, HPLC high performance liquid chromatography, LC-MS liquid chromatography mass spectrometry, LH luteinizing hormone, LPS lipopolysaccharide, MDA malondialdehyde, NPs nanoparticles, PE petroleum etherRR reviewed region, SOD superoxide dismutase, STZ

streptozotocin, TAC total antioxidant capacity, TFC total flavonoid content, TG triglycerides, TLC thin layer chromatography, TPC total phenolic content, TTC total tannins content.

1. INTRODUCTION: Taxonomy and Ethnomedicine

The genus *Urtica* (Nettle, قُرّاص in Arabic and סְרָפָד in Hebrew) is part of the Urticaceae plant family. The number of species included in this genus is highly debated, even though these plants are very common in the "Old World". However, the number of species ranges from 30 to 80.[1] According to the website of "Plants of Israel and Adjacent Areas", four species of *Urtica* grow wild in the RR: *Urtica kioviensis, Urtica membranacea, Urtica pilulifera* and *Urtica urens.*[2]

Of all four species, *Urtica pilulifera* is most published by modern research and it is also most documented and published for ethnopharmacological uses. M.S. Ali-Shtayeh ahc reported that traditional medicine of the RR used this species to treat rheumatism, arthritis, wound healing, inflammation and cancer.[3] O. Said ahc found in their ethnopharmacological survey of the RR that *U. pilulifera* is used by local communities for treatment of cancer, stomach, intestine pain, inflammation, liver diseases and circulatory system.[4] A detailed recipe is provided. W. El Yaagoubi ahc reported that the middle Atlas communities of Morocco extensively use *U. pilulifera* roots, stems and flowers decoctions or powders for treatment of diarrhea, abdominal pain, eczema and diabetes, and to lesser extent, they also use *Urtica urens.*[5] A. Alimari ahc reports also that in the RR *U. pilulifera* is used to treat nephritis, haematuria, jaundice, menorrhagia, arthritis and rheumatism.[6] M. Hussain from Pakistan reviewed the traditional uses of thirty species of *Urtica*, including *U. kioviensis*, *U. pilulifera* and *U. Urens.*[7] He lists detailed ethnomedicinal uses of these species in the RR and Eastern Europe. These uses are all mentioned above, combined.

In a comprehensive article, A.Y. Atat presents the ethnobotany and the ethnomedicine of *Urtica pilulifera* in Arab culture, along with selected findings of modern research, including phytochemistry (structures).[8] The article presents the taxonomy of this species, natural habitat, side effects, contraindications and commercial formulations. But the very special part of this work is that it cites almost all ancient medical scholars that mentioned *Urtica pilulifera* in their writings, from Dioscorides and Galen to eleven Arab physicians, including Ibin Sina (Avicenna), Ibin Albeetarand Dawood Alantaki. As an example for what the ancient physicians wrote about *U. pilulifera*, Ibin Sina wrote in his book "The Canon of Medicine" that Alanjara (الأنجرة, *U. Pilulifera*) has no less than thirty-two medical uses.

2. Selected Publications about *Urtica* Species of Israel and Palestine

Modern research publications about *Urtica* species of the RR, reveal notable differences in the number of articles about each one of these species. In the following text a summary of these findings is presented.

General notice: percentages of solvent mixtures are given with volume per volume (v/v), unless stated otherwise.

Urtica kioviensis

Leaves (fresh, dried, roasted and boiled) were extracted with methanol and the extracts were analyzed for TFC and TPC, and tested for antioxidant activity using DPPH method.[9]

Urtica membranacea

Whole plant 50% aqueous ethanolic extract was tested against fourteen cancer cell lines resulting strong effect. *Artemesia monosperma* and *Origanum dayi post* were also studied in this research.[10]

Leaves methanolic extract had weak activity against three viruses. It was analyzed (HPLC) for chemical composition affording forty-two compounds including Secoisolariciresinol, Scopoletin (**Figure 1**) and Caffeoylquinic acid.[11]

Aerial parts 50% aqueous ethanolic extract was analyzed for TFC, total hydroxycinnamic acids content and TPC, and tested for antioxidant activity with ABTS, DPPH and FRAP methods. The extract was tested for anti-inflammatory activity in LPS-induced Raw 264.7 cells. Effect was measured by nitric oxide production inhibition. The extract was chromatographed yielding 31 known compounds.[12]

