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Phytochemical Profiling and Biological Activity of Tamarix Nilotica Leaves Extract

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Abstract

Tamarix nilotica. (Tamaricaceae) is one of the most used plants in Sudan folkloric medicine. The powdered leaves of *Tamarix nilotica* were extracted using 80% methanol; the methanolic extract was then fractionated by Petroleum ether, Chloroform and Ethyl acetate. Phytochmical profiling was carried for all fractions of *Tamarix nilotica* leaves by using Thin Layer Chromatography (TLC). The TLC chromatogram revealed the presence of Flavonoids, Phenolic acids, Coumarins, Terpenoids and Alkaloids. However, the ethyl acetate fraction was the richest fraction with polyphenols. The radical scavenging activity of *Tamarix nilotica* leaves was screened by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), the antioxidant results were expressed as concentration of inhibition (IC50) of the free radical DPPH. The extract give highest antioxidant activity 82±0.01% with IC50 0.0093∓0.09µg/ml. compared to the control standard Gallic acid. The scavenging activity of *Tamarix nilotica* leaves fractions increases correspondingly with the increasing polyphenolic content. The aforementioned extract was screened for antimicrobial activities against five standard bacteria *(Bacillus subtitles, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*, one fungi *(Candida albicans)* using in vitro agar well diffusion method. The methanolic extract at a concentrations (5mg/ml) was significantly active against *Staphylococcus aureus* (20 mm), *Bacillus subtilus* (18 mm), *Protues vulgaris* (20 mm), *Pseudomona. aeruginosa*,(21 mm) and *Escherichia coli* (14 mm). Furthermore, this extract at the same concentration possessed activity against *Candida albicans* (20 mm). Methanolic extracts of *Tamarix nilotica* showed stronger action against urease activity, parentage of inhibition (loss of enzyme activity) reported as > 70%.

Keywords: Antimicrobial activity; DPPH method; Urease inhibitory activity; *Tamarix nilotica*.

1. Introduction

Tamarix nilotica belonging to the Tamaricaceae family. This plant has diverse and potential medicinal uses in traditional herbal medicine for treating relieve headache, draw out inflammation, and as an antiseptic agent. *Tamarix nilotica* have been occurs in Lebanon, Palestine, Egypt, Sudan, Somalia, Ethiopia and Kenya [1]. Phytochemical investigation revealed that the major chemical constituents of *Tamarix nilotica* are flavonoids, tannins and phenolics. The hydro-alcoholic extracts of the leaves of *T. nilotica* exhibited significant antioxidant, anti-tumor, hepatoprotective and antiviral activities. It is now widely recognized that gastric and duodenal ulcers are generally caused by *H. pylori*, which survives and grows in acidic environments [2, 3]. This organism releases urease that converts urea into ammonia and the released ammonia protects it from the acidic environment of the stomach. It has been demonstrated that a urease-negative mutant does not cause gastritis due to difficulties in colonization, therefore, specific inhibition of urease activity has been proposed as a successful strategy to eliminate the organism in the body [4]. The cases of *H. pylori*-related infections are increasing in the developing countries, while in some parts of the World more than 50% of the population is reported to be infected with *H. pylori* [2, 3]. Triple therapy, comprising a proton pump inhibitor and any of the two antibiotics such as amoxicillin (AMX), clarithromycin (CLA), metronidazole (MNZ) and tetracycline (TET), is frequently employed to cure *H. pylori* infections [3]. In recent years, the search for phytochemicals possessing antimicrobial properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes.

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Several species of plants belonging to the genus Tamarix have been employed in traditional medicine. *Tamarix nilotica* common traditional uses shown in various reports for some plant species of the genus are as a diaphoretic, diuretic and hepatotonic and to treat liver disorders, relieve headache, ease prolonged or difficult labor, and cure sores and wounds besides being an astringent and employed for tanning and dyeing purposes [5-9].

The present study was aimed to evaluate phytochemical profiling, antimicrobial, antioxidant, cytotoxic and Urease Inhibition Activities of *Tamarix nilotica* Leaves Extracts.

