Egypt. J. Chem. **Vol. 67** No. 9 pp. 1 - 12 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

UPLC-ESI-MS Analysis of Secondary Metabolites from *Tamarix Aphylla* With Its *In-Vitro* Anti-Inflammatory and Anti-Microbial Activities

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Abstract

Tamarix aphylla is considered one of the known species of the genus Tamarix. It has been shown to has a various pharmacological property. In our investigation, we study the different extracts of Tamarix aphylla [85% methanol (MeOH), petroleum ether (PE), ethyl acetate (EtOAc), butanol (BuOH), and water (Aqueous) extract] as anti-inflammatory and antimicrobial with the identification of the chemical profile of the most effective extracts. The obtained fractions were subjected to the total phenolic content determination using Folin-Ciocalteu method, the total Flavonoid content was achieved using Aluminum chloride, anti-inflammatory activity was measured by Cell Culture (Seeding and Treatment), and antimicrobial activity was premeditated. Qualitative determination of compounds by Ultra performance liquid chromatography (UPLC-ESI-MS). Our results showed that; the EtOAc and BuOH extracts have the highest phenolic content between the examined extracts. EtOAc extract antimicrobial activity ranged from 27 mm against Escherichia coli, to 19 mm against Aspergillus niger. Moreover, BuOH, EtOAc, and Aqueous extracts proved anti-inflammatory efficacy as induced marked inhibition of NO production by (60-70%) as compared to LPS. UPLC-ESI-MS analysis of the butanol extract in the negative-ion Mode gave compounds such as Gallic acid, while in the positive-ion mode of the butanol extract contains metabolites like syringic acid. Analysis of the Ethyl acetate extract in the negative-ion Mode was 2,3-Dihydroxybenzoic acid and others. Analysis of the ethyl acetate extract in the positive-ion Mode revealed some natural compounds within gallic acid methyl ester. However, these bioactive compounds are responsible for promising inhibition of Nitric oxide (NO) production, antimicrobial and antibacterial activities.

"Keywords:T. aphylla; Total phenolic content; Total flavonoid content; Anti-inflammatory; Antibacterial activity."

1. Introduction

Herbs played a crucial role in social health throughout human history [1-3]. In history, WHO expected that 80 % of the world's inhabitants depends on traditional drugs of plant origin to provide health treatment, exclusively in developing countries [4-6]. Tamarix aphylla is a participant of the family Tamaricaceae. It has several names including saltcedar (English), Farash (India), Tamaris (French), Tamariske (German), Taray (Spanish) and Woestyn tamarisk (Afrikaans) [7]. It is a patterned tree, its leaves, bark, stem, and twigs are used for treating several diseases without side effects. The plant is a safe and cheap actual medicinal agent that acts as a potential alternate for controlling infectious diseases [8]. Tamarix aphylla has predictably been used in folk medicine to treat various illnesses such as hepatitis, eczema, rheumatism and skin diseases such as tinea capititis and syphilis [9]. The remedial activities of plants are due to the secondary metabolites in them as alkaloids, tannins, flavonoids, phenolic, etc. These secondary metabolites are operative in inflammation and pathogenic microbes as well as other catching diseases. Inflammation is an

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Received date 05 March 2024; revised date 17 May 2024; accepted date 20 May 2024
DOI: 10.21608/EJCHEM.2024.271590.9403

energetic process that is produced in response to mechanical damages, burns, microbial infections, and other noxious incentives that may impend the well-being of the host [10]. Host defense mechanism comprises several mechanisms including cell-mediated and tissue exchanges as a response to destructive stimuli and various infections. Studies in the last decades described that inflammation is regulated by a large number of pro and inflammatory mediators [11]. Moreover, morbific microbes have a wide variety of microbial species including Gram-positive bacteria, Gram-negative bacteria, mycobacteria, molds, yeasts, and dermatophytes [12]. From our data, infectious diseases caused by bacteria, fungi, viruses, and parasites are still a major threat to public health.
Moreover, their impression is particularly large in developing states due to the relative unapproachability of medicines and the development of widespread drug resistance [13]. From this idea, researchers in the last two periods concerned with drugs from medicinal plant extract [14]. In our investigation, we study the different extracts of *Tamarix aphylla* as anti-inflammatory and antimicrobial with the identification of the chemical profile of the most effective extracts.

2. Experiment

2.1. Plant Material

Leaves and stem of Tamarix aphylla were collected during December 2020 from Egypt-Alexandria dessert road, Giza, Egypt. The plant was identified by Dr. Rim Samir Hamdy, Prof. of Plant Taxonomy, Botany Dept, Faculty of Science, Cairo University. After the collection, leaves were air-dried under shade and grinded into fine powder by the grinder. Then, the powder obtained was stored for consequent study. Voucher specimen was dropped in the laboratory of Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

2.2. Extracts Preparation

1 kg of *Tamarix aphylla* (TA) was extracted with 85% methanol (MeOH) for one week then filtered using *Whatman* paper No. 1 and then evaporated, at 40 °C, under vacuum until comprehensive dryness. The crude MeOH extract was defatted and fractionated by petroleum ether (PE), ethyl acetate (EtOAc), butanol (BuOH) and water (Aqueous). The obtained fractions were filtered through *Whatman* paper No. 1 and then evaporated, at 40 °C, under vacuum to dryness completely, then the extracts stored in laboratory for further study

2.3. Total phenolic content

The total phenolic content was measured using Folin-Ciocalteu according to Attard, 2013 [15]. A stock solution of Gallic acid was used as standard at 1mg/ml in methanol. 9 serial dilutions were arranged in concentrations of (12.5, 25, 50, 100, 200, 400, 500, 800, 1000 µg/ml). MeOH, PE, EtOAc, BuOH and Aqueous extracts were prepared at concentrations 5 mg/ml in methanol. The found results were regarded by a microplate reader FluoStar Omega. Each of 9 standards and tasters were pipetted in the plate wells in 6 replicates. The absorbance of total phenolic content was measured at 630 nm and the average of 6 replicates reading was reserved. The consequence data were expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract [16].

2.4. Total flavonoid content

The total Flavonoid content was achieved using Aluminum chloride affording to Kiranmai *et al*. [17]. A stock solution of Rutin of 1mg/ml in methanol was used as standard. 10 serial dilutions were arranged in concentrations of (6.5, 12.5, 25, 50, 100, 200, 400, 500, 800, 1000 µg/ml). MeOH, PE, EtOAc, BuOH and Aqueous extracts were equipped at concentrations 5mg/ml in methanol. The obtained results were observed by a microplate reader FluoStar Omega. Each of 10 standards and trials were pipetted in the plate wells in 6 replicates. The absorbance of total phenolic content was measured at 415 nm and the average of 6 replicates reading was taken. The result data were expressed as mg/g of rutin equivalents in milligrams per gram (mg QE/g) of dry extract.

