



(RESEARCH ARTICLE)



Taxonomic study of a cultivated plant of genus *Zanthoxylum* l. 1753 (Rutaceae) in Iraq

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Abstract

Zanthoxylum L. 1753 belongs to the family Rutaceae. The plant, with its forest green color and with its distinctive fragrance attracted the attention of the study, specially it was an exotic to the environment of Iraq. *Zanthoxylum* is an exotic plant produced to Iraq and planted in gardens as an ever-green aromatic plant named (BUKHUR HINDI), as it is known among sellers and farmers in Iraq, because of the aromatic smell that emanated from the leaves and resembles Indian incense. The plant adapted to environmental conditions and succeeded in growing and blooming. It was examined and diagnosed for the first time in Iraq as *Zanthoxylum beechyanum* K. Koch. (Chinese pepper tree). Morphological characteristics and anatomical characteristics for leaves (considering that their variations are reliable and taxonomically important) were provided. The phytochemical screening showed that the leaves contain alkaloids, tannin, flavonoids, terpenes and saponins where the quantity of active constituent vary, the highest concentration was 3.939 phenols follows by flavonoids, alkaloids 0.159 and terpenoids 0.111 mg/g dry weight.

Keywords: Chinese pepper tree; Exotic; Iraq; Rutaceae; *Zanthoxylum*

1. Introduction

The flora of Iraq submitted that the order Rurales is of 4 families, much the largest being the Rutaceae, the only one with native Iraq representation; the genera *Ruta* L. and *Haplophyllum* Adr. Juss. which belong to the subfamily Rutoideae and genus *Citrus* L. belongs to the subfamily Aurantioideae, these three genera are native to Iraq [1]. Rutaceae family was one of many plant families that researchers sensed might pose problems in terms of delimitating its relationships with other angiosperm families, [2]. A phylogenetic study referred that the families Simaroubaceae and Meliaceae are the out-groups closest to Rutaceae [3]. The Rutaceae plants produce a great diversity of secondary metabolites, much are very characteristics or unique for it [4]. [5] in his encyclopedia 'The families and genera of vascular plants' states that Rutaceae comprises about 2100 species in approximately 154 genera and its best known for the economically important genus *Citrus* L. the *Zanthoxylum* plants have drawn attention by researchers because of some chemical compound that is consist of and its used as insecticidal [6, 7]. [8] referred to *Zanthoxylum* as a genus belongs to the subfamily Rutoideae. There are 200 or more species belong to it, these species are pantropical distributed in E. Asia and E. North America. Forty-one of these species grown in China; 25 are endemic. [9].

New York Botanical Garden in [10] described *Zanthoxylum* L. as shrubs or trees, prickly stems; leaves alternate, odd-pinnately compound; flowers are small greenish or whitish, unisexual (dioecious plant); stamens as many as the petals, stamens alternate with petals; 2-5 carpels, ovaries not united (polycarpous), style coherent from above, it contains 2 collateral ovules, pendulous; fruit fleshy follicle, dehiscent across the top; 1 or 2 seeds. Some species have undifferentiated perianth of 5-9 tepals in series others have with differentiated perianth of 2 series with 4-5 sepals and 4-5 petals, fruit distinct or basally connate, seed globose – ovate [9].

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The current study aimed to examine, identify and recorded *Zanthoxylum beechyanum* K.Koch. 1853. for the first time in Iraq as an exotic plant adapted and succeeded in growing in the environment of Iraq.

2. Material and methods

2.1. Identification

The study identified the plant in e-flora of China and India [9, 10] and compared the morphological characteristics with the herbarium specimens of GBIF org. gallery online [11] and check the acceptance of its binomial scientific name with the World Flora Online [12, 13]

2.2. Morphological study

Different parts (vegetative and reproductive) were collected from cultivated plant grown in scientific garden in college of science for women/ University of Baghdad and some other public gardens, to examine the morphological characteristics by dissecting microscope.