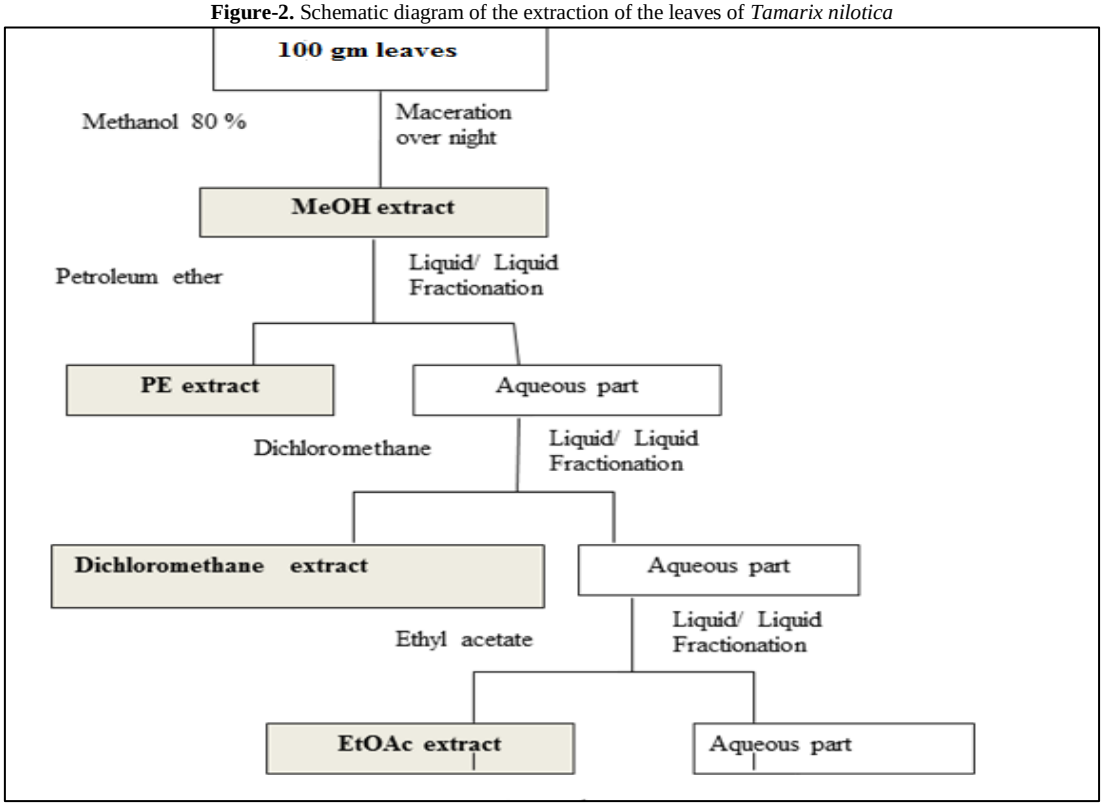
2. Material and Methods

2.1. Plant Material Preparation and Extraction

Tamarix nilotica leaves (Fig.1) were collected from North Kordofan State, taxonomical identification was made at the Medicinal and Aromatic Plant Research Institute (MAPRI), the fresh leaves were rinsed with tap water then they were shade dried and grounded. Hundred grams of dry powdered leaves were macerated in 80% ethanol overnight, the extract is filtered and additional volume of 80% ethanol is added .The ethanolic extract was then collected together and the solvents were removed and the extracts were concentrated to dryness by evaporating the rest of solvents at room temperature in the cover fume. The crude extract obtained was subjected to further biological and phytochemical profiling (fig. 2).