Urtica pilulifera

Leaves aqueous and ethanolic extracts ameliorated Bisphenol A-induced hypersensitivity in rat skin.[13]

Whole flowering plants were extracted with 70% aqueous methanol and the extract was active against Ehrlich Ascites cancer in mice. The extract was fractionized and chromatographed yielding known compounds including 3,5-Dicaffeoylquinic acid (**Figure 2**), 2,4-Dicaffeoylquinic acid (? Carbon number 2 of Quinic acid is not attached to hydroxyl

group and can not produce a ester with Caffeoyl group), Apigenin and Genstein glycosides (the structures presented in this article are incorrect).[14]

Leaves were separately extracted with *n*-hexane, methanol and water, and the resulting extracts were tested against HeLa cancer cells. The *n*-hexane extract (most active) was partitioned and analyzed (GC-MS) resulting the identification of 19 compounds including Bis(2-ethylhexyl) phthalate (**Figure 2**) and long carbon chain alcohols.[15]

Leaves ethanolic extract was active against Ehrlich Ascites cancer in mice, in combination with Cisplatin. The extract was analyzed for TFC and TPC, and tested for antioxidant activity using DPPH and TAC methods.[16]

Lectin that was isolated from seeds was active in STZ-induced diabetic rats. Effect was measured with several parameters including blood glucose level and body weight.[17]

Leaves methanolic extract significantly increased the expression of HSP70 (heat shock) protein in kidney tissue of Alloxan-induced diabetic rats.[18]

Follow up of previous study: pre-diabetic rats were treated with leaves methanolic extract. The results were lowering of blood glucose, cholesterol and TG, along with positive effect on GSH and MDA oxidative stress biomarkers.[19]

Leaves methanolic extract was administered to STZ-induced diabetic male rats. The results were increase of body weight, decrease of blood glucose levels, increasing the testis and epididymis weights, testosterone levels, sperm count and motility.[20]

Leaves methanolic extract was administered to Alloxan-induced diabetic rats. The result was positive effect on several biomarkers including urea, uric acid and creatinine levels.[21]

Seeds PE extract treated Carrageenan-induced paw edema in rats, with Indomethacin as a standard drug.[22]

Leaves methanolic extract treated Complete Freund's Adjuvant-induced arthritis in rats.[23]

Male patient with twelve years seminal fluid infection drank leaves tea (200 mL) for three months every morning, and 200 mL of *Alhagi graecorum* tea. The illness was cured.[24]

Seeds aqueous and methanolic extracts were analyzed for TFC and TPC, and were tested against Formalin-induced paw edema in rats.[25]

Aerial parts methanolic extract was partitioned with *n*-hexane, chloroform and EtOAc, and the fractions were chromatographed yielding five major compounds: β-Sitosterol, β-Amyrin, Ursolic acid, β-Sitosterol-3-O-glucoside and Caffeic acid. The three fractions were tested for antidiabetic (mainly blood glucose levels), anti-inflammatory (mainly Serum C-reactive protein) and antioxidant (CAT, GSH, MDA and SOD levels); in rats with type II diabetes induced by high fat diet and low dose of STZ.[26]

Leaves, roots and seeds were separately extracted with methanol and water, and the extracts were tested for antimicrobial activity against 16 strains. They were also analyzed for TPC and tested for antioxidant activity using DPPH method. *Urtica dioica* was also studied in this research.[27]

Aerial parts EO (hydrodistillation) was active against *S. aureus* and *E. coli* with Gentamicin as a reference. The EO was analyzed (GC-MS) yielding 45 compounds with 7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin (**Figure 2**) with highest percentage (19.6).[28]

Aerial parts, roots and seeds were separately extracted with PE and 20% aqueous methanol. The six extracts were supplemented to mice resulting activity increase of liver antioxidant enzymes and decrease of lipid peroxidation.[29]

Flowers, leaves, roots and seeds were separately extracted with 80% aqueous methanol. The extracts were analyzed for TPC and tested for antioxidant activity using seven methods.[30]

Seed gum was analyzed for GCC, total sugar content, monosaccharides, uronic acids and tested for antioxidant activity with DPPH method. Other parameters of the gum were determined.[31]

