

## Pharmacognostic and preliminary phytochemical standardization of *Tamarix aphylla* and *Ziziphus nummularia* growing in Saudi Arabia

Hasan Soliman Yusufoglu<sup>1\*</sup>, Aftab Alam<sup>1</sup>, Abdullah Al-Howeemel<sup>2</sup>

1 Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O.Box 173, Al-Kharj-11942, KSA.

2 Undergraduate Student, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O.Box 173, Al-Kharj-11942, KSA.

E-mail : hasanagazar@hotmail.com, h.yusufoglu@sau.edu.sa

Contact No. : 00966-11-588-6012

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### Abstract

*Tamarix aphylla* (Family: Tamaracaceae), commonly known as Athil and farash an evergreen tree. The natives it used for treatment of jaundice, bad evils, rheumatism, wound and abscesses. *Ziziphus nummularia* (Family: Rhamnaceae), commonly known as Sidar or Jharber, is a thorny bush and shrub. It is used for wound and abscesses, scabies and other skin disease. These plants are widely used by Arabs. However, no detailed scientific information is available to identify and authenticate these local plants. In the present study, the macroscopical and microscopical characters along with physicochemical parameters and phytochemical screening were carried out in order to identify and authenticate the leaves of both plants. The physicochemical parameters such as moisture content, total ash, water-soluble, acid-insoluble ash and values of petroleum ether, alcohol and water extractives were documented for both the plants. The results of phytochemicals screening suggested that the active compounds present in both plants could be beneficial in treatment of various ailments.

Key words : Anatomical, Morphological, Physico-chemical, *Tamarix aphylla*, *Ziziphus nummularia*

### INTRODUCTION

*Athil* (*Tamarix aphylla*, F. Tamaricaceae) or related plant species are mentioned in the Quran, Ahadith and Islamic literature for the folklore use in various ailments<sup>[1]</sup>. People in several parts of Qassim area of Saudi Arabia apply butter and Vaseline base formulation of this plant powder, once or twice daily for 3-7 days, over lesions to cure mycotic or allergic dermatitis of camels<sup>[2]</sup>. The use of this plant as antimicrobial or antifungal agent, and to control leishmaniasis, mycotic or allergic dermatitis, eczema and fever has been reported<sup>[3]</sup>. The active constituents of *T. aphylla* are alkaloids, flavonoids, tannins, triterpene and other polyphenolic compounds<sup>[4-6]</sup>. The anti-inflammatory, antioxidant and wound healing activities of the plant were studied<sup>[7]</sup>. *Z. nummularia* family Rhamnaceae is one of five species belonging to the genus *Ziziphus* that are native of Saudi Arabia. *Z. nummularia* is a very common, generally occurring as branched and thorny shrub attaining the height of 1-2 m and having light colored bark<sup>[8]</sup>. The leaves are antipyretic and reduce obesity<sup>[9]</sup>. The fruits have tonic, digestive, laxative aphrodisiac and removes biliousness, thirst, vomiting and burning sensations<sup>[10]</sup>. It is routinely used in traditional medicine of many Asian countries. It is commonly known in Arabic as sidr, and was mentioned Sura Saba (4 time) as earth tree while in other Suras it is mentioned as a Paradise tree. Many hadiths recommended mixing ZPS leaves with warm water for giving disinfectant bath and the use of the oil from the resin as deodorant. Leaves and seeds of this plant are used by ethno veterinarians at Al-Qassim region, Kingdom of Saudi Arabia for treatment of several of camel ailments<sup>[2]</sup>. Fruits of *Z. nummularia* are used as laxative and astringent, while its leaves are used to treat scabies, boils<sup>[11]</sup> and as expectorant<sup>[12]</sup>. They are also used internally in treatment of a range of conditions including loss of appetite, chronic fatigue, diarrhea, pharyngitis, bronchitis, burns, anemia, irritability, hysteria<sup>[13]</sup>. The recent study showed both the plants have good

anti-inflammatory, antioxidant and wound healing activities<sup>[7]</sup>. Phytochemical reports on *Z. nummularia* have revealed the presence of polysaccharides, a pectin composed of L-rhamnose, D-galacturonic acid, D-galactose, L-arabinose, peptide alkaloids, cyclopeptides, flavonoides, saponins, triterpenoides, fatty acids and dodeca acetyl prodolphinidin B3<sup>[14]</sup>. The compounds nummularogenin<sup>[15]</sup> and zizynummin<sup>[16]</sup> have been isolated from the plant.