2.5. Anti-inflammatory Activity

2.5.1. Cell Culture (Seeding and Treatment)

The ATCC as long as the RAW 264.7 macrophage cell line (American type culture collection). The cells were grownup in RPMI 1640 medium with 1% pen/strep and 10% heat-inactivated fetal bovine serum (Roswell Park Memorial Institute). The cells were subculture twice before the testing and incubated in a moistened incubator at 37 °C in a 5% CO_2 atmosphere.

2.5.2. Procedure

All the subsequent steps were disseminated in a clean area with a biosafety class II level Laminar flow cabinet (Baker, SG403INT, Sanford, ME, USA). In RPMI media, RAW 264.7 cells were propagated. For the tests, 1×10^5 cells per well (in 96 well plates) were sowed after 24 h and incubated for

24 h. The cells were then cultivated for 1 h with the samples at doses of 100, 50, 25, and 12.5, µg/ml. They were left for 24 h with 10 µg/mL Lipopolysaccharide (LPS). The supernatant was then allocated to a new 96-well plates for nitric oxide (NO) measurement, while the cells in the former plate were used for viability testing using the MTT method. The samples were added in the medium were dissolved in DMSO. The mitochondrial reliant on reduction of yellow MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to purple formazan was used to determine cell viability [18]. Giving to the below formula, the percentage change in viability was computed.

((Extract reading / Negative control reading) -1) x 100

2.5.3. Nitric Oxide Assay

The generation of nitric oxide (NO) in the supernatants of cultivated RAW 264.7 cells was calculated. Through minor modification, the measurement was carried out as formerly described [19]. The quantity of nitrite, a stable metabolite of NO generation in the culture medium, was estimated using the Griess reagent after pre-incubation of RAW 264.7 cells (1×10^5 cells/ ml) with LPS (10 µg/ ml) for 24 h [sulfanilamide (1%) and naphthylethylenediamine dihydrochloride (0.1%) in phosphoric acid (2.5%)].

50 µl of cell culture media were mixed with 50 µl of Griess reagent. The mixture was then incubated at room temperature for 15 min before being measured in a micro plate reader at 540 nm. A blank of fresh culture medium was included. A sodium nitrite standard curve was used to calculate the quantity of nitrite, as given away in the equation:

$$\text{Nitric Oxide inhibition (\%)} = \frac{(\text{control} - \text{Test})}{\text{Control}} \times 100$$

2.6. Antimicrobial activity

Antimicrobial activity was done in accordance with [20,21]. MeOH, PE, EtOAc, BuOH and Aqueous samples were equipped by dissolving 2 mg in 2 ml of DMSO and 100 µl (containing 100µg) was used to estimate their antimicrobial activities by cup plate diffusion method using the assessment microbes *Staphylococcus aureus* (G+ve), *Escherichia coli* (G-ve), *Candida albicans* (yeast), *Aspergillus flavus* (fungus) and *Aspergillus niger* (fungus). Nutrient agar plates were deeply injected uniformly with 100 µl of 10^5 -10^6 cells/ml in case of bacteria and yeast. Potato Dextrose agar plate broadcasted by 100 µl the fungal inoculum was used to assess the antifungal activities. 1cm – diameter hole was made in media by gel cutter (Cork borer) in sterilized form. One drop of melted agar was transferred into the bottom of the hole and permitted to solidify to make a base layer. 0.100 µl of softened samples were poured into the hovels. The plates were kept at low temperature (4 °C) for 2 – 4 h. to allow maximum dissemination. The plates have been incubated at 37 °C for 24 h. for bacteria and at 30 ^oC for 48 h. in vertical position to avoid scattering of liquid samples and permit maximum growth of the organisms. The antimicrobial activity of the tested samples was inspected by measuring the diameter of the inhibition zones articulated in millimeter (mm). The testing was carried out twice and mean of reading was recorded [3].

2.7. Quantitative determination of compounds by Ultra performance liquid chromatography (UPLC-ESI-MS)

ESI-MS Negative and Positive ion achievement mode was carried out on a XEVO TQD triple quadruple instrument. Waters Corporation, Milford, MA01757 U.S.A, mass spectrometer. Column: ACQUITY UPLC - BEH C18 1.7 µm - 2.1 × 50 mm Column, Flow rate: 0.2 mL\min, Solvent system: consisted of (A) Water having 0.1 % formic acid. (B) Methanol having 0.1 % formic acid.The sample (100 µg/mL) solution was prepared using high performance liquid chromatography (HPLC) analytical grade solvent of/MeOH, filtered using a membrane discfilter (0.2 µm) then endangered to LC-ESI-MS analysis. Samples injection volumes (10 µL) were injected into the UPLC device prepared with reverse phase C-18 column (ACQUITY UPLC - BEH C18 1.7 µm particlesize - 2.1 × 50 mm Column). Sample mobile phase was equipped by filtering using 0.2 µm filter membrane disc and degassed by sonication previously injection. Mobile phase elution was completed with the flow rate of 0.2 mL/min using gradient mobile phase containing two eluents: eluent A is H_2O acidified with 0.1% formic acid and eluent B is MeOH acidified with 0.1% formic acid. Elution was accomplished using the above gradient. The restrictions for investigation were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were spotted in the ESI negative ion mode between m/z 100–1000. The peaks and spectra were handled using the Maslynx 4.1 software and tentatively recognized by comparing their retention time (Rt) and mass spectrum with informed data.

2.8. Statistical Analysis

Microsoft Excel and GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) were used for statistical analysis of *in-vitro* cell line studies. All data were done in triplicate and were expressed as the mean ± SD from three different experiments.

3. Results and Discussion

3.1. Total phenolic and Flavonoid contents

The primary components of medicinal plants are phenolic and flavonoid chemicals, which have been shown to have antimicrobial and therapeutic effects against a wide range of illnesses [2]. The hydroxyl groups present in that substance are responsible for its ability to scavenge free radicals [22]. The Folin-Ciocalteu reagent has been used in this investigation to quantify the phenolic content. The results were reported in gallic acid equivalents (GAE) per gram dry extract weight and were derived using a calibration curve of gallic acid (12.5-1000 µg/ml) (y = 0.0036x – 0.1174, R2 = 0.9917). As shown in Figure 1, the maximum phenolic content was found in the BuOH and EtOAc fractions (181.66 ± 17.96 and 127.07 ± 7.37 mg GAE/g dry extract, respectively). MeOH extract and aqueous fractions came in second and third, with 145.63 ± 12.16 and 103.28 ± 9.29 37 mg GAE/g dry extract, respectively). In parallel, a colorimetric approach utilizing aluminum chloride was used to determine the total flavonoid content of that specific plant. Rutin (6.25-1000 µg/ml) was used as the calibration reference for the results, which were reported in rutin acid equivalents (GAE) per gram dry extract weight (y = 0.0014x + 0.0660, R2 = 0.9986). The findings **(Figure 1)** showed that the flavonoid content of the PE and EtOAc fractions is the highest (48.81 ± 4.84 mg RE/g dry extract and 99.02 ± 7.62 mg RE/g, respectively), followed by BuOH part and MeOH extract (33.38 ± 2.59 and 15.50 ± 2.41 mg RE/g dry extract, respectively). Phenolics and flavonoids compounds have biological assets such as anti-allergic, antiangiogenic, anti-cancer, antimicrobial, anti-inflammatory, and antioxidant [23,24]. Our results are in full contract with earlier studies that demonstrated the high phenolic and flavonoid contents for different *Tamarix aphylla* extracts [25].