Seeds 70% aqueous ethanolic extract was supplemented to female mice resulting positive effects on body parameters, fertility hormones (FSH, LH, and Estradiol), as well as MDA and SOD levels. GC-MS analysis of *n*-hexane extract showed that the major components were fatty acids and sterols, with Vanicol ($CH_3(CH_2)_{16}CO_2H$) 6.06%.[32]

Seeds methanolic extract reduced alleviated copper-induced oxidative damage in tomato seedlings. *Corchorus olitorius* was also studied in this research.[33]

Seeds oil was prepared by extraction with *n*-hexane, and it was analyzed for fatty acids composition, partial GCC, total carotenoids content, total chlorophyl content and sterols composition. *Urtica dioica* was also studied in this research.[34]

Seeds 80% aqueous ethanolic extract inhibited in three doses for cyclic adenosine monophosphate-Phosphodiesterase (cCAMP-PDE) *in vitro*. Researchers concluded that the extract can be used to treat ED.[35]

Benzoic acid and its three monohydroxy derivatives (previously isolated from this species) were subjected to esterification with 2-Phenoxyethylbenzoate (**Figure 3**). The resulting esters had antioxidant (DPPH method) and antimicrobial (bacteria and fungi) activities.[36]

Follow up of the study cited in reference 29 using the same extracts: effect on immunoglobulin G and M and hormones in rats, where the production of both immunoglobulins was increased and reduction in cortisol and increased in thyroid hormones. Aqueous methanolic extract was most active.[37]

Seeds supplementation to broilers had very limited positive effect.[38]

Seeds mucilage was used in cake preparation resulting higher firmness of te cake and longer storage time.[39]

Leaves 70% aqueous methanolic extract ameliorated cadmium-induced hepatotoxicity in rats. Effect was approved by testing inflammatory and oxidant-antioxidant biomarkers. The extract was analyzed for TFC, TPC, saponin content, anthocyanin content, total steroids content, and tested for antioxidant activity using DPPH, TAC and Fe^{+2} chelating methods. GC-MS analysis of the extract afforded 17 compounds including Oxiraneoctanoic acid,3-octyl-, methylcyclohexyl ester (7.18%, **Figure 2**).[40]

Whole plant aqueous extract significantly reduced the fertility of *Culex pipiens* mosquito.[41]

Leaves 70% aqueous methanolic extract was analyzed for TFC, TPC, TTC and GCC. It was tested for antioxidant (DPPH method), antibacterial (against five strains) and antihemolytic

activities. The extract was chromatographed yielding known compounds including Colneleic acid (**Figure 2**).[42]

Leaves and stems were combinedly extracted with water and the extract was analyzed for mineral content, total sugars content, TPC and tested for antioxidant activity using Prussian Blue method.[43]

Urtica urens

Aerial parts 50% aqueous ethanolic extract was analyzed for TFC, total hydroxycinnamic acids content and TPC, and tested for antioxidant activity with ABTS, DPPH and FRAP methods. The extract was tested for anti-inflammatory activity in LPS-induced Raw 264.7 cells. Effect was measured by nitric oxide production inhibition. The extract was chromatographed yielding 29 known compounds.[12]

Leaves 70% aqueous methanolic extract was analyzed for TFC, TPC, TTC and GCC. It was tested for antioxidant (DPPH method), antibacterial (against five strains) and antihemolytic activities. The extract was chromatographed yielding known compounds including Azelaic acid (**Figure 4**).[42]

Leaves and stems were combinedly extracted with water and the extract was analyzed for mineral content, total sugars content, TPC and tested for antioxidant activity using Prussian Blue method.[43]

Aerial parts 80% aqueous ethanolic extract was tested for antinociceptive **(**writhing, formalin and hot-plate tests) and anti-inflammatory (TPA-induced ear edema and carrageenan-induced paw edema) activities in mice. The extract was chromatographed yielding Chlorogenic acid as major ingredient.[44]

Leaves aqueous extract was active against four human cancer cell lines: HCT-116, A549, MCF-7 and HSF.[45]

Aerial parts methanolic extract was tested for anticancer (against HepG2, MCF-7, PC3 and HCT cell lines), antidiabetic (in STZ-induced diabetic rats) and antioxidant (determined by CAT, GHS, MDA and SOD levels in the animals blood serum) activities. The extract was partitioned and chromatographed affording 8 major components including β-Adenosine and Tryptophan (**Figure 4**).[46]