Figure-1. *Tamarix nilotica* leaves

Figure-2. Schematic diagram of the extraction of the leaves of *Tamarix nilotica*



2.2. Chromatographically Analysis

2.2.1. Thin Layer Chromatography (TLC)

Aluminium silica gel plates 60 F254 (Merck 5554) or pre-coated TLC plates were used in carrying out TLC of the different plants extracts. Standard chromatograms of the plant extracts were prepared by applying 20 µl solution (5 mg/ml) to a silica gel plate and developing it in Toluene: EtoAc: HCOOH (5:4:1) solvent system. Chromatograms were detected under UV light (254 and 366) and sprayed with diagnostic reagents which include: vanillin-H_2SO_4 reagent, potassium hydroxide and Natural Product Reagent (NPR).

2.2.2. Antimicrobial Activity of Crude Extracts

The extracts of *Tamarix nilotica* were tested *in vitro* for their antibacterial and antifungal activities against difference pathogenic organisms. Plant extracts were tested against *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) *Salmonella typhi* (ATCC 0650), *Staphylococcus aureus* (ATCC 25923), *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596) using agar diffusion method [10] with minor modifications.

2.2.3. Antioxidant Activity (Free Radical Scavenging Assay)

Free radicals are involved in a number of pathological conditions such as inflammatory diseases, atherosclerosis, cerebral ischemia, AIDS, and cancer [11]. This method was carried out according to that described by Shyur, *et al.* [12] with some modifications. Stock solution was prepared by dissolving 1mg of the sample in 1ml of absolute ethanol (99%). 0..9ml Tris-HCL and 1ml of 0.1 mM DPPH in methanol solution were added to each 0.1ml of sample and incubated at room temperature in the dark for 30 minutes. The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity using the formula below:-

$$\text{Scavenging activity (\%)} = \frac{(\text{A control} - \text{A sample}) \times 100}{\text{A control}}$$

solution of 0.9 ml Tris-HCL+ 0.1ml absolute ethanol+ 1ml absolute ethanol was used as blank , while solution of 0.9ml tris-HCL+0.1ml absolute ethanol+1ml DPPH was used as a control.
A freshly prepared DPPH solution exhibits a deep purple colour with a maximum absorbance at 517 nm. The purple colour disappears when an antioxidant is present in the medium. Thus, the change in the absorbance of the reduced DPPH was used to evaluate the ability of test compound to act as free radical scavenger. Furthermore, the "Inhibitory concentration" or IC50 value (the concentration of antioxidant that causes 50% loss of the DPPH activity) was also used to assess the antioxidant activity of the plant extract compared to the standard gallic acid.

2.3. Urease Inhibitory Activity

In-vitro urease inhibition assay was done according to Weather Burn [13] with some modifications.