2.3. Anatomical study

The study examined the orthogonal section of the blades and their petioles, with wax sectioning method following [14, 15] to examine the anatomical characteristics by compound light microscope. The study also followed [16] for peeling method to examine the stomata system.

2.4. Chemical study

Plant extract: *Zanthoxylum* leaves were dried in shade and grinding then extracted with methanol in soxhlet apparatus at ratio (1:10) (plant: solvent), for 6-8 h at 60-80 C°, the extract filtered and dry. Phytochemical analysis: The crude extract was introduced to phytochemical determine to detect active compounds (alkaloids, tannins, flavonoids, terpenes and saponins) this test done according to the study [17]

- Detection of alkaloids: Mayer and Wagner reagent was used to indicate the presented of alkaloids
- Detection tannin: $FeCl_3$ and Lead acetate adding to extract
- Detection flavonoids: Magnesium crystals and 1% hcl and concentrate H_2SO_4
- Detection terpenes: Chloroform then H_2SO_4 sequentially and Anace aldehyde
- Detection of saponins: Foam formation and $HgCl_2$

2.4.1. Quantitative analysis of some active compounds

- Total phenolic determination: The amount total phenolic amounts were determined alcoholic extract, with folin- ciocalteu reagent as a stander. The mixture reaction consists from 100 μ l of extract, folin- ciocalteu reagent (500 μ l) and about 1.5 ml of 20% sodium carbonate, the mixture introduces to vortex apparatus for 1 min, then distilled water added to reach 10 ml, then let the mixture to react at room temperature for 2h and the absorbance at 765 nm was detected, according to garlic acid calibration curve and total phenolic amount calculated according to mg garlic acid equivalent per g dry weight. The test exam was done in triplicate [18].
- Total flavonoids determination: The amount of flavonoids in *Zanthoxylum* crude extract determine using aluminum chloride colorimetric method, which contain, 50 μ l of 1 gm/ml extract added to methanol 1ml, 4 ml distilled water and 0.3 ml of $NaNO_2$ were mixed and incubated for 5 min at room temperature, then 0.3 ml of 10% $AlCl_3$ solution was added to the mixture and let it to rested for 6 min, after that added 2 ml of 1 mol/L of NaOH, then diluted with double- distilled water until the final volume were 10 ml, let the mixture for 5 min, and measured the absorbance at 510nm the total flavonoid amount calculated from calibration curve and expressed the result as mg rutin equivalent per g dry weigh [19].
- Total alkaloids determination: To determine the quantity of alkaloids in plant extract, alkaloids separation through dissolve the extract in 2N HCl and mixed, 1ml of the mixture placed in separated funnel and wash with chloroform 10ml three time, adjusting solution PH to 7 using 0.1N of NaOH, after that 5ml of Bromocresol Green (BCG) reagent solution and 5ml phosphate buffer with PH 4.7 were added. The stander curve of alkaloid Atropine, parts precisely measured (0.4, 0.6, 0.8, 1 and 1.2ml) of stander Atropine solution (1mg/10ml D.W), where added separately, each mixture were divided and extracted with 1, 2, 3, 4 chloroform and shaking vigorously for 1 min, then completes the volume to 10 ml with chloroform in volumetric flask, after that the absorbance spectrum measured at 470 nm compared with mixture solution contain from above solution without Atropine which considered as blank [20].

Total terpenoids determination: The amount of total terpenoids calculated by adding 7ml of methanol and acetonitrile to 1.5 g of *Zanthoxylum* extract and shaking by shaker apparatus for 30 min then incubated in dark at room temperature for 24h then centrifuge the mixture at 6000 rpm/min, the supernatant (5 ml) collected and 1.5 ml of chloroform and 0.5 ml of concentrate H₂SO₄ were added, mixed for 1 min then methanol added to have final 10 ml volume, linalool stander used in different concentration, the absorbance recorded at 538nm and the percentage of terpenoids calculated [21]