Leaves EtOAc extract was analyzed for TFC and TPC, and tested for antioxidant activity using DPPH and hydroxyl scavenging method. It was also tested against six cancer cell lines: PC3, HEPG-2, MCF-7, MDA-MB-231, HCT-116 and HaCaT. The extract was chromatographed affording 31 major compounds including Carvacrol (**Figure 4**).[47]

Aerial parts 80% aqueous methanolic extract alleviated depression-like model in mice induced by tail suspension and forced swimming tests. The extract was analyzed for GCC.[48]

Aerial parts 80% aqueous methanolic extract was analyzed for TPC and chromatographed for single compounds affording Chlorogenic acid as major component. The extract was active against LPS-induced inflammation in keratinocytes, where the effect was measured mainly by nitric oxide production inhibition.[49]

Aerial parts 70% aqueous ethanolic extract was analyzed for GCC, TFC, TTC and *o*-Bisphenol (**Figure 4**) content. It was tested for antioxidant (TAC method, CAT, GPx and SOD levels) and anti-inflammatory (Carrageenan-induced paw edema in rats) activities. Chromatography of the extract yielded Chlorogenic acid as major component.[50]

Leaves, roots and stems were separately extracted with water and the resulting extracts were tested for antimalarial activity using β-Hematin inhibition method: leaves extract was most active. LC-MS analysis yielded known phenolics.[51]

Leaves and stem-bark were separately extracted with *n*-hexane, chloroform, EtOAc and methanol, and roots were extracted with chloroform and methanol, obtaining a total of ten extracts. These were tested against six bacterial and two fungal strains showing a wide range of activity from weak to very strong, except against *Penicillium digitatum* fungus where none of the extracts was active.[52]

Leaves ethanolic extract was active against *Staphylococcus aureus* and healed paw wounds in rabbits.[53]

Leaves were consecutively extracted with *n*-hexane, DCM, methanol and water, and the extracts were tested against seven bacterial species: activities ranged from inactive to moderately active. *Urtica dioica* and *U. Mexicana* were also studied in this research.[54]

Leaves were separately extracted with acetone methanol and water, and the extracts were analyzed for GCC, TFC, TPC, several micro- and macro-elements and total anthocyanins. The extracts were tested for antioxidant (ABTS, DPPH and FRAP methods) and antibacterial (against ten bacteria species) activities. Antibacterial activity ranged from inactive to moderately active. *Argemone subfusiformis* was also studied in this research.[55]

Aerial parts were separately extracted with acetone, methanol and water, and the extracts were analyzed for TFC, TPC, TTC, total anthocyanin content and total carotenoids content. The extracts were tested for antibacterial (against *E. coli*, *S. typhimurium*, *E. faecium* and *E. faecalis*) and antioxidant (DPPH and TAC methods) activities.[56]

Follow up of the study cited as reference 50: leaves were separately extracted with 70% aqueous ethanol and water, and the extracts were analyzed for TFC, TPC and vitamins C, D and E contents. The extracts were tested for antibacterial (against 8 bacterial strains) and antioxidant (ABTS, βCBM, DPPH and FRAP methods) activities.[57]

Follow up of the study cited as reference 56: aerial parts aqueous extract was used to treat mice infected with *Salmonella typhimurium* (antibacterial activity), and that were injected with hydrogen peroxide (antioxidant activity which was measured by GSH and MDA levels in blood serum of the animals).[58]

Leaves and roots were separately extracted with 80% aqueous ethanol and water. The extracts were analyzed for TFC, TPC and TTC. The extracts were tested for antimicrobial (three bacteria and one fungi species) and antioxidant (βCBM and DPPH methods).[59]

Flowers, leaves and stems were separately extracted with water, and the extracts were analyzed for minerals, TFC, TPC and TTC. The extracts were tested for antibacterial (against *S. aureus*, *B. cereus*, *E. coli* and *P. aeruginosa*), antioxidant (DPPH and FRAP methods) and insecticidal (against *Aphis gossypii*). Eaves extract was chromatographed affording known phenolics.[60]

