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ANTIOXIDANT AND CHEMICAL CONSTITUENTS OF ETHYL ACETATE EXTRACT OF *TAMARIX APHYLLA* LEAVEAS IN SAUDI ARABIA

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Abstract

The purpose of this study was to determine the phytochemical analysis, antioxidant activity and chemical components of ethayl acetate extract of *T. aphylla* from Saudi cultivar. The objective of this study was to characterize the extract for its phytochemical composition and antioxidant activity. Phytochemical analysis of crude extracts for *T. aphylla* detected the presence of alkaloids, flavonoids, tannins, and saponins, whereas, both steroids and triterpenoids were absent. The maximum total phenol content was 39.3 mg gallic acid / g dry extract. The antioxidant activity was evaluated by DPPH assay; concentration of *T. aphylla* 1, 5, 10, 15, 20 and 25 mg/ml corresponed to scavenging activities of ~22.1%, 31.4%, 39.2%, 53.5, 60.0 and 66.3%, respectively. While for ascorbic acid (Standard), the scavenging activities were 33.1, 46.2, 51.6, 58.2, 67.3 and 72.2% at the same concentrations. IC50 value of *T. aphylla* was 14.0 mg/ml whereas it was 9.0 mg/ml for ascorbic acid. Addition GC-MS chromatogram of T. *aphylla* ethyl acetate extract were presented twenty-three constituents.

Key wards: *Tamarix aphylla*, Ethyl acetate, Antioxidant, Chemical constituents, GC-MS.

Introduction

The genus *Tamarix* composed of about 60 species of flowering plants in the family Tamaricaceae (Bughioa, *et al*., 2017). In Europe, 14 species of this plant, including African tamarisk, were identified as (*Tamarix africana*), Bove's tamarisk (*T. boveana*), French tamarisk (*T. gallica*), Canary Islands tamarisk (*T. canariensis*), Dalmatian tamarisk (*T. dalmatica*), (Brotherson *et al*, 1986; Allred 2002; González 2004; Heywood *et al.*, 2007) and have been used in folk traditional medicine to cure various ailments including hepatitis, eczema and skin diseases such as tinea capititis (Panhwar & Abro, 2007; Yusufoglu & Alqasoumi, 2011) and syphilis. Some species of Tamarix are cultivated as ornamental plants in gardens or shade trees or as windbreaks (Orabi *et al.*, 2011, Sharma & Parmar, 1998). Several studies have identified the different types of secondary metabolites found in *T. aphylla* like flavonoids, phenolics (Souliman *et al.*, 1991; Ksouri *et al*., 2009; Meot-Duros *et al.*, 2008), hydrolysable tannins (Orabi *et al.*, 2015), and alkaloids (Souliman *et al*., 1991; Yusufoglu e*t al.*, 2015). Alcohol extract from *T. aphylla* leaves has been shown to have anti-inflammatory, antioxidant and wound healing effects (Shafaghat, 2010). The researchers proposed that the existence of known active phytochemicals such as flavonoids and polyphenols would explain the effects reported (Yusufoglu & Alqasoumi, 2011; Abdullah, 2016).

Materials and Methods

Plant materials: *T. aphylla* leaves were obtained from the district of Riyadh, Saudi Arabia. Samples were washed thoroughly with distilled water and dried at room temperature then ground to fine powder, and kept for analysis**.** Extraction was performed on a weighed amount of *T. aphylla* leaves (10 g) with 200 ml of ethyl acetate at room temperature for 2 h under magnetic stirring (Mahfoudhia *et al.*, 2014).

Phytochemical screening: Phytochemical screening was performed using the methods described by (Goveas & Abraham, 2014; Joanne *et al*., 2016). Tannins: by added five drops of iron (III) chloride and blended well. A black precipitate formation indicates the presence of tannins.

Alkaloids: Two drops of Wagner reagent were applied to 2 ml of extract and blended well by the Wagner reagent sample. A reddish color indicated the presence of alkaloids.

Saponins: 5 ml of extract and 2.5 ml of water are applied to a test tube, shaken vigorously and held for 10 minutes, suggesting the presence of Saponins when we experience frowning persistence.