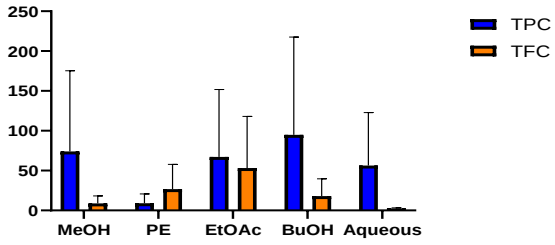


Figure 1. Total phenolic and flavonoid content of *Tamarix aphylla*

3.2. Anti-inflammatory Activity

3.2.1. Effect of Different extracts on Nitric Oxide levels in LPS-stimulated RAW 264.7 macrophages:

The outcome of different extracts on levels of Nitric Oxide **(Figure 2)** in LPS-stimulated RAW 264.7 cells was investigated. All the cells were treated with different extracts along with LPS or LPS alone for 24 h.

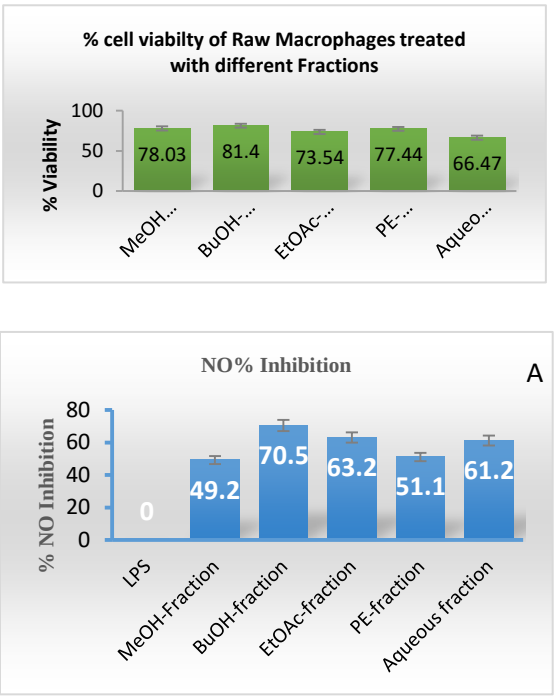


Figure 2. A: Effects of different extracts on the inhibition of nitric oxide production in LPS-stimulated RAW264.7 macrophages. Cells were treated with different compounds plus LPS (1µg/mL) or LPS alone for 24 h. NO production was estimated using the Griess reagent. Values are expressed as the means ±SD (n=3) p<0.05 versus LPS alone, B: Effect of different extracts on viability of Raw-264.7 macrophages at concentrations (100 µg/ml). Cells were exposed to compounds for 24 h.Values are expressed as the means ±SD (n=3).

Using Griess reagent, we computed the amount of nitrite released into the culture medium in order to calculate the amount of NO generation. Consequently, LPS by itself significantly increased the production of NO as divergent to the control. However, pretreatment with different extracts precious levels of NO significantly produced in LPS-stimulated RAW 264.7 cells as shown in **(Figure 2)**. Moreover, BuOH, EtOAc and Aqueous fractions encouraged marked inhibition on NO production by (60-70%) as compared to LPS. In a similar experiment, to study the cytotoxicity of different extracts on RAW 264.7 cells, the cells were

preserved with each extract for 24 hours with and without LPS, and cell viability was measured by the MTT assay. The outcomes displayed that cell viability was not affected by treatment with diverse extracts as designated by the MTT reduction assay. These results specify that the anti-inflammatory activity in LPS-stimulated RAW 264.7 macrophages was not owing to loss of cell viability.

Accumulating suggestion indicates that NO is a critical mediator of inflammation. NO plays a pivotal role in several body roles; however, its overproduction, mostly in macrophages, can lead to cytotoxicity, inflammation, and autoimmune disorders [20]. These discoveries proved the ant-inflammatory activity of Tamarix aphylla fractions (BuOH, EtOAc, and Aqueous), This perhaps ascribed to their phenolic and flavonoid content in those fractions. Our marks in consent with prior reports that proved the anti-inflammatory effect of Tamarix aphylla [26,27]. Furthermore, Karker et al [28] proved that Tamarix africana has anti-inflammatory effect against nitric oxide (NO) overproduction in LPS-stimulated RAW 264.7 macrophages.

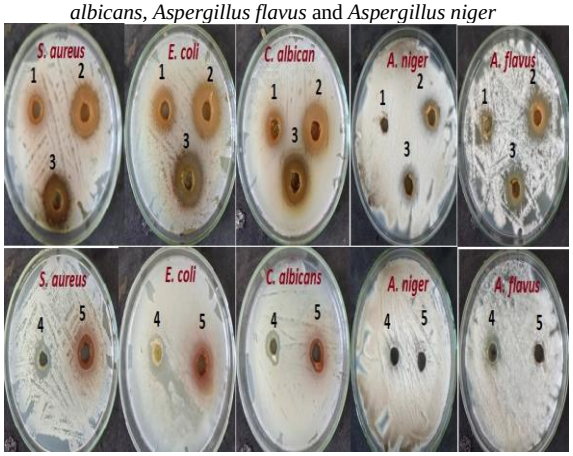
3.3. Antimicrobial activity

The results of the antimicrobial activity of Tamarix aphylla against Gram-positive, Gram-negative and fungi were confirmed in (Table 1 and Figure 3). The plant extract and different fractions verified diverse inhibition activity. The highest inhibition activity was conveyed for EtOAc fraction followed by BuOH fraction while, the moderate inhibition activity was for MeOH extract and the minimum inhibition activity was for Aqueous fraction. EtOAc antimicrobial activity ranged from 27 mm against Escherichia coli, to 19 mm against Aspergillus niger. While, BuOH antimicrobial activity ranged from 27 mm against Escherichia coli to 16 mm against Staphylococcus aureus. Also, MeOH extract showed inhibition area ranging from 22 mm against Escherichia coli to 14 mm against Aspergillus flavus Whereas, no activity was recorded against Aspergillus niger. PE fraction showed inhibition area neither high nor low against tested bacteria and fungi spp. Moreover, Aqueous fraction demonstrated antibacterial activity ranged from 17 mm against Staphylococcus aureus and Escherichia coli to 15 mm against Candida albicans but all fungi strains showed resistance against Aqueous fraction. Conferring to the resistance of several pathogens to synthetic antibiotics so, alternative natural drugs were vital especially from plant origin [29]. Our data showed that the plant had promising antimicrobial activity which decided with other preceding reports [4] However, Bakht et al., (2014) [30], Sautron and Cock (2014) [31] and Shakeri et al., (2015) [32] verified that EtOAc and BuOH fraction have effective antimicrobial activity. The higher activities by the ethyl acetate and BuOH fractions as compared to other against tested pathogens may be accredited to the high concentration of phenolic and flavonoid contents. Also, Prakash et al. [33] reported that MeOH extract was found to be more effective against pathogenic bacterial spp.