3. Results and discussion

3.1. Identification

Zanthoxylum belongs to the order Rutales perleb 1826 that is a synonym of Sapindales Dumort 1829 [22]. *Zanthoxylum* belongs to the family Rutaceae, it is one of the many plants imported to Iraq for ornamental purposes. It's cultivated in gardens as an evergreen aromatic plant named locally 'BUKHUR HINDI' among sellers and farmers, due of the aromatic scent emitted by leaves, which is reminiscent of Indian incense. *Zanthoxylum* L. reported as an accepted name and listed 10 synonyms by World Flora Online [12-a], shown in the list below:

- *Blackburnia* J.R.Forst. & G.Forst.
- *Fagara* L.
- *Fagaras* Kuntze
- *Lacuris* Buch.- Ham.
- *Ochroxylum* Schreb.
- *Pohlana* Nees & mart.
- *Pseudopetalon* Raf.
- *Tipalia* Dennst.
- *Xanthophyllon* St. - Lag.

During studying the morphological characteristics to identify the species, the study submitted it as *Zanthoxylum beechyanum* K.Koch. 1853. The common name is: Chinese pepper tree [23] it was planted in Doha city in 2016 and the flora submitted this species as an exotic species with four synonyms. The Catalogue of Life Checklist listed seven synonyms [11] and [13- a, b] mentioned six synonyms. While [12-b] listed two synonyms for *Zanthoxylum beechyanum* K.Koch.; they are: *Fragara alata* var. *beecheyana* (K.Koch).M.Hiroe and *Zanthoxylum arnotianum* Maxim.

The plant adapted well to the environmental conditions and established itself vegetatively in Iraq. The study noted that the plant thrived in medium shade rather than direct sunlight and required moderate irrigation. Additionally, the study observed that the plant is unisexual (dioecious). The flowers symmetrical, actinomorphic, tricyclic, 4- 5 merous, imperfect, apetalous (no corolla), pistillate and that is why no fruits, the ovary start to increase in size and change color but, it aborted because there were neither pollination or fertilization. that is why the study did not find any fruits and seed.

3.2. Morphology Characteristics

Perennial, evergreen shrubs, stem erect, branched from base till top, woody up to 2 meter height, leaves compound, forest green, 50mm. x 18mm.; multifoliolate (9- 13)- 15 leaflets, imparipinnate; leaflet or pinna blades are elliptic-ovate- obovate, (12- 18)- 20mm. x 5- 7mm., semi-sessile; rachis is winged, (0.8- 1)mm. in width and (35- 50)-55mm. in length; margins of leaflets and the wings of rachis are entire with yellow dots represented by sessile glands; leaflets blades apex are obtuse, blades base are cuneate- attenuate; venation is pinnately reticulate with one main vein (figuer.1).

Inflorescences are cymose, polychasium- simple dichasium- umbelliform; 2- 3- 5 florets; peduncles and pedicel are light green- reddish brown; peduncles are 3- 4 mm. in length and the pedicels are very short ≤1mm.; florets are female red apetalous, calyx green changed to red with time, nine sepals basally connate, less than 1 mm. teeth of sepals are triangular, apex attenuate, margin entire, 1mm. in length. Gynoecium is polycarpous, 4-5 pistils, connate at base only; ovaries dark red, style is apical, yellow; stigma light yellow, capitate; the ovaries and the stigma curved outward during growing; pistils are ± 2 mm. (figure. 2).