Leaves were separately extracted with *n*-hexane, DCM, methanol and water. The extracts were tested for antibacterial (seven bacterial species) and hematopoietic (in femur and spleen cells isolated from female mice) activities.[61]

Aerial parts were soaked in 50% aqueous ethanol, *n*-hexane and water (combined) and the extract was separated to organic and aqueous. The two resulting extracts were tested for antiobesity activity by inhibition of pancreatic lipase. *Portulaca oleracea*, *Brassica napus* and *Lathyrus hierosolymitanus* were also studied in this research.[62]

Aerial parts were separately extracted with methanol and water. The extracts were analyzed for GCC, TFC and TPC, and was tested for antioxidant activity using ABTS, DPPH and FRAP methods. *Mercurialis annua* was also studied in this research.[63]

Aerial parts methanolic extract was chromatographed yielding six flavonoids where Patuletin (**Figure 4**) was the major component. It was tested against tested against Aflatoxin B1-induced toxicity in rats. The positive effect was measured using several biomarkers including GPx and SOD levels in blood serum of the animals.[64]

Leaves were separately extracted with PE, chloroform, ethanol and water, and the extracts were analyzed for extended GCC, TFC and TPC. The extracts were tested for antioxidant activity using DPPH method.[65]

Aerial parts 80% aqueous ethanolic extract inhibited egg phospholipid peroxidation induced by UV radiation and tyrosinase. Mechanism of action is proposed for tyrosinase inhibition. The extract was chromatographed resulting Chlorogenic acid (**Figure 4**) as major component.[66]

Aerial parts methanolic extract had anxiolytic effect in mice that were tested using hole broad-, rota rod- and light-dark-test. Diazepam was reference drug.[67]

Leaves powder was prepared for animal nutrition use, and was analyzed for extensive GCC, nutritional values, amino acids-, fatty acids- and mineral-composition.[68]

Aerial parts powder was fed to broilers (chicks) resulting positive effect that was measured (among other parameters) by growth performance, blood composition and short chain aliphatic acids.[69]

Seeds *n*-hexane extract alleviated Carbon tetrachloride-induced hepatotoxicity in rats. The effect was measured by measuring six biomarkers of enzymes activities.[70]

Follow up of the studies cited as references 50 and 57: aerial parts 70% aqueous ethanolic extract ameliorated Imidacloprid-induced hepatotoxicity and osteoporosis in rats. The positive effect was measured in the liver by using CAT, GSH, SOD and vitamin E levels; and for osteoporosis by bones x-ray. The extract was chromatographed affording 31 compounds including 6-Nitro-cyclohexadecane-1,3-dione.[71]

Another follow up by the same group of previous study: aerial parts 70% aqueous ethanolic extract ameliorated Imidacloprid-induced nephrotoxicity and osteoporosis in rats. Positive effect was detected by decrease of deaths compared with control group, kidney parameters and Creatinine levels in serum and urine.[72]

A follow up of the study cited as reference 71: treatment of osteoporosis (induced by bilateral ablations of the ovaries) by aerial parts 70% aqueous ethanolic extract separately or in combination with Bisphosphonate.[73]