Table 1. The antimicrobial activities of *Tamarix aphylla* against *Escherichia coli, staphylococcus aureus, Candida albicans, Aspergillus flavus* and *Aspergillus niger*

No.	Sample name	Clear zone (ϕmm)				
		Staphylococcus aureus	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*	*Aspergillus flavus*
1	MeOH	15	22	16	0	14
2	Butanol	16	27	22	18	25
3	EtOAc	23	27	23	19	20
4	Pet	15	18	14	15	15
5	Ether	17	17	15	0	0
	H_2O					

Figure 3. Inhibition zone of *Tamarix aphylla* against against *Escherichia coli, staphylococcus aureus, Candida albicans, Aspergillus flavus* and *Aspergillus niger*



The size of the zone of inhibition is usually related to the level of antimicrobial activity present in the sample or product – a larger zone of inhibition usually means that the antimicrobial is more potent.

3.4. Qualitative determination of compounds in Tamarix aphylla extracts using UPLC/MS/MS.

UPLC/MS/MS-based techniques are anticipated to be particularly important in plants. Due to the very diverse biochemistry of plants, which includes many semi-polar chemicals, including important secondary metabolite groups, which can best be separated and

detected by MS/MS approaches. Analysis was carried out to assess the efficacy of various MS/MS procedures and devices. The butanol and ethyl acetate *T. aphylla* extracts underwent the first analysis utilizing which produced promising results for the detection of secondary metabolites. The outcomes demonstrate how the instrument and environment

choices affect the outcomes of the analysis. The analysis was hampered by the comparatively high energy, which caused the extract's components to be extensively fragmented. This results in the identification of only the chemicals that are present in large amounts. The 31 phytocompounds were characterized and tentatively identified in the comparison of the constituent mass spectra with the Literature for the butanol and ethyl acetate extracts, these are follows phenolic acids, flavonoids, and coumarins. (Orfali, 2005) [34] reported the compositions of various Tamarix species; *T. nilotica* from Saudi Arabia had bi cyclo octan-2-one as the main constituent (46.09%). Nonetheless, three substances were noted as important components of *T. boveana*: hexadecanoic acid (in aerial parts and stems), 2,4- nonadienal (in flowers), and germacrene D (in leaves) [35]. Nonaromatic hydrocarbons resembled the prevalent group of *T. aphylla* aerial parts in *T. chinensis*, but fatty esters and fatty acids are the majors in *T. boveana* leaves [35]. In contrast, *T. aphylla* and *T. boveana* both have high levels of hydrocarbon sesqui terpenes [35].

3.5. Analysis of the butanol extract in the Negative-ion Mode

The fragmentation patterns in negative electrospray ionization of compounds in the *T. aphylla* have been rarely reported. Since it appeared more selective and more sensitive for liquid chromatography-mass spectrometry analysis of natural compounds in -ve mode (**Table 2 and Figure 4**).

Compound (1) was Gallic acid, The deprotonated molecular ion $[M–H]^-$ of GA, *m/z* 169, and its MS/MS gives only a fragment ion at *m/z* 125 corresponding to the loss of a CO_2 group from the carboxylic acid moiety followed by the successive losses of neutral fragments at (*m/z* 97) due to CO and (*m/z* 81) for C_2H_4O moiety losses, respectively [36].

Compounds (2) were identified by the in-house database as Oxacyclododecan-2-one; with pseudomolecular ion peaks $[M-H]^-$ at m/z 183 [23].

Compounds (3) showed a pseudomolecular ion peak $[M-H]^-$ at *m/z* 205 followed by a peak at m/z 174 corresponding to $[M-H-31]^-$ due to loss of OCH_3, so it was identifed as Acetyl eugenol [37].

Compound (4) was identified as 5-(3'-Methoxy-4'-hydroxyphenyl)-gamma -valerolactone with pseudomolecular ion peaks $[M-H]^-$ at *m/z* 221 [37].

Compound (5) showed a pseudo molecular ion peak $[M-H]^-$ at *m/z* 192 and was identified as Scopoletin [37].

Compound (6) showed a pseudomolecular ion peak $[M-H]^-$ at *m/z* 211, followed by a peak at m/z 180, corresponding to $[M-H-31]^-$ due to loss of CH_3O related to 2,3-Dihydroxy-1-Guaiacylpropanone compound [37].

Compound (7) is Gallocatechin, from the spectrum it was seems that a pseudomolecular ion peak $[M-H]^-$ at *m/z* 305, followed by a peak at 271, corresponding to $[M-H-34]^-$ due to loss of two OH [38].

Compound (8) showed a pseudo molecular ion peak $[M-H]^-$ at *m/z* 492 followed by a peak at m/z 447, due to loss of COOH unit, corresponding to $[M-H-45]^-$, so the compound was isorhamnetin-3-*O*-glucuronide [39].

3.6. Analysis of the butanol extract in the Positive-ion Mode

The analysis was positively impacted by this comparatively low energy, which improved the identification of flavonoids, phenolic acids, and their glycosides. Mass spectra obtained from the analysis allowed tentative identification (**Table 2 and Figure 4**).

The mass spectra of compound (9) was typical for 5-(3',4',-dihydroxyphenyl)--valerolactone.

The pseudo molecular ion peak $[M+H]^+$ at *m/z* 209 was revealed by The mass fragmentation. The peak locations followed by a peak at m/z 192, due to loss of OH unit, corresponding to $[M+H-17]^+$ [37].

The mass spectrum showed a molecular ion peak $[M+H]^+$ at *m/z* 135 indicating that the compound (10) is malic acid [40].

The peak at *m/z* 167, $[M+H]^+$, is characteristic of 3-Hydroxyphenylpropionic Acid (11) followed by a peak at m/z 150, corresponding to $[M+H-17]^+$, due to the loss of one OH unit attached to the acid and a peak at m/z 105, corresponding to $[M+H-17-45]^+$, due to the loss of COOH unit [37].

The peak at *m/z* 147 $[M+H]^+$ is characteristic of Coumarin (12) followed by a peak at m/z 119, corresponding to $[M+H-28]^+$, due to the loss of CO unit [37].

Compound (13) represents the pseudomolecular ion peak appeared at m/z 179, corresponding to $[M+H]^+$ of Mellein and the molecular ion peak at m/z 135 due to the loss of CO_2 unit $[M+H-44]^+$ [37].

The chromatogram revealed a peak at m/z 181, $[M+H]^+$, which characteristic of caffeic acid (14) followed by a peak at m/z 136, corresponding to $[M+H-45]^+$, due to the loss of COOH unit [41].