3. Results and Discussion

3.1. Thin Layer Chromatography (TLC) of Tamarix Nilotica Leaves

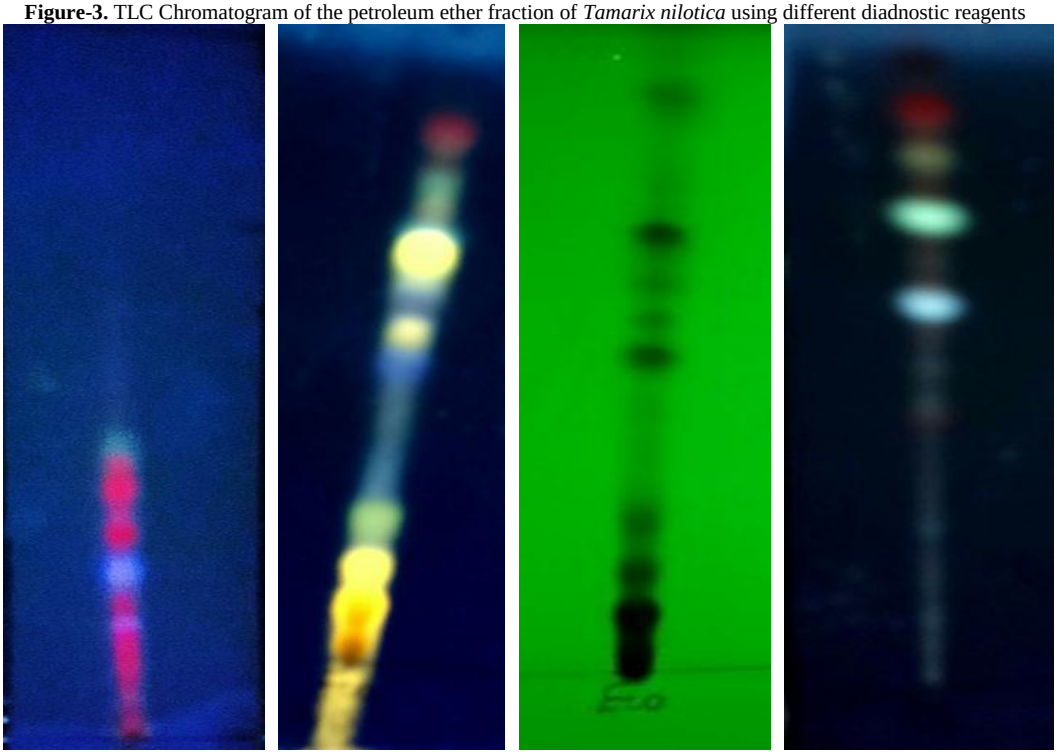
T. nilotica is a rich source of different classes of natural products with varying structural patterns. Many compounds have been isolated from *T. nilotica* including carbohydrates, phenols, flavonoids, terpenoids, steroids, tannins, and cardiac glycosides [14]. TLC profiles of the petroleum ether, chloroform and ethyl acetate fractions are conducted

TLC profiles of the different fractions of the petroleum ether fractions of *Tamarix nilotica* using different diadnostic reagents are presented in Tables (1) and Figures (3). Wagner, *et al.* [15], is used as a reference in color detection of the different phytochemical compounds.

Table-1. TLC Profiles of the petroleum ether fractions of *Tamarix nilotica* using different diadnostic reagents

Comp. Spot	R_f Values	UV Reaction		Compounds Colour Reactions to DiagnosticSpray Reagents			Expected Metabolites (+/-)
		254nm	366nm	NPR 366nm	Vanillin H_2S0_4	drag	
1	0.023	quenching	Pink	Red	violet	-ve	chlorophyll ,terpenoid
2	0.05	-ve	Blue	Red	-ve	-ve	terpenoid
3	0.09	quenching	Pink	Red	-ve	-ve	chlorophyll
4	0.12	quenching	Pink	Red	violet	-ve	chlorophyll ,
5	0.14	-ve	Pink	Red	-ve	-ve	terpenoid
6	0.17	quenching	Blue	Red	-ve	-ve	Flavonoid
7	0.19	quenching	Pink	yellow	violet	-ve	chlorophyll , flavonoid
8	0.23	-ve	Pink	yellow	-ve	-ve	flavonoid
9	0.25	quenching	Blue	yellow	violet	-ve	Flavonoid,terpenoid
10	0.28	quenching	Pink	yellow	violet	-ve	flavonoid
11	0.31	quenching	Pink	Red	-ve	-ve	chlorophyll
12	0.35	quenching	Red	Red	violet	-ve	Alkaloid, chlorophyll
13	0.37	quenching	Orange	orange	violet	brown	Alkaloid,chlorophyl
14	0.40	quenching	Pink	orange	violet	brown	alkaloid, chlorophyll
15	0.43	quenching	Pink	yellow	violet	-ve	Flavonoid, terpenoid
16	0.46	-ve	Blue	yellow	violet	-ve	Flavonoid, terpenoid
17	0.52	-ve	Blue	pink	violet	-ve	Terpenoid

Figure-3. TLC Chromatogram of the petroleum ether fraction of *Tamarix nilotica* using different diadnostic reagents