The external leaf characters such as shape, margin, hairs, etc. are important for identifying plant species. The type of leaves and their arrangement on the stems is usually characteristic of a species. Microscopic authentication refers to observing cell structure and internal features using light or sometimes electron microscope, polarized light microscope and fluorescence microscope to expand the number of features available for identification. For example, it has been found that starch grains, crystals of calcium oxalate, stone cells, vessels and fibers have stable and special polariscopic characteristics<sup>[17]</sup>. Phytochemical screening is a process of tracing plant constituents.

There are many reports on the geographical distribution, habitat, and morphological characters of the plant in Saudi Arabia. The documented information of plant of folklore used are lacking in Saudi Arabia. The present study aims to document the pharmacognostical, phytochemical and physicochemical standard parameters of *Athil* and *Sidr* leaves.

### MATERIAL AND METHODS

Chemicals: Phloroglucinol, glycerin, hydrochloric acid, ferric chloride, Ammonium hydroxide, chloral hydrate, potassium hydroxide, ammonium hydroxide, lead acetate, copper sulfate and all other chemicals used in the study were of analytical grade. All the solvents were of analytical grade. Plant material collection and authentication: The leaves of *Athil* (*T. aphylla*) and *Sidr* (*Z. nummularia*) were obtained from Al-Kharj area in central region

of Saudi Arabia. The collected samples were washed to remove dirt and impurities and kindly identified by Dr. Mohammed Yusuf, Taxonomist at the College of Pharmacy, King Saud University (KSU), Riyadh. The leaves were removed and dried under shade and then powdered by using an automated grinder. They were stored in tight container and used as samples for the present study. The Pharmacognostic evaluation was accounted by the evaluation of macroscopical and microscopical characters. For microscopical evaluation, the dried powdered leaves was separately treated with alcoholic phloroglucinol solution then conc. HCl then mounted in glycerin<sup>[18, 19]</sup> and was observed by Motic digital microscope (Motic instrument Inc, Canada) fitted with camera. Physicochemical evaluation such as moisture and ash content was determined according to reported method<sup>[20]</sup>. The extractive value, of both plants, was determined according to Banso & Adeyemo<sup>[21]</sup>. The phytochemical screening of the chemical constituents (alkaloids, carbohydrates, glycosides, saponins, steroid, terpenoids, fat and oils, tannins, flavonoids, amino acids and proteins) was carried out following the procedures present in Trease & Evans and Harborne<sup>[18, 19]</sup>.

The leaves *Z. nummularia* (Figure A) are alternate, glabrous, ovate and green in color and having a pubescence adaxial surface, measuring 2-5 cm wide and 2-3 cm broad with three conspicuous veins at the base, and a finely toothed margin.