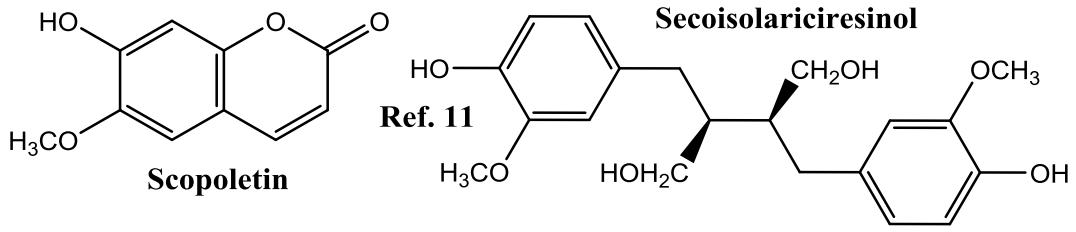

Figure 1. Natural products isolated from *Urtica membranacea.*

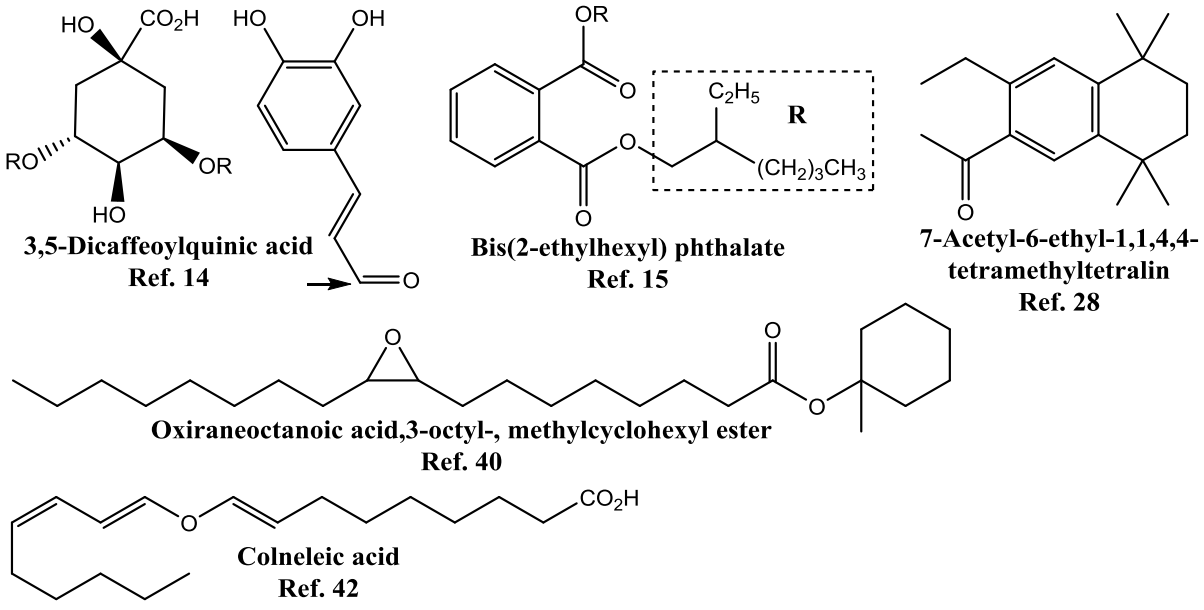

Figure 2. Natural products isolated from *Urtica pilulifera.*

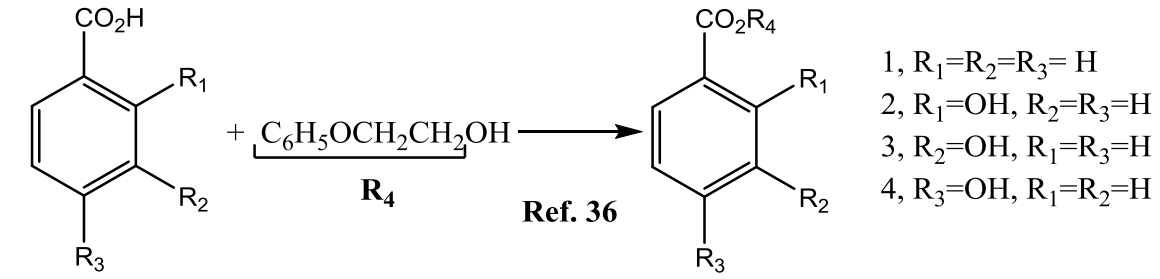

Figure 3. Esterification of Benzoic acids isolated from *Urtica pilulifera*[36]