3.2. Antimicrobial Activity of Tamarix Nilotica Leaves Extracts

The family Tamaricaceae have proven to be a promising source of antimicrobial growth inhibitors and bactericidal/fungicidal agents. Traditional healers in all regions of the world use species of Tamaricaceae for a wide range of medicinal purposes including the treatment of abdominal pain and other abdominal disorders, constipation, coughs and colds, conjunctivitis, constipation, coronary diseases, diarrhoea, dysentery, earache, fever, headache, infertility, inflammation and swelling, jaundice, leprosy, parasites, pneumonia, sexually transmitted infections (STI's), sore throats, toothache and ulcers [16]. Some of these conditions are caused by bacteria and fungi. The obtained methanolic extract of *Tamarix nilotica* leaves were screened for their antimicrobial activities against five standard bacteria (*Bacillus subtitles, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli*, two fungi (*Candida albicans and Aspergillus niger*) using in vitro agar well diffusion method. The methanolic crude extract at a concentrations (5mg/ml) was significantly active against *Staphylococcus aureus* (20 mm), *Bacillus subtilus* (18 mm), *Protues vulgaris* (20 mm), *Pseudomona. aeruginosa*,(21 mm) and *Escherichia coli* (14 mm). Furthermore, this extract at the same concentration possessed activity against *Aspergillus niger* (16 mm) and *Candida albicans* (20 mm) Table (2).

The genus Tamarix was reported to have anti-microbial in addition to its pharmacological activity, [17] results of antimicrobial assays showed that all tested extract were active against all tested microbial species including gram positive and negative bacteria.

The antifungal activity of *Tamarix nilotica* was also reported by Abouzid and Sleem [18], eight extracts of various polarities from the stem wood and bark were screened for their growth-inhibitory effects against filamentous fungi commonly causing fruit, vegetable, grain and wood decay, as well as infections in the immunocompromised host. Ethyl acetate extracts of the stem wood and bark gave the best antifungal activities, with MIC values of 250 µg/mL against *Nattrassia mangiferae* and *Fusarium verticillioides*, and 500 µg/mL against *Aspergillus niger* and *Aspergillus flavus*. Aqueous extracts gave almost as potent effects as the ethyl acetate extracts against the *Aspergillus* and *Fusarium* strains, and were slightly more active than the ethyl acetate extracts against *Nattrassia mangiferae.* The results obtained indicated that the leaves extract of *Tamarix nilotica* grown in Sudan exhibited remarkable antimicrobial activity by inhibiting the growth of tested microorganisms.

Table-2. Antimicrobial activity of the leaves extracts of *Tamarix nilotica*

Extract concentration	**Extract**	**Measurement of inhibition zones diameter (mm) Bacteria (MIZD) fungi**						
		***Bacteria**					***Fungi**	
		S.a	***B.s***	***P.v***	***P.a***	***E.c***	***A.n***	***C.a***
5mg/ml	ethanolic crude extract	20	18	20	21	14	16	20

B. s = *Bacillus subtitles.* S. a = *Staphylococcus aureus,* E.c = *Escherichia coli,* P. a = *Pseudomonas aeruginosa,* A. n = *Aspergillus niger,* C. a = *Candida albicans,*MIZD (mm): > 18 mm: Sensitive; 14— 18 mm: intermediate; < 14 mm: Resistant.

The radical scavenging activity of the extract was screened by using 1, 1-diphenyl-2- picrylhydrazyl (DPPH), the antioxidant results were expressed as concentration of inhibition (IC50).The extract give highest activity 82±0.01% with IC50 **9.3** µg/ml Table (3)

Table-3. Antioxidant activity of *Tamarix nilotica* leaves Extract

Sample	Activity%S∓/D	IC50 S∓D
Methanolic extract	82 ∓.0.01	0.0093∓0.09

The enzyme activity and inhibition was measured through catalytic effects of urease on urea by measuring change in absorbance in the absence and in the presence of inhibitor at 625nm using UV spectrophotometer. In the study, methanolic extracts of *Tamarix nilotica* showed stronger action against urease activity, parentage of inhibition (loss of enzyme activity) reported as > 70% figures (4) and figure (5). An overview on the medicinal uses of *Tamarix nilotica* showing anti-urease activity may predict their possible alternative use for stomach problems. This study may help to explain the beneficial effects of this plant against stomach infection associated with pathogenic strains of *H. pylori* as Urease is the most prominent protein component of *H. pylori*