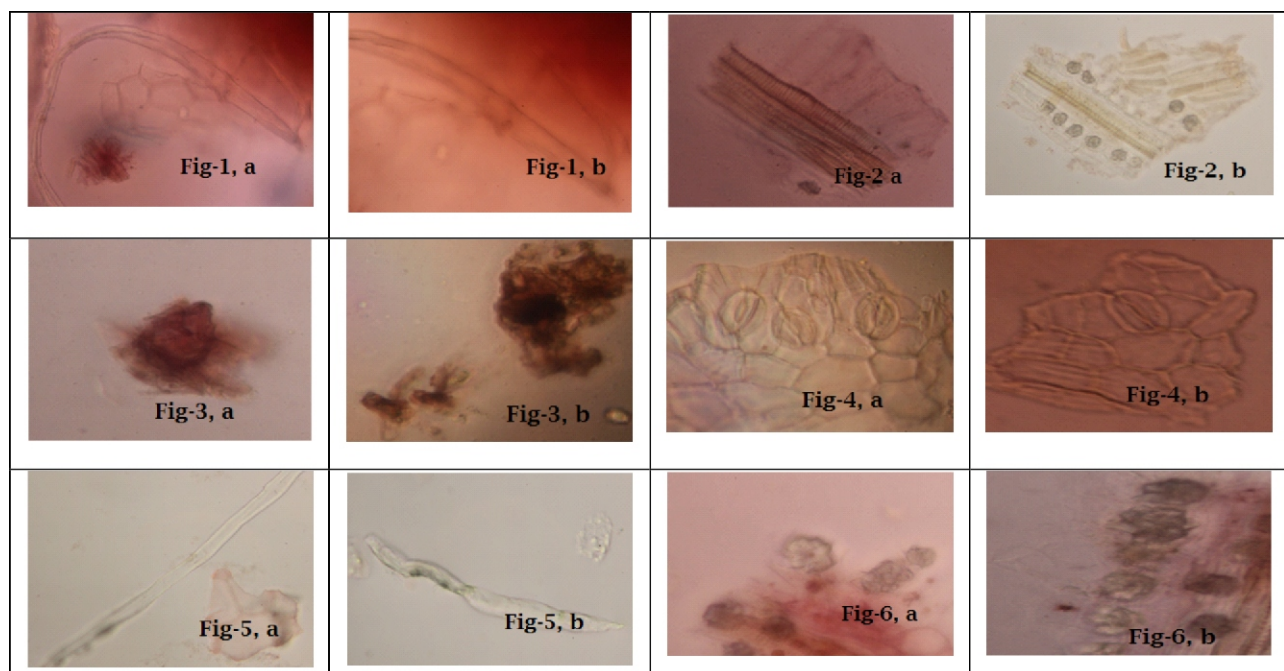
Microscopic studies of powdered leaves of *Z. nummularia* are shown in Figure 1-6. The powder is characterized by the presence of fragments of covering multicellular uniseriate trichomes (Fig. 1a & 1b), parenchymatous cells underlying gannular to spiral xylem vessel (Fig. 2a & 2b), and tannin bodies (Fig. 3a & 3b). The epidermal cells are polygonal with straight anti-clinal walls and covered with thin smooth cuticle and showing anomocytis tomata (Fig. 4a & 4b). Lignified fibers (Fig. 5a & 5b) and androsettes crystals of calcium oxalate (Fig. 6a & 6b) are abundant.

*T. aphylla* leaves (Figure B) are greyish-green in color,

alternate, and reduced to sheathing scales 1.5-4 mm long and 1 mm in diameter on twigs, abruptly truncate and with minute point, with salt-excreting glands, larger branchlets with persistent scale-like leaves. The morphological characters of *T. aphylla* are shown in figure 1 and listed in table 1.