Terpenoids: 1 ml of conc. H_2SO_4 has been added to 2 ml of extract and well mixed. A reddish brown color suggests terpenoid presence.

Steroids: (Leibermann Burchard reaction): two milliliters of acetic anhydride and 2 ml of conc. H_2SO_4 has been added and blended well to 2 ml of extract. A dark bluish green color formation indicated the presence of steroids.

Flavonoids: Added five milliliters of dilute ammonia solution to 5 ml of extract, followed by added conc. H_2SO_4. That's it. Yellow color appearance indicated the presence of flavonoids.

Determination of total phenolic content: Total phenolic content was measured using a spectrophotometric method in the plant ethyl acetate extract (Liu *et al*., 2002). The method started with, 0.5 mL of Folin-Ciocalteu's reagent and 1 mg of extract were separately combined with 10 mL of distilled water for 3 minutes, followed by the addition of 1 mL of sodium carbonate solution for up to 25 mL. The samples put in the dark place were held for 1 h and the absorbance was measured at 750 nm wavelength. For each analysis, the samples were prepared in triplicate and the mean of value was obtained.

A calibration curve was prepared for different concentrations of gallic acid (5, 10, 20, 40, 60, 80, 100 and 150 mg mL−1). Mg gallic acid / gram extract was expressed in the total phenolic content (Ayoola *et al*., 2006).

Evaluation of antioxidant activity: UV spectrophotometry determined the antioxidant activity of the ethyl acetate plant extract against radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (model: biochrom libra 22) at 518 nm (Testing, 2006; Juan & Chou, 2010). Different plant extract concentrations (1, 5, 10, 15, 20 and 25 µg mL−1) are prepared. 1 mL of each extract and 3 mL of methanol were combined in methanol with 0.5 mL of 1.0 mM DPPH and allowed for 30 minutes of reaction at room temperature. In order to prepare the blank solution, the same amount of methanol and DPPH were mixed. Vitamin C has been used as the standard for antioxidants. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The following equation was used to quantify the radical scavenging activity:

$$\% \text{ Inhibition} = Ab/Aa\text{-}Ab \times 100$$

In which Ab is absorption of the blank sample and Aa is absorption of the extract.

Extract concentration providing 50% inhibition (IC_{50}) was calculated as IC_{50}; mg/ml.

GC-MS analysis: GC-MS analysis of the ethyl acetate extract of tamarisks was performed using a Perkin–Elmer GC Clarus 500 system (Gas Chromatograph Mass Spectrometer) (GC-MS). For detection of GC-MS, an electron ionization system with an ionization energy of 70 eV was operated in electron impact mode. Helium gas (99.999%) was used as a carrier gas with a constant flow rate of 1 ml / min and an injection volume of 2 µl (10:1 split ratio) was used. The temperature of the injector was maintained at 250°C, the temperature of the ion source was 200°C, the temperature of the oven was programmed at 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, finishing with an isothermal temperature of 9 minutes at 280°C. Mass spectra were taken at 70 eV; 0.5 s scanning intervals and 45 to 450 Da fragments. The solvent delay was 0 to 2 min, with a total run time of 30 minutes for GC / MS. (Ezhilan & Neelamegam, 2012).

Identification of components: The unknown component spectrum was compared to the spectrum of known components contained in National Institute Standard and Technology (NIST) library database with more than 62,000 patterns.

Results and Discuusion

The chemical content of the various fractions is considered to be secondary metabolite components known to be biologically active ingredients, and these secondary metabolite components have been isolated from the polar extract (Hatipoglu *et al*., 2013).

Table 1. Phytochemical screening of ethyl acetate *T. aphylla* extract.

Group	Presence
Tannins	+
Alkaloids	+
Flavonoids	+
Saponins	+
Terpenoids	-
Steroids	-
Total phenol contents	39.3 mg gallic acid/ g extract

Table 2. Percentage of inhibition of DPPH and IC50 for ethyl acetate extract of *T. aphylla* at different concentrations (mg/mL) compared with vitamin C.