Compound (15) was identified as syringic acid with pseudomolecular ion peaks m/z 199, $[M+H]^+$, that followed by a peak at m/z 168, corresponding to $[M+H-31]^+$, due to the loss of OCH_3 and a peak at m/z 123, corresponding to $[M+H-31-45]^+$, due to the loss of COOH unit [41]

Table 2. Identification of the major secondary metabolites in the Butanol extract of *T. aphylla* applying the UPLC/MS/MS Negative-ion and Positive-ion Mode technique

Proposed Compounds	Mode of Ionization (-ve mode /+ve mode)	MS/MS (*m/z*)	Ref.
1- Gallic acid	$[M–H]^-$ at *m/z* 169	125, 97, 81	[36]
2- Oxacyclododecan-2-one	$[M-H]^-$ at *m/z* 183		[23]
3- Acetyl eugenol	$[M-H]^-$ at m/z 205	174	[37]
4- 5-(3'-Methoxy-4' hydroxyphenyl)-gamma -valerolactone	$[M-H]^-$ at *m/z* 221		[37]
5- Scopoletin	$[M-H]^-$ at *m/z* 192		[37]
6- 2,3-Dihydroxy-1 Guaiacylpropanone	$[M-H]^-$ at *m/z* 211	180	[37]
7- Gallocatechin	$[M-H]^-$ at *m/z* 305	271	[38]
8- Isorhamnetin-3-*O*-glucuronide	$[M-H]^-$ at *m/z* 492		[39]
9- 5-(3',4',-dihydroxyphenyl)--valerolactone	$[M+H]^+$ at *m/z* 209	192	[37]
10- Malic acid	$[M+H]^+$ at *m/z* 135		[40]
11- 3-Hydroxyphenylpropionic Acid	$[M+H]^+$ at *m/z* 167	150, 105	[37]
12- Coumarin	$[M+H]^+$ at *m/z* 147	119	[37]
13- Mellein	$[M+H]^+$ at *m/z* 179	135	[37]
14- Caffeic acid	$[M+H]^+$ at *m/z* 181	136	[41]
15- Syringic acid	$[M+H]^+$ at *m/z* 199	168,123	[41]

3.7. Analysis of the Ethyl acetate extract in the Negative-ion Mode

The chemical profiling of 8 phenolic compounds from Ethyl acetate extract using the UPLC/MS/MS method were carried out and thus, the results are shown in **Table 3 and Figure 4**.

Compound (16) is 2,3-Dihydroxybenzoic acid, it showed a pseudomolecular ion peak $[M-H]^-$ at *m/z* 153, followed by a peak at m/z 136, corresponding to $[M-H-17]^-$ due to loss of OH and group and a peak at m/z 119, corresponding to $[M-H-17-17]^-$ due to loss of another OH group from the compound [37].

The peak at *m/z* 163 is characteristic of 4-hydroxycinammic acid (17), followed by a peak at m/z 146, corresponding to $[M-H-17]^-$, due to the loss of one hydroxyl unit at position 4 attached to the cinammic acid. The molecular ion peak at m/z 118, corresponding to $[M-H-17-28]^-$ due to loss of CO_2H [38].

The mass spectrum showed a molecular ion peak $[M-H]^-$ at m/z 133 indicating that the compound is malic acid (18) isomer of compound 10 [40].

The pseudomolecular ion peak appeared at m/z 149, corresponding to $[M-H]^-$ of Thymol (19), this peak followed by ion peak at m/z 134, corresponding to $[M-H-15]^-$ due to the loss of CH_3 group then a molecular ion peak at m/z 92, corresponding to $[M-H-15-43]^-$ because of $H_3C\text{-}CH\text{-}CH_3$ loss [37].

Compound (20), was 3,4-Dihydroxyphenylglycol, The deprotonated molecular ion $[M–H]^-$, *m/z* 169, and its MS/MS gives a fragment ion at *m/z* 135 corresponding to the loss of two OH groups $[M–H-17-17]^-$, followed by the successive loss of at (*m/z* 106) due to CHOH $[M–H-17-17-29]^-$ [37].

Compound (21) Methyl gallate showed a pseudo molecular ion peak $[M-H]^-$ at *m/z* 183, followed by a peak at m/z 166 responsible for the loss of OH group $[M–H-17]^-$. A base peak at m/z 135 was observed, corresponding to the loss of OCH_3 fragment $[M–H-17-31]^-$ [42].

The compound (22) was identified as Syringic acid, it was showed pseudo molecular ion peak [M-

$H]^-$ at *m/z* 197, followed by a peak at m/z 180, due to loss of 17 amu from the compound $[M-H-17]^-$ responsible for the loss of OH unit and a peak at m/z 149, $[M-H-17-31]^-$ responsible for the loss of OCH_3 group and it is an isomer of compound 15 [38].

Compound (23) showed a pseudomolecular ion peak $[M-H]^-$ at *m/z* 217, followed by a peak at m/z 173, corresponding to $[M-H-44]^-$ due to loss of CO_2 attached to Benzoic acid, cyclohexylmethyl ester [43].

3.8. Analysis of the ethyl acetate extract in the Positive-ion Mode

UPLC/MS/MS profiling of *T. aphylla* revealed 8 main peaks, which were tentatively identified (**Table 3 and Figure 4**).

Compound (24) showed a pseudo molecular ion peak $[M+H]^+$ at *m/z* 181 was observed, corresponding to caffeic acid, followed by a peak at m/z 164 due to OH loss $[M+H=17]^+$. A peak at m/z 119, corresponding to loss of COOH group and it is an isomer of compound 14 [44].

Compound (25) was identified as 4-Hydroxybenzaldehyde due to its pseudomolecular ion peak $[M+H]^+$ at m/z 123 followed by a molecular ion peak at m/z 106 corresponding to the loss of hydroxyl group $[M+H-17]^+$ and finally the loss of CHO group revealed a peak at m/z 77, $[M+H-29]^+$ [37].

The peak at m/z 147 $[M+H]^+$ is characteristic of Coumarin (26) followed by a peak at m/z 119, corresponding to $[M+H-28]^+$, due to the loss of CO unit and it is an isomer of compound 12 [37].

Compound (27) was identified as Esculetin due to its pseudo molecular ion peak $[M+H]^+$ at *m/z* 179, an additional fragment ion at m/z 162 was detected for the compound due to loss of OH group $[M+H-17]^+$ and a peak at m/z 145 due to loss of another OH group $[M+H-17-17]^+$ [37].

The Peak $[M+H]^+$ at *m/z* 199, yield fragments at m/z 182 $[M+H-17]^+$ and m/z 165 $[M+H-17-17]^+$ responsible for the loss of two hydroxyl groups and Loss of methoxy group was observed m/z 134 $[M+H-17-17-31]^+$ followed by a peak at m/z 119 indicates the loss of methyl for ester linkage of 5-methoxy gallic acid methyl ester (28) [45].

The tentative mass spectrum for Hydroxy-trimethoxy-methylchromen-4-one (29) showed the protonated molecule $[M+H]^+$ at *m/z* 267, the major fragment ions produced by the analysis were m/z 250 and 219, corresponding to loss of OH and Methoxy groups $[M+H-17]^+$ and $[M+H-17-31]^+$, respectively, from the precursor ion [46].