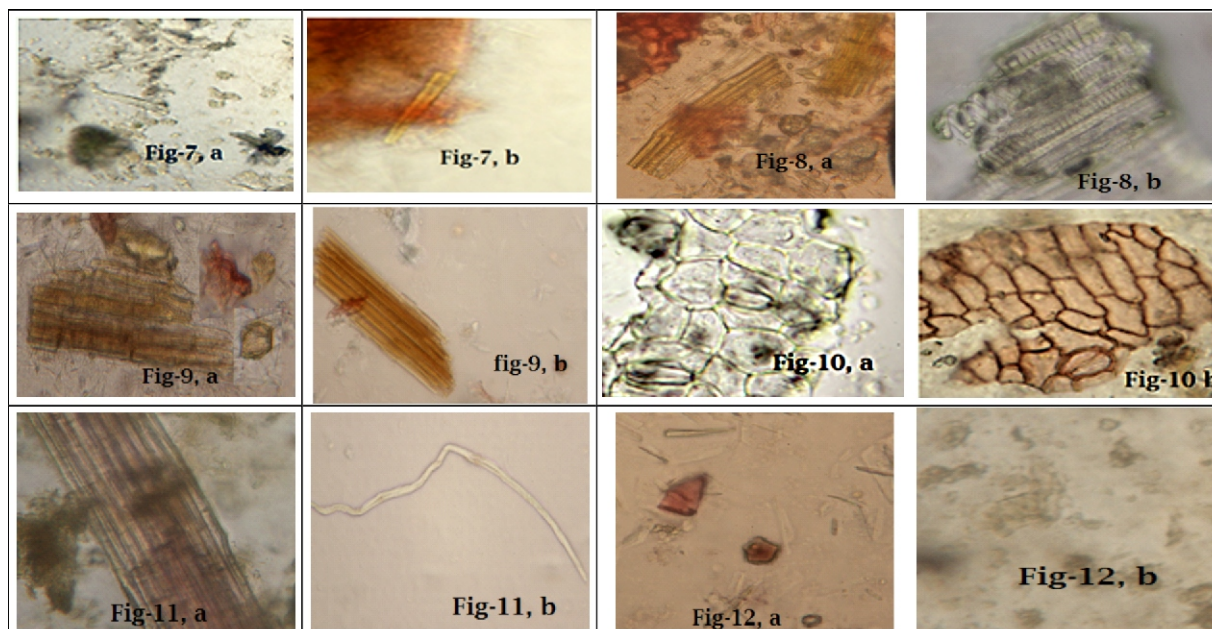
Powdered shoots of *T. aphylla* (Figure 9-14) are characterized by the presence of few and small covering trichomes (Fig. 7a & 7b), parenchymatous cells underlying spiral xylem vessel (Fig. 8a & 8b), reddish tannin bodies (Fig. 9a & 9b) and epidermal cells with slightly wavy anti-clinal walls and covered with thin smooth cuticle and showing anomocytis tomata (Fig. 10a & 10b). Lignified fibers from the stem (Figure 11a & 11b) and prismatic crystals of calcium oxalate (Figure 12a) were also observed.

Physicochemicals parameter of both plants such as moisture content, ash value (total ash, water soluble ash, acid insoluble ash) and percentage extractive values (Petroleum ether (60-80), Methanol, and water Extracts) are cited in table 2. In present studies, the result showed that *Z. nummularia* (6.94%) was comparatively lower moisture contents than *T. aphylla*. The water soluble ash is lesser than acid insoluble ash and amount of total ash were nearly double of their water soluble ash. The total ash values of *Z. nummularia* and *T. aphylla* were 3.063 and 2.629 respectively. The acid insoluble and water soluble ash of *Z. nummularia* was 2.439 and 0.328 respectively while acid insoluble and water soluble ash of *T. aphylla* was 2.176 and 0.418 respectively. The present studies show that aqueous extracts of both the plants *Z. nummularia* and *T. aphylla* were higher 18.2 and 16.12% w/w respectively while petroleum ether extract was lower 2.6 and 0.73% w/w for *Z. nummularia* and *T. aphylla* respectively. Preliminary phytochemical analysis of powdered of plants of *Z. nummularia* and *T. aphylla* were showed in Table-3 and revealed the presence of alkaloids, carbohydrates, glycosides, steroids and triterpenoids, fats and oils, Phenols and tannins, flavonoids, proteins and amino acids.



**Figure** of *Z. nummularia* leaves powdered, (40 x), under light microscope. **1 (a & b)**; Showed long covering trichome, **2 (a & b)**; Showed helical to spiral shaped xylem vessel, **3 (a & b)**; Showed tannin contents, **4 (a & b)**; Showed stomata of leaves powdered, **5 (a & b)**; Showed fiber and **6 (a & b)**; Showed ca-oxalate crystals.





**Figure: 7 (a & b);** Showed long covering trichome, **8 (a & b);** Showed helical to spiral shaped xylem vessel, **9 (a & b);** Showed tannin contents, **10 (a & b);** Showed stomata of *Z. nummularia* leaves powdered, **11 (a & b);** Showed fiber and **12 (a & b);** Showed calcium oxalate crystals of *Athil (T. aphylla)* leaves powdered, (40 x), under light microscope.