Concentration (mg/mL)	% Inhibition by *T. aphylla*	% Inhibition by vitamin C
1	22.1	33.1
5	31.4	46.2
10	39.2	51.6
15	53.5	58.2
20	60.0	67.3
25	66.3	72.2
IC_{50} (mg/ml)	14.0	9.0

They are directly responsible for various activities such as antimicrobial, antioxidant, and anticancer (Anyasor *et al*., 2010). Data in Table (1) showed that phytochemical screening revealed the presence of tannins, Alkaloids, flavonoids and saponins, while both triterpenoids and steroids were absent in the crude extracts for *T. aphylla*. In this work, the total phenolic content of the crude extract and its derived fractions from *T. aphylla* leaves was determined, and the total phenolic values were recorded at 39.3 and expressed in terms of mg gallic acid / g extract. Many researchers have reported in plants that phenolic compounds and flavonoids have biological effects such as antiangiogenic, anti-allergic, anti-cancer, anti-inflammatory, antimicrobial and antioxidant. Phenolic compounds and their derivatives are also generally considered to be primary antioxidants or free radical scavengers (Liu *et al*., 2011).

Previous studies have shown that phenolic compounds including flavonoids are associated with strong antioxidant activity and have advantages (Sakanaka *et al*., 2005).

Evaluation of antioxidant activity: Determination of plant extract antioxidant activity was used by the effect of phenolic compounds on radical DPPH. DPPH can donate hydrogen when it reacts with an antioxidant compound and the color changes from deep violet to light yellow. DPPH radical is very sensitive in very short time to active ingredients, this technique is often used to measure radical scavenging activity of various plant extracts (Sultana *et al*., 2007). Concentration of *T. aphylla* 1, 5, 10, 15, 20 and 25 mg/ml corresponds approximately to scavenging activities of 22.1%, 31.4%, 39.2%, 53.5, 60.0 and 66.3%, respectively, while for ascorbic acid (Standard), the scavenging activities were 33.1, 46.2, 51.6, 58.2, 67.3 and 72.2% at the same previous concentrations (Table 2 and Fig. 1). DPPH residual scavenging activity (%) was applied to various sample extract concentrations and a linear regression curve was developed to measure IC 50, which is the sample quantity

required to reduce DPPH free radical absorption by 50%. IC50 values of *T. aphylla* was 14.0 mg/ml whereas it was 9.0 mg/ml for ascorbic acid. Relative to plant extract, ascorbic acid has the lowest IC50 quality. Vitamin C is commonly used as an antioxidant and has a powerful DPPH scavenging ability (Tona *et al*., 1998). The lower the IC50 in the DPPH assay, the better the ability to scavenge the radicals (Lim *et al*., 2007).

The IC50 value of Nigella sativa seed methanol extract was found to be 168.8 compared to the standard ascorbic acid value of 8.6 µg/ml. Various experiments were carried out to recognize the property of plant extracts in order to scavenge free radicals (Pavithra & Vadivukkarasi, 2015).

A linear relation between antioxidant activity and phenolic content of plant extracts has been demonstrated in several studies (Pensec *et al.,* 2016).

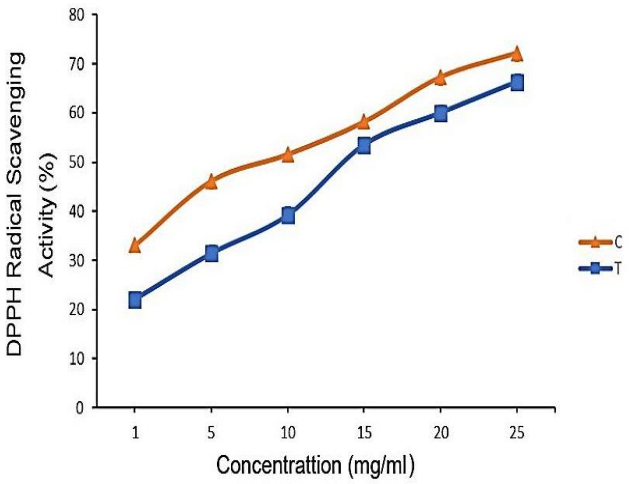

Fig. 1. DPPH free radical scavenging activity of ethyl acetate *T. aphylla* extract (standard ascorbic acid ◄, and *T. aphylla* extract □).