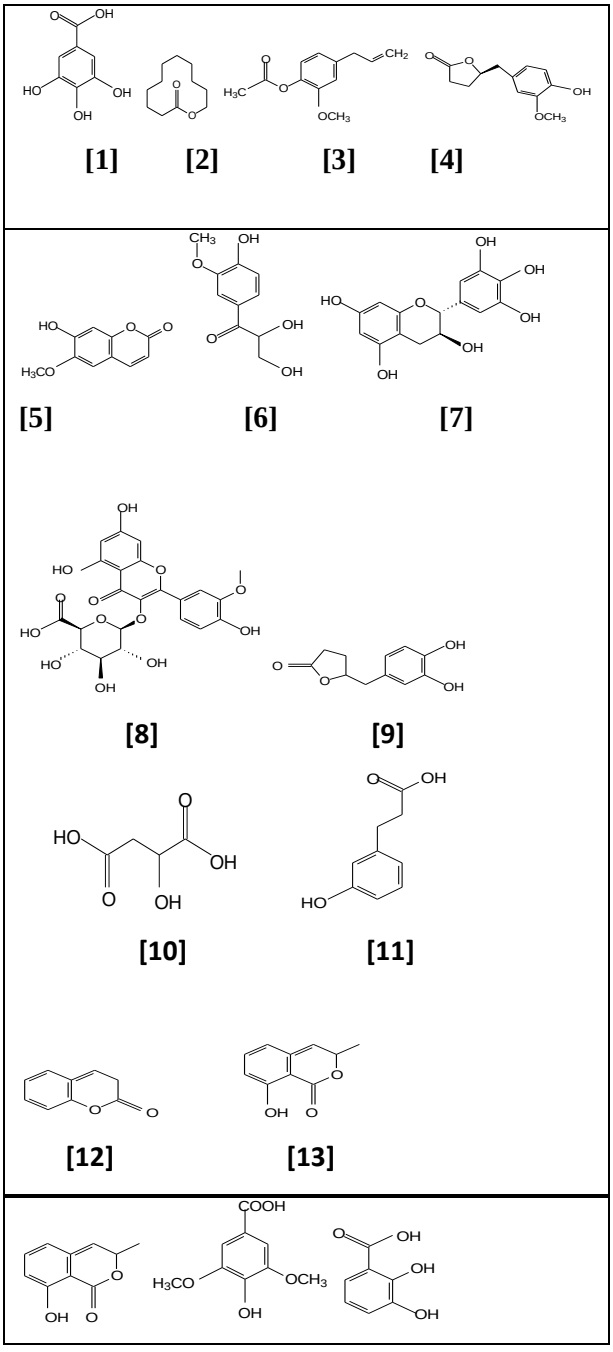
Loss of hydroxy group was observed in the molecular ion peak $[M+H-17]^+$, *m/z* 210 and $[M+H-17-45]^+$ exhibiting a fragment mass at m/z 165 and was identified as Dihydrosinapic acid (30) within a peak at $[M+H]^+$ at *m/z* 227 [37].

The peak with $[M+H]^+$, at *m/z* 315 yielded fragment at m/z 298 $[M+H-17]^+$, assigned that the compound (31) is 2',7-Dihydroxy-4',5'-Dimethoxyisoflavone [37].

Table 3: Identification of the major secondary metabolites in the Ethyl acetate extract of *T. aphylla* applying the UPLC/MS/MS Negative-ion and Positive-ion Mode technique

Proposed Compounds	Mode of Ionization (-ve mode /+ve mode)	MS/MS (*m/z*)	Ref.
16- 2,3-Dihydroxybenzoic acid	$[M-H]^-$ at *m/z* 153	136, 119	[37]
17- 4-hydroxycinammic acid	$[M-H]^-$ at *m/z* 163	146, 118	[38]
18- Isomer of compound 10 (malic acid)	$[M-H]^-$ at m/z 133		[40]
19- Thymol	$[M-H]^-$ at *m/z* 149	134, 92	[37]
20- 3,4-Dihydroxyphenyl glycol	$[M–H]^-$ at *m/z* 169	135, 106	[37]
21- Methyl allate	$[M-H]^-$ at *m/z* 183	166, 135	[42]
22- Syringic acid	$[M-H]^-$ at *m/z* 197	180, 149	[38]
23- Benzoic acid, cyclohexylmethyl ester	$[M-H]^-$ at *m/z* 217	173	[43]
24- Isomer of compound 14 (caffeic acid)	$[M+H]^+$ at *m/z* 181	164, 119	[44]
25- 4-Hydroxybenzaldehyde	$[M+H]^+$ at *m/z* 123	106, 77	[37]
26- Isomer of compound 12 (Coumarin)	$[M+H]^+$ at *m/z* 147	119	[37]
27- Esculetin	$[M+H]^+$ at *m/z* 179	162, 145	[37]
28- 5-methoxy gallic acid methyl ester	$[M+H]^+$ at *m/z* 199	182, 165, 134, 119	[45]

29- Hydroxy-trimethoxy-methylchromen-4-one	$[M+H]^+$ at *m/z* 267	250, 219	[46]
30- Dihydrosinapic acid	$[M+H]^+$ at *m/z* 227	210, 165	[37]
31- 2',7-Dihydroxy-4',5'-Dimethoxyisoflavone	$[M+H]^+$, at *m/z* 315	298	[37]