**Table 1:** Macroscopic studies of fresh Sidr (*Ziziphus nummularia*) and Athil (*T. aphylla*) plant.

S.N	Microscopic characters	Sidr ( <i>Ziziphus nummularia</i> )	Athil ( <i>T. aphylla</i> )
1	Phyllotaxy	Alternate	Leaves alternate, reduced to sheathing scales
2	Lamina	Ovate	scale-like
3	Size	15-40mm long	1.5-4 mm long and 1 mm in diameter
4	Surface	Glabrous	NA
5	Margin	Entire/ Toothed (not clear)	NA
6	Apex	Obtuse/ Acute	NA
7	Venation	Palmate	overlap
8	Colour	Green	grey-green foliage
9	Taste	Characteristics	Characteristics

\* Average of 10 leaves, NA (not applicable)

## DISCUSSION

Different plant in different location documented for different uses and the same plant are required for correct treatment hence needs a reproducible product. The proper control of starting material is highly essential for achieving a similar product. Hence, the routine standardization of medicinal plants such as macroscopic, microscopic, physicochemical and phytochemical properties are required. According to World Health Organization the macroscopic and microscopic report of a medicinal plant is the primary step towards establishing its quality and should be carried

out before any tests are taken [22]. The macroscopic analysis exposed that the leaves of both have different characters. Both the plants were easily identified macroscopically. The macroscopically *Z. nummularia* has different and a unique phyllotaxy, lamina, surface, color and taste are easily identified with other plant. The microscopic of the *Z. nummularia* have a unique trichomes, calcium oxalate, fibers, helical to spiral shaped xylem vessel and epidermal cells with anomocytic stomata. Morphologically *T. aphylla* showed different structure easily identified from other plants. The microscopically *T. aphylla*

**Table 2:** Physicochemical parameters of Athil (*T. aphylla*) and Sidr (*Z. nummularia*) leaf powdered.

Parameter	Physicochemical parameters	Athil	Sidr
Ash value	Total Ash*	2.629	3.063
	Water soluble*	0.418	0.328
	Acid insoluble*	2.176	2.439
Percentage moisture content	Moisture content*	11.7 % w/w	6.94 % w/w
Percentage extractive value	Petroleum ether(60-80) Extract *	0.73 % w/w	2.6 % w/w
	Methanol*	15.73 % w/w	15.9 % w/w
	Distilled water*	16.12 % w/w	18.2 % w/w

\* Average values of leaf powdered (n=3)

**Table 3:** Phytochemical studies of Athil (*T. aphylla*) and Sidr (*Ziziphusnummularia*) plant leaf samples.

Phytoconstituents	Reagent	Sidr ( <i>Z.nummularia</i> )	Athil ( <i>T.aphylla</i> )
Alkaloids	Mayer's	-	+
	Dragendroff's	+	+
Carbohydrates	Molisch's	+	+
	Fehling's	+	+
Glycosides	Killer-Killani test	-	-
Saponins	Foam Test	+	+
Steroids and Triterpenoids	Liebermann Burchard's	+	+
Fats & oils	Stain Test	+	-
Tannins	Ferric Chloride Test	+	-
Flavonoids	Lead acetate Test	+	+
Proteins &	Ninhydrin Test	+	+
Amino acids	Biuret Test	-	+

\* +/- (Presence/Absence)

showed few and small covering trichomes, ca-oxalate (prism shaped), xylem vessel and anomocytic stomata. The phytochemicals (secondary metabolites) of medicinal plants report for their medicinal value. The determination of physical constants analyses of crude drugs can facilitate the detection of improper handling of the plant material. During the storage of natural drugs, excess of moisture content may lead to the breakdown of essential constituents and the growth of micro-organism<sup>[23]</sup>. Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along

with the drug. The percentage of total ash, acid insoluble ash and water soluble ash are carried out and determination of ash value gives an idea of the earthy material or inorganic composition and other impurities present along with the drug. Extractive values are primarily useful for the determination of exhausted or adulterated drugs<sup>[24]</sup>. Phytochemical constituents are responsible for medicinal activity of plant species. Hence in the present preliminary phytochemical screening of *Z. nummularia* and *T. aphylla* a medicinal plants was carried out, qualitative phytochemical analysis of this plant confirm the presence of

various secondary metabolites like alkaloids, glycosides, tannins, saponin, flavonoids, steroid, triterpenes and phenol.

## CONCLUSION

In conclusion, the present study gave useful information of pharmacognostical, phytochemical and physiochemical standardization parameters for authentication and carry out the further investigation for its use in different ailment.

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