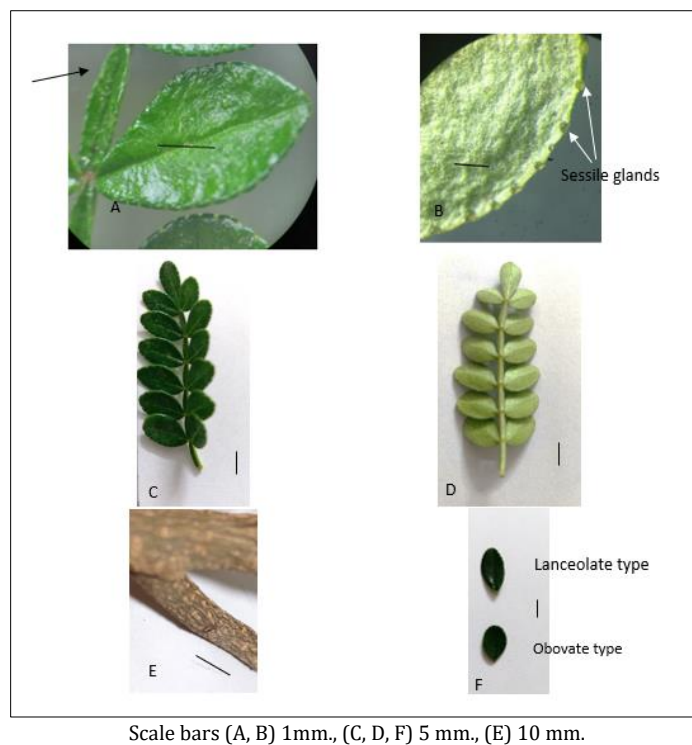


Figure 1 *Zanthoxylum beechyanum*; (A): winged rachis, (B): entire margin with glands, (C): adaxial surface of the leaf, (D): abaxial surface of the leaf, (E): part of the brunch, (F): Types of leaflets

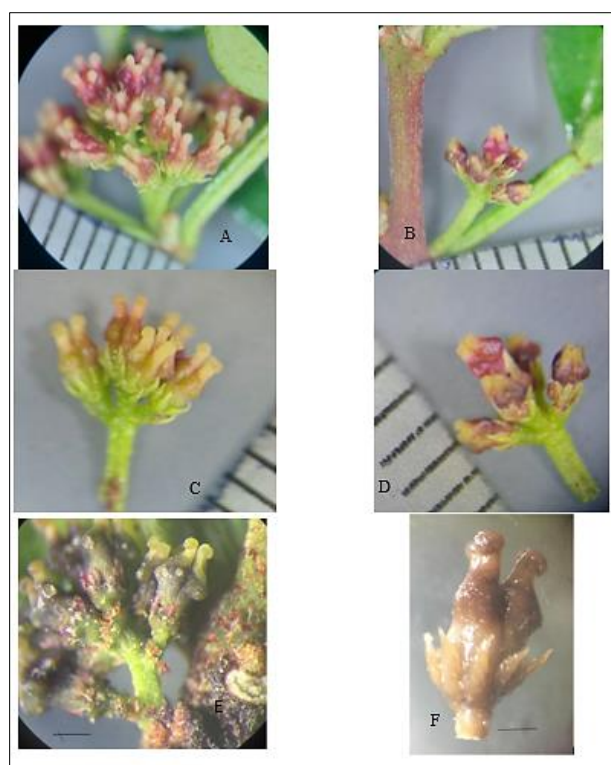


Figure 2 *Zanthoxylum beechyanum*; (A): Compound Inflorescence, (B): Simple Inflorescence, (C): light brown ovaries with yellow style and stigma, (D): reddish brown ovaries with yellow styles and stigma, (E): ovaries become black and aborted, (F): the ovary and the stigma curved outward

3.3. Anatomical Characteristics

Leaflets are dorsiventral. The orthogonal section of the blade appears bifacial. The adaxial epidermis cells arranged in one row, square-rectangle in shape, cells of adaxial epidermis are smaller than the cells of abaxial epidermis. Epidermis cells arranged in two rows near the midrib. The palisade cells are thin elongated rectangular, arranged in one row; in some places appear to be in two rows of shorter cells, but still, the free surfaces end in one plane.

Glandular sessile peltate trichomes distributed among the epidermis cells and appeared in the sections as small cavities located in different spots at both epidermises, surrounded by 2- 3 rows of thin and transverse rectangular parenchymatic cells, the main big cavity of the multicellular peltate trichomes appeared at the lateral end of the rachis wings and the margins of the leaflets, its surrounded by 4-5 rows of parenchymatic cells.