Figure-4. Anti-urease activity of leaves extracts of *Tamarix nilotica*

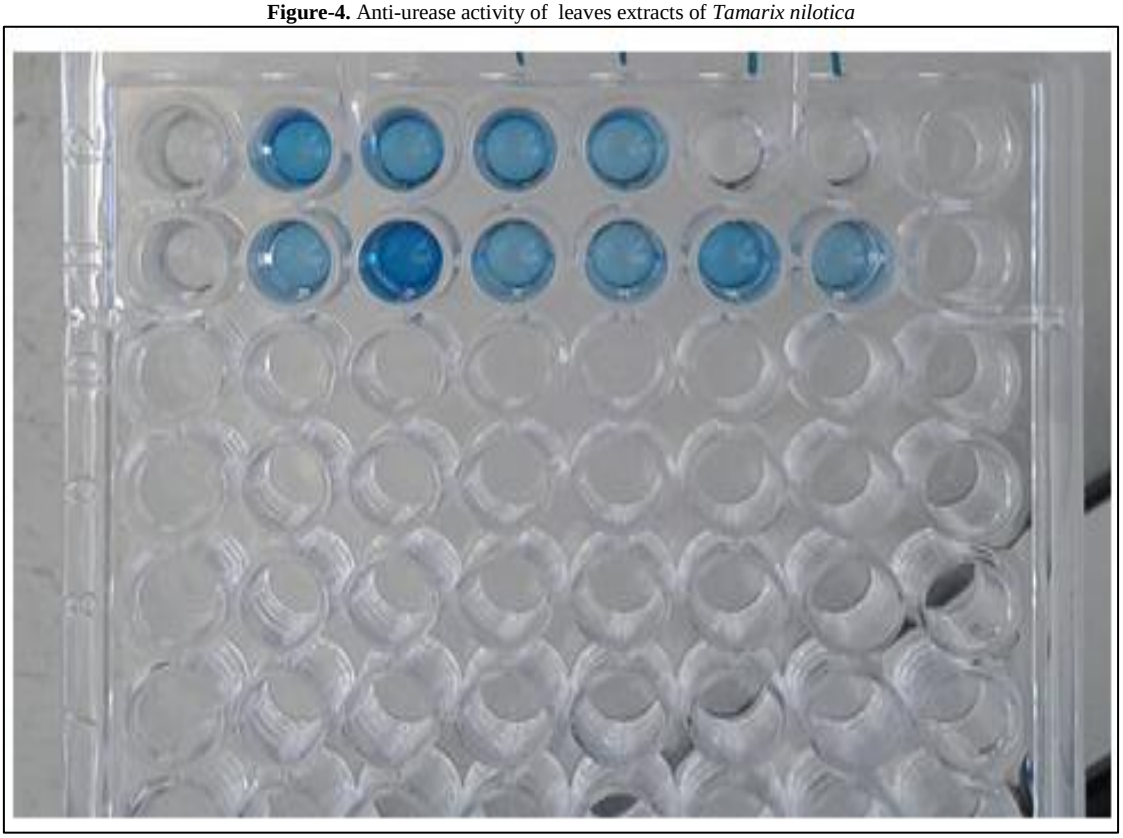


Figure-5. Enzyme Kinetic of *Tamarix nilotica*

4. Conclusion

-The current study showed that *Tamarix nilotica* leaves extract fractions contain Polyphenols, Flavonoid, Terpinoids and Alkaloids. .

- The extracts of the leaves of *Tamarix nilotica* were found to be significantly active against tested standard human pathogenic bacteria and fungi.

- This study also revealed the antioxidant potential of *Tamarix nilotica* leaves.

- Our findings may help to explain the beneficial effects of this plant extracts against stomach infection associated with pathogenic strains of *H. pylori*

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