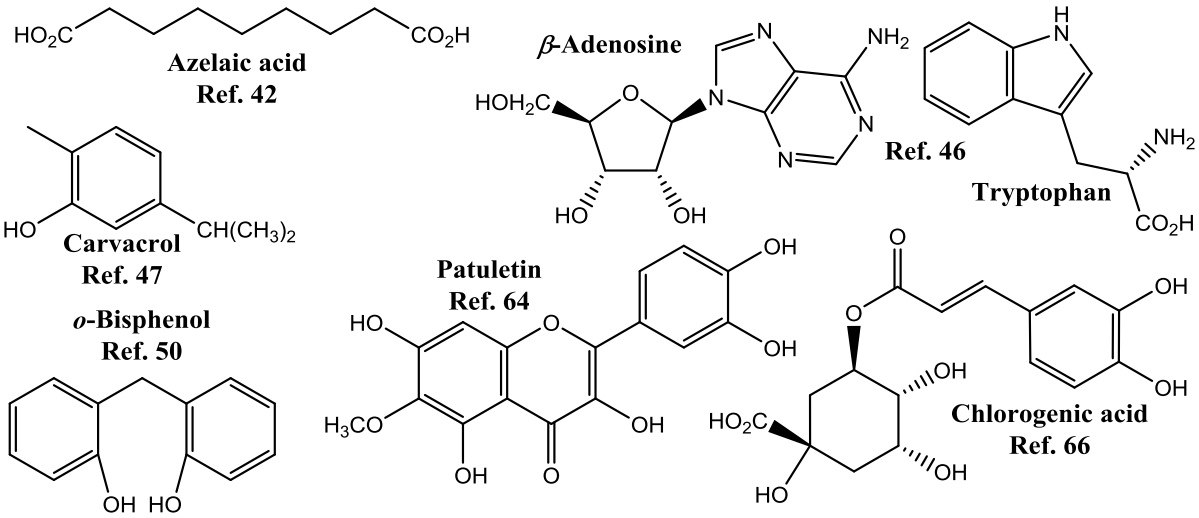

Figure 4. Natural products isolated from *Urtica urens.*

3. DISCUSSION

In this short discussion, special focus will mainly be on several publications that concern *Urtica pilulifera* but could not be included in the previous part of this minireview article: growth conditions. To make these publications easy for readers access, we summarized them in **Table 1**.

Table 1: Published Articles about Growth Conditions of *Urtica pilulifera.*

Author(s)	Growth Conditions; Impact; Reference
I. Dogan ahc	Influence of Al^{+3}; mixed impact on minerals uptake and accumulation [74]
I.I. Ozyigit ahc	Influence of Al^{+3}; mixed impact on antimicrobial activity [75]
S. Sancaktaroglu & E. Bayram	Influence of Bornova (Turkey) conditions; general decrease of the plant and seed physical parameters and oil yield [76]
I.I. Ozyigit & Ş. Akinci	Influence of Al^{+3}, Cd^{+2} and drought; mixed impact except in case of severe drought where a clear negative impact was recorded [77]
H.E. Wahba ahc	Effect of nitrogen fertilizers; general enhancement, ammonium sulfate was best, increase of caffeic acid and derivatives [78]
H.E. Wahba ahc	Follow up of previous study using foliar and focusing on amino acids content; increase [79]

I. Dogan ahc	Influence of Cd^{+2}; severe damage on growth parameters, material content and genotoxicity transferred to the next generation [80]
S. Ghazouani ahc	Influence of salinity; growth and some phytochemicals levels decrease but increase of antioxidant enzymes [81]

In addition to the eight reports in **Table 1**, an interesting report was published about the preparation of ZnO NPs using aqueous extract of *Urtica pilulifera*.[82] J.N. Hameed ahc also reported anticancer activity of these NPs. To the best of our literature search, this is a unique report of preparing metal/metal-oxide NPs using extracts of *Urtica* species of the RR.

To conclude this discussion, we will present two very important studies. N.A. Jaradat reported a detailed method of standardization of crude extracts of all four *Urtica* species of Israel and Palestine.[83] The importance of this work emerges from the fact that these extracts, organic and aqueous, are the starting materials of tested medicinal and other activities-properties of these species. The standardization of the crude extracts is the basis of uniformity and consistency of the results of the tests and for preparations of formulations.

Urtica urens is widely used in homeopathy, stated S. Patel ahc.[84] Based on this fact, they conducted a study which studied the morphological properties of aerial parts powder and analyzed it for GCC and chemical composition (TLC). Their findings are interesting in the context of comparison to commercial products of this plant.

4. CONCLUSIONS

1) Among the four species of *Urtica* growing wild in the RR, *U. kioviensis, U. Membranacea* were hardly published for medicinal and other properties-activities.

2) There is an urgent and intensive research of *U. kioviensis, U. Membranacea.*

3) The chemical compositions of all species are not complete.

4) There is a need to complete these compositions, especially alkaloids, which are repeatedly reported in GCCs.

5) Compared with uses of these species in ethnomedicine, modern research still needs to find out whether these uses are applicable for studies using modern methods.

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