Cuticle layer covered both epidermises. The sponge layer consists of different size of parenchyma thin walled filled the mesophyll area. The vascular bundle is collateral. Secondary vascular bundles are small regularly located in the sponge layer proximal each other, consist 4- 5 columns of transporting elements arranged in small arch.

The main vascular bundle at the midrib has a horseshoe shape, consist of 37- 40 columns of transporting elements. The section shown that there was a small second vascular bundle above the main one (under the upper surface of the blade), it consists of 5- 6 columns of transporting elements. Parenchyma tissue filled the area between these two vascular bundles, which revealed that the vascular bundle will further be closed circle in shape (leptocentric), as it shown in [24], peltate trichomes not found at the sections and that was unlike the results in the former study, that studied *Zanthoxylum armatum*. The study found some druses crystal in some of the parenchyma cells near the small vascular bundles, but it was very rare. (figure 3).

Stomata complex is hypostomatic and anomocytic. The stomata surrounded with 5-6 hexagonal epidermal cells. Adaxial surface almost lack stomata complex, while abaxial surface is rich with. The stomata were at the same level with the epidermal cells. (figure 4).

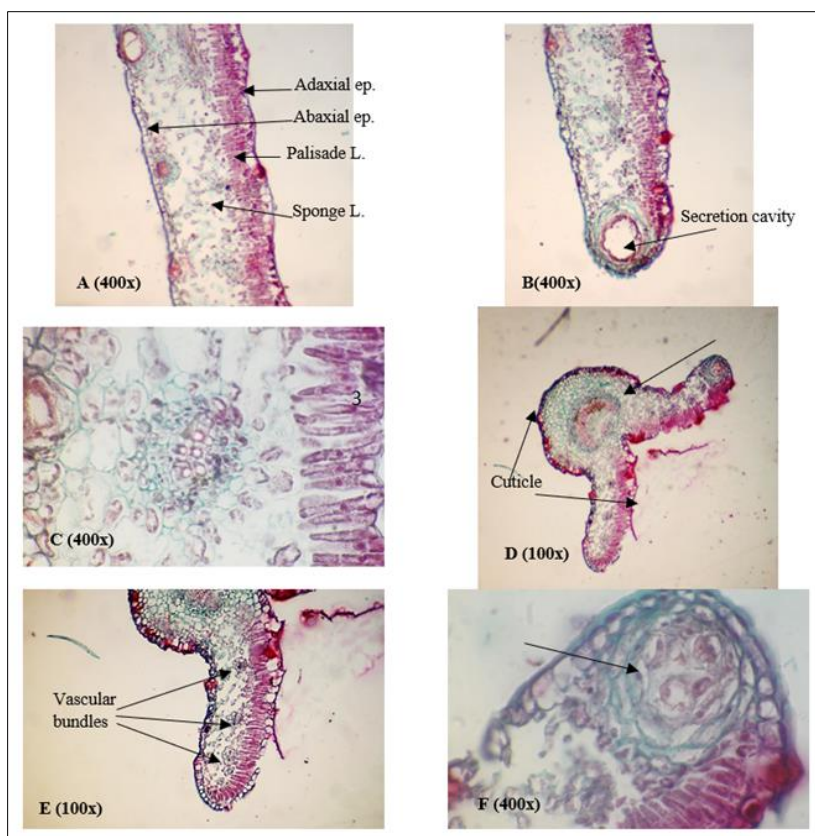


Figure 3 *Zanthoxylum beechyanum*; sMicrographs of orthogonal sections of leaf. (A) leaf blade, (B) lateral end of the blade, (C) lateral vascular bundle, (D) winged rachis, (E) lateral secondary vascular bundles, (F) lateral multicellular gland