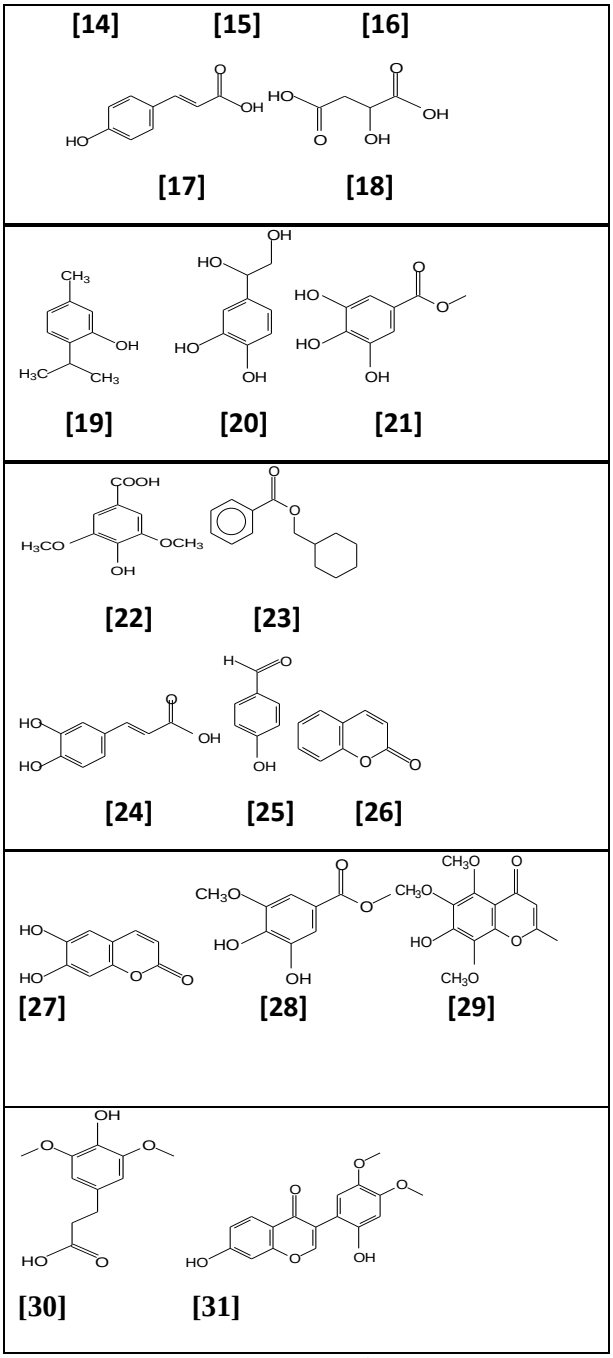


Figure 4. Phenolic compounds identified in *Tamarix aphylla*.

4. Conclusion

Our findings demonstrated that the various fractions of Tamarix aphlla exhibited antibacterial and anti-inflammatory properties commensurate with their overall phenolic and flavonoid concentrations. The presence of bioactive chemicals identified by UPLC-ESI-MS analysis may be the cause of these results. As a result, this plant has therapeutic uses for infectious disorders. Furthermore, the findings showed that the plant has medicinal value, indicating

the necessity for additional research to create pharmaceutical products.

5. Financial Support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

6. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

7. Acknowledgement

We acknowledge the team of research for help and supporting the work to be achieved as best as we can.

8. References

[1] Schippmann, U., Leaman, D.J., Cunningham, A.B. (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. Biodivers ecosyst. *App Agric, For Fish*. 2002.

[2] Mohamed, M.A., Hamed, M.M., Ahmed, W.S., Abdou, A.M. (2011). Antioxidant and cytotoxic flavonols from *Calotropis procera*. *Z. Naturforsch*. 66C: 547-554.

[3] Hamed, M.M., Refahy, L.A., Abdel-Aziz, M.S. (2017a). Assessing the bioactivity and antioxidative properties of some compounds isolated from *Abutilon hirtum* lam.. *Asian J Pharmaceut Clin* Res. 10(3): 333-340.

[4] Alrumman, S.A. (2018). Phytochemical and antimicrobial properties of *Tamarix aphylla* L. leaves growing naturally in the Abha Region, Saudi Arabia. *Arab J Sci Engi*. 2016; 41: 2123-2129.

[5] Mohamed, G.S., Hamed, M.M., Moatamed, S.A. (2018). Studies of the biological activity of *Cassia fistula*. *Pharmacology online*. 1: 75-85.

[6] Abdel-Hady, H., Morsi, E.A., El-Wakil, E.A. (2022). *In-Vitro* antimicrobial potentialities of *Phylanthus emblica* leaf extract against some human pathogens. *Egyptian J Chem*. 65(7): 401-707.

[7] Qadir, M. I., Abbas, K., Hamayun, R., Ali, M. (2014). Analgesic, anti-inflammatory and anti-pyretic activities of aqueous ethanolic extract of *Tamarix aphylla* L.(Saltcedar) in mice. *Pakistan J Pharm Sci.* 27(6): 1985-8.

[8] Adnan, M., Tariq, A., Bibi, R., Abd Elsalam, N. M., Rehman, H., Murad, W., Aziz, M.A. (2015). Antimicrobial potential of alkaloids and flavonoids extracted from *Tamarix aphylla* leaves against common human pathogenic bacteria. *African J Trad, Compl and Alter Med*. 12(2): 27-31.

[9] Marwat, S.K., Khan, M.A., Ur-Rehman, F., Bhatti, I.U. (2009). Aromatic plant species mentioned in the holy Qura'n and Ahadith and their ethnomedicinal importance. *Pak Nutr.* 8(9): 1472-1479.

[10] Wiart, C. (2007). Anti-inflammatory plants. Ethnopharmacology of medicinal plants. *Asia Pac*. 1-55.

[11] Abo-Dola, M.A, Lutfi, M.F., Bakhiet, A.O., Mohamed, A.H. (2015). Anti-inflammatory, analgesic, antipyretic and the membrane-stabilizing effects of *Tamarix aphylla* ethanolic extract. *European J Med Plants*. 5(4): 341-348.

[12] Rogers, S.O., Starmer, W.T., Castello, J.D. (2004). Recycling of pathogenic microbes through survival in ice). *Med hypoth*. 63(5): 773-777.

[13] Zampini, I.C., Cuello, S., Alberto, M.R., Ordóñez, R.M., D'almeida, R., Solorzano, E., Isla, M.I. (2009). Antimicrobial activity of selected plant species from "The Argentine Puna" against sensitive and multi-resistant bacteria. *J Ethnopharmacol.* 124(3): 499-505.

[14] Arya, V., Yadav, S., Kumar, S., Yadav, J.P. (2010). Antimicrobial activity of *Cassia occidentalis* L (leaf) against various human pathogenic microbes. *Life Sci Med Res*. 9(1): e12.

[15] Attard, E. (2013). A rapid microtitre plate Folin-Ciocalteu method for the assessment of polyphenols. *Open Life Sci*. 8(1): 48-53.

[16] Hamed, M.M., Abdalla, A.M., Ghareeb, M.A., Saleh, S.A. (2017c). Chemical constituents, *in vitro* antioxidant activity, oral acute toxicity and LD_{50} determination of *Moringa oleifera* leaves). *Int J Pharm Pharmaceut Sci*. 9 (5): 240-247.

[17] Kiranmai, M., Kumar, C.M., Mohammed, I. (2011). Comparison of total flavanoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. *Res J Pharmaceut Biol Chem Sci*. 2(3): 254-261.

[18] EL-Shahid, Z.A., Abd EL-Hady, F.K., Fayad, W., Abdel-Aziz, M.S., Abd EL-Azeem, E.M., Ahmed, E.K. (2021). Antimicrobial, Cytotoxic, and α-Glucosidase Inhibitory Potentials Using the One Strain Many Compounds Technique for Red Sea Soft Corals Associated Fungi' Secondary Metabolites and Chemical Composition Correlations). *J Biol Act Prod Nat*. 11,5-6: 467-489.