Chemical constituents of ethayl acetate *T. aphylla* extract by GC-MS: The ethayl acetate extract of *T. aphylla* GC-MS chromatogram analysis showed 23 peaks indicating the presence of 23 phytochemical components (Fig. 2). The 23 phytocompounds were characterized and identified in comparison of the constituent mass spectra with the NIST library. The *T. aphylla* ethayl acetate GC-MS analysis resulted in 23 components being identified predominantly by Acetophenone, Tetrazole, Pyridine, 2,4,6-trimethyl, 1-undecanol, Oxacyclododecan 2- one, Digitoxin, Ricinoleic acid, Gibberellic acid, Oxacyclododecan 2- one, Octadecanol, Colchicine, Trifluoromethyl, Vobassan1-17-oic acid, 4 dimethyl-3-oxo-methyl ester, Phenylmercuric salicylate, pentadecanol, Cholesteryl benzoate, Gamabufotalin, Beclomethasone, Gamabufotalin and Prednisolone acetate (Table 3 and Fig. 2). *Tamarix* species have been reported different compositions by (Orfali, 2005), *T. nilotica* of Saudi Arabia have bi cyclo octan-2-one as the major compound (46.09%). In *T. chinensis* fruit, Hexadecanoic acid methyl ester was reported as the major principle (Mahemuti *et al*., 2015). Hexadecanoic acid (in aerial parts and stems), 2,4-nonadienal (in flowers), and germacrene D (in leaves) were, however, reported as majors of *T. boveana* (Saidana *et al*., 2008). Like in *T. chinensis*, nonaromatic hydrocarbons resembled the abundant group of *T. aphylla* aerial parts, while in *T. boveana* leaves, fatty esters and fatty acids are the majors (Saidana *et al.*, 2008). On the other hand, hydrocarbon sesqui terpenes are prevalent in *T. aphylla* and *T. boveana* (Saidana *et al.,* 2008), respectively. The GC–MS analysis revealed that the leaves contain ten different chemical compounds; beta-d-mannofuranose (23.04 %) and propenoic acid (28.99 %) are found to be dominant in *Tamarix aphylla* L. leaves Growing in, Saudi Arabia (Abdullah, 2016).

Table 3. Chemical constituents of ethayl acetate *T. aphylla* extract by GC-MS.

No.	Rt.	Compound	Molecular formula	Molecular weight
1.	3.27	Acetophenone	C_8H_7CLO	154
2.	3.61	Tetrazole	CH_2N_4	70
3.	5.01	Pyridine,2,4,6-trimethyl	$C_8H_{11}N$	121
4.	5.22	Tetrazole	CH_2N_4	70
5.	7.22	1-undecanol	$C_{11}H_{24}O$	172
6.	9.13	Oxacyclododecan 2- one	$C_{11}H_{20}O_2$	184
7.	9.73	Digitoxin	$C_{41}H_{64}O_{16}$	764
8.	10.86	Ricinoleic acid	$C_{18}H3_4O_3$	298
9.	11.85	Gibberellic acid	$C_{19}H_{22}O_6$	346
10.	12.04	Oxacyclododecan 2- one	$C_{11}H_{20}O_2$	184
11.	12.62	Octadecanol	$C_{18}H_{38}O$	270
12.	12.76	Colchicine	$C_{22}H_{25}NO_6$	399
13.	13.45	Trifluoromethyl	$C_{13}H_{17}F_3N_4O_4$	350
14.	14.12	Vobassan1 -17-oic acid,4 dimethyl-3-oxo-,methyl ester	$C_{20}H_{22}N_2O_3$	338
16.	15.30	Phenylmercuric salicylate	$C_{13}H_{10}HGO_3$	416
17.	16.19	pentadecanol	$C_{15}H_{32}O$	228
19.	17.45	Cholesteryl benzoate	$C_{34}H_{50}O_2$	490
20.	19.50	Gamabufotalin	$C_{24}H_{34}O_5$	402
21.	21.51	Beclomethasone	$C_{22}H_{29}CLO_5$	408
22.	23.44	Gamabufotalin	$C_{24}H_{34}O_5$	402
23.	25.29	Prednisolone acetate	$C_{23}H_{30}O_6$	402