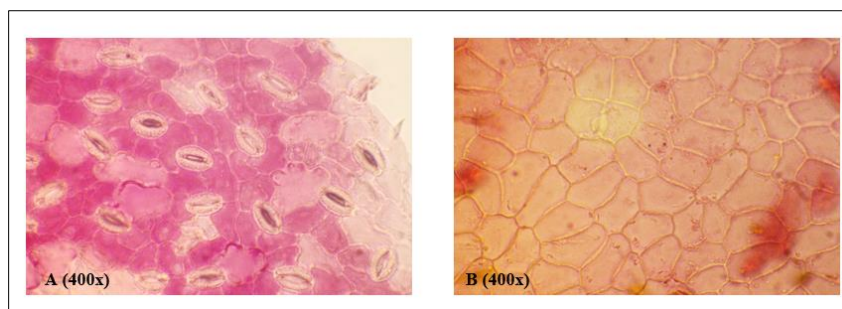


Figure 4 *Zanthoxylum beechyanum*; stomata complexes, (A) abaxial surface, (B) adaxial surface

3.4. Chemical study

3.4.1. Phytochemical analysis

The table (1) shows the phytochemical estimation of qualitative analysis of some active compounds presented in crude *Zanthoxylum* leaves extract. The result indicate te present of alkaloids, tannin, flavonoids, terpenes and saponins in *Zanthoxylum* extract, this result were agreed with [25] which refer that the plant contains Alkaloids, phenolics and terpenoids, while they didn't point the present of flavonoids and saponins. Flavonoids, alkaloids and terpenes indicate its presence from [26], [27] indicate the presence of two type of alkaloids. Where [28] refer to presence of alkaloids, tannin, flavonoids, terpenes and saponins in all plant parts.

Table 1 Phytochemical analysis of *Zanthoxylum* leaves active compounds

Active compounds	Reagent type	result
alkaloids	Mayer	+
	Wagner	+
tannin	FeCl ₃	+
	Lead acetate	+
flavonoids	Magnesium crystals and 1% HCl	+
	H ₂ SO ₄	+
terpenes	Chloroform and H ₂ SO	+
	Anace aldehyde	+
saponins	Foam	+
	HgCl ₂	+

(+) the presence of compound

3.4.2. Quantitative analysis of some active compounds

The result in table (2) consists the quantity of active metabolite per 1 gram of dry *Zanthoxylum* leaves, that indicated the presence of phenols, flavonoids, alkaloids and terpenes which their percentage vary, where the highest percentage was phenols followed by flavonoids then alkaloids where is the lowest percentage was for terpenes. The high percentage of phenols (3.939mg/ g) were is 0.159 mg/g alkaloids, this result agrees with [29] which have the highest concentration of phenols than alkaloid in *Zanthoxylum* leaves. The high amounts of phenols refer to ability of plant to tolerant the biotic and abiotic stress as a defense appliance [30]. Flavonoids follow the phenols in their quantity which agree with [31] that indicated that the phenols amount higher than flavonoids in *Zanthoxylum*. Variation in quantities of secondary metabolite and their gradients may return to effect of environment condition, nutrient abundance and the characterization of geographic reign where the plant grow can take place in result explain which all that can affect the active compounds production [29].

Table 2 Total quantitative analysis of *Zanthoxylum* leaves active compounds

No	Active compound	Concentration mg/ g dry weight
1	Total phenolic content	3.939
2	Total flavonoid content	2.775
3	Total alkaloid content	0.159
4	Total terpinoid content	0.111

4. Conclusion

Zanthoxylum beechyanum K.Koch. 1853 is an exotic plant grow in Iraq vegetatively, up to this study, there were no fruits or seeds because there were only female plants. There were no negative biological effects nor on the other nearby plants or the farmers who is working nearby. The study suggests to do some phylogenetic researches to found the relationship of this plants with other species of Rutaceae in Iraq.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have no conflicts of interest to declare.

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<https://efloraindia.bsi.gov.in/eflora/taxonList.action?id=630&type=2>
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