[19] Eid, M.M., Ismail, A.M., Elshahid, Z.A., Abd Elzaher, FH, Mahmoud, K. El-Manawat, M. (2022). Plasmonic Superparamagnetic SPION@Ag@chitosan Core-shell: Uptake and

Nitric Oxide Inhibition by Colorectal Cell Lines. *J Inorg Organomet Polym*. 23(3): 931-940.
[20] Barry, A.L. (1976). The antimicrobial susceptibility test. *Prin Prac, Lea Feb*.
[21] Abdel-Hady, H., El-Sayed, M.M., Abdel-Gawad, M.M., El-Wakil, E.A., Abdel-Hameed, E.S.S., Abdel-Lateef, E.E.S. (2018). LC-ESI-MS analysis, antitumor and antioxidant activities of methanolic extract of Egyptian). *J Appl Pharmaceut Sci*. 8(7): 085-092.
[22] Hamed, M.M., Mohamed, M.A., Ahmed, W.S. (2017b). Antioxidant potential, antitumor activity and phenolic profile of *Phoenix dactylifera* linn. *Int J Pharm Pharmaceut Sci*. 9 (5): 130-136.
[23] Al-Othman, M.R., Alkhattaf, F.S., Abd El-Aziz, A.R. (2020). Antioxidant and chemical constituents of ethyl acetate extract of *Tamarix aphylla* leaves in Saudi Arabia. *Pak J Bot*. 52(6): 2257-2261.
[24] Mohammedi, Z., Atik, F. (2011). Impact of solvent extraction type on total polyphenols content and biological activity from *Tamarix aphylla* (L.) Karst. *Int J Pharm Bio Sci*. 2(1): 609-615.
[25] Mahfoudhi, A., Prencipe, F.P., Mighri, Z., Pellati, F. (2014). Metabolite profiling of polyphenols in the Tunisian plant *Tamarix aphylla* (L.) Karst. *J pharmaceut Biomed Anal*. 99: 97-105.
[26] Gadallah, A.S., Yousuf, S., Jabeen, A., Swilam, M.M., Khalifa, S.A., El-Seedi, H.R., Choudhary, M.I. (2020). Anti-inflammatory principles from *Tamarix aphylla* L.: A bioassay-guided fractionation study. *Molecules*. 25(13): 2994.
[27] Almatroodi, S.A., Khan, A.A., Aloliqi, A.A., Syed, M.A., Rahmani, A.H. (2022). Therapeutic Potential of *Tamarix aphylla* in the Prevention of Lung Injury through the Regulation of Inflammation, Oxidative Stress and Cell-Signaling Molecules. *Appl Sci.* 12(19): 9925.
[28] Karker, M., De Tommasi, N., Smaoui, A., Abdelly, C., Ksouri, R., Braca, A. (2016). New sulphated flavonoids from *Tamarix africana* and biological activities of its polar extract. *Plant Med*. 82(15): 1374-1380.
[29] Khulbe, K., Sati, S.C. (2009). Antibacterial activity of *Boenninghausenia albiflora reichb*. (Rutaceae). *African J Biotech*. 8(22): 6346-6348.
[30] Bakht, J., Khan, S., Shafi, M. (2014). *In vitro* antimicrobial activity of *Allium cepa* (dry bulbs) against Gram positive and Gram-negative bacteria and fungi. *Pak J Pharm Sci*. 27(1): 139-145.
[31] Sautron, C., Cock, I.E. (2014). Antimicrobial activity and toxicity of *Syzygium australe* and *Syzygium leuhmannii* fruit extracts. *Pharmacogn Commu*. 4(1): 53-60.
[32] Shakeri, A.R., Farokh, A. (2015). Phytochemical evaluation and antioxidant activity of *Verbascum sublobatum Murb*. Leaves. *Res J Pharmacogn*. 2(3): 43-47.
[33] Prakash, V., Rana, S., Sagar, A. (2016). Studies on antibacterial activity of *Verbascum Thapsus*. *J Med Plants Stud*. 4(3): 101-113.
[34] Orfali, R.S. (2005). Phytochemical and biological study of *Tamarix nilotica* growing in Saudi Arabia. *MSc. thesis, King Saud Univ, Riyadh*.
[35] Saidana, D., Mahjoub, M.A., Boussaada, O., Chriaa, J., Chéraif, I., Daami, M., Helal, A.N. (2008). Chemical composition and antimicrobial activity of volatile compounds of *Tamarix boveana* (Tamaricaceae). *Microbiol Res*. 163(4): 445-455.
[36] Lee, R.J., Lee, V.S., Tzen, J.T., Lee, M.R. (2010). Study of the release of gallic acid from (–)-epigallocatechin gallate in old oolong tea by mass spectrometry. *Rapid Commun Mass Spectrom*. 24(7): 851-858.
[37] Tang, J., Dunshea, F.R., Suleria, H..A. (2019). Lc-esi-qtof/ms characterization of phenolic compounds from medicinal plants hops and juniper berries and their antioxidant activity. *Foods*. 9(1): 7.
[38] Tenfen, A., Siebert, D.A., Spudeit, D., De Cordova, C.M.M., Micke, G.A., Alberton, M.D. (2017). Determination of phenolic profile by HPLC-ESI-MS/MS and antibacterial activity of *Eugenia platysema* against mollicutes strains. *J Appl Pharmaceut Sci*. 7(5): 007-011.
[39] Parejo, I., Jauregui, O., Sánchez-Rabaneda, F., Viladomat, F., Bastida, J., Codina, C. (2004). Separation and characterization of phenolic compounds in fennel (*Foeniculum vulgare*) using liquid chromatography−negative electrospray ionization tandem mass spectrometry. *J Agric Food Chem*. 52(12): 3679-3687.
[40] Bingol, M.N., Bursal, E. (2018). LC-MS/MS analysis of phenolic compounds and *in vitro* antioxidant potential of *Stachys lavandulifolia vahl*. var. brachydon boiss. *Int Lett Nat Sci*. (72): 28-36.
[41] Hossain, M.B., Rai, D.K., Brunton, N.P., Martin-Diana, A.B., Barry-Ryan, C. (2010). Characterization of phenolic composition in Lamiaceae spices by LC-ESI-MS/MS). *J Agric Food Chem*. 58(19): 10576-10581.
[42] Chernonosov, A.A., Karpova, E.A., Lyakh, E.M. (2017). Identification of phenolic compounds in *Myricaria bracteata* leaves by high-performance liquid chromatography with a diode array detector and liquid chromatography with tandem mass spectrometry. *Rev Brasileira Farmacogn*. 27: 576-579.
[43] Sen, I., Shandil, A.., Shrivastava, V.S. (2011). Determination of benzoic acid residue from fruit juice by gas chromatography with mass

spectrometry detection technique. *Arch Appl Sci Res*. 3: 245-252.
[44] Sajjadi, S.E., Ghanadian, M., Haghighi, M. (2017). Isolation and identification of two phenolic compounds from a moderately cytotoxic fraction of *Cousinia verbascifolia* Bunge. *Adv Biomed Res*. 6: 66.
[45] Li, C., Seeram, N.P. (2018). Ultra-fast liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry for the rapid phenolic profiling of red maple (*Acer rubrum*) leaves. *J Sep Sci*. 41(11): 2331-2346.
[46] Fayek, N.M., Farag, M.A., Abdel Monem, A.R., Moussa, M.Y., Abd-Elwahab, S.M., El-Tanbouly, N.D. (2019). Comparative metabolite profiling of four citrus peel cultivars via ultra-performance liquid chromatography coupled with quadrupole-time-of-flight-