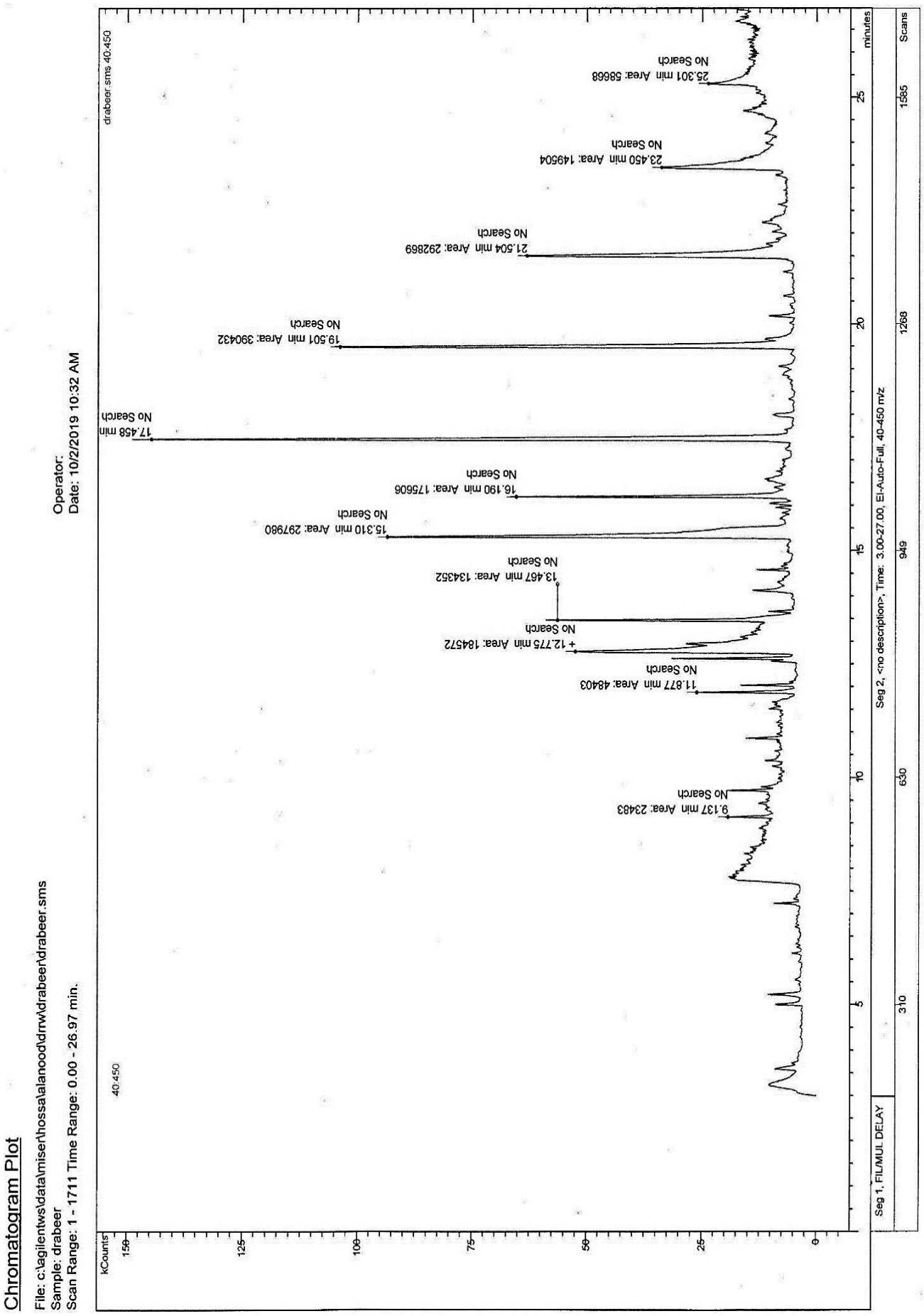

Fig. 2. GC-MS chromatogram of *T. aphylla* ethayl acetate extract.

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