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RESEARCH ARTICLE

PHARMACOGNOSY

IMPACT OF SOLVENT EXTRACTION TYPE ON TOTAL POLYPHENOLS CONTENT AND BIOLOGICAL ACTIVITY FROM *TAMARIX APHYLLA* (L.) KARST.

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ABSTRACT

Secondary metabolites of an arabo-saharan tree *Tamarix aphylla* (L.) Karst. Were extracted from dry powdered leaves with different solvents: aqueous methanol; aqueous ethanol, aqueous acetone and distilled water. Total phenolic content of the four extracts were determined by Folin Ciocalteu method and their antioxidant activity was assayed through in vitro radical scavenging activity using $DPPH^{\cdot}$ assay. The results showed that mixture water-methanol was better than water-ethanol, water-acetone and water for extraction bioactive compounds in particularly total polyphenols from *Tamarix aphylla* leaves. The average total phenol content of aqueous methanol extract was 262.26mg GAE/100g dry weight lyophilized extract. However, all extracts were free-radical inhibitors but aqueous acetone extract was more potent than aqueous ethanol extract and two others extracts. In order of effectiveness (EC50), the potent inhibitors were from water-acetone extract (0.080mg/ml) > water-ethanol extract (0.140mg/ml)>water extract (0.173mg/ml)> Water-methanol (0.911mg/ml).

KEYWORDS

Tamarix aphylla, polyphenols, free radicals, DPPH, antioxidant activity

INTRODUCTION

Research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems[1]. The medicinal value of these plants is related in their phytochemical components which produce definite physiological actions on human body. The most important of these components are alkaloids, tannins, flavonoids and phenolic compounds[2]. Several herbs have been reported to exhibit antioxidant activity[3-6] and a great potential source of antioxidant are polyphenols[7]. Solvent and process variables must be carefully chosen to optimize their extraction. The extraction yield and antioxidant activity of the extracts highly depend on the solvent polarity, which determines both quantitatively and qualitatively the extracted antioxidant compounds[7].

Polyphenols content and phytochemical compounds in Algerian *Tamarix aplylla* are not investigated. This is the first report on the polyphenols content and biological activity from an Algerian Saharan tree; *Tamarix aphylla* (L.) Karst. This species is called by the local population “Tarfa” or “Athl”; it is a fast growing evergreen tree, popular salty deserts and it is widely distributed in Algerian sahara. *T. aphylla* is used as herbal medicines such as diuretic, carminative, anti-inflammatory and for treatment internal hematomas. The seed named “Takormest” which is the Galle of a small butterfly was used to tan skins. In this paper, we studied the extraction of polyphenols and the antioxidant activity of ethanolic, methanolic, acetonic and water extracts from *Tamarix aphylla* leaves and discussing some results of polyphenols extraction yields and DPPH$^{\cdot}$ scavenging activity, as affected by several solvents type. Our study was conducted to optimize the extraction conditions for total phenolic contents using Folin Ciocalteu method.

MATERIAL AND METHODS

Plant material

The leaves of *Tamarix aphylla* (L.) Karst. (Family: Tamaricaceae) were collected from *Adrar*; a Saharan area in South Algeria.

Extraction

The leaves were oven dried and milled into uniform dry powder. Extracts were prepared using four solvents: ethanol (70%), methanol (70%), acetone (70%) and distilled water. Briefly for each solvent, 100g dry powder was extracted with 1000ml solvent by maceration at room temperature for 48 hours. Then, two filtration of each mixture through N°1 whatman paper and filter paper (0.45µm porosity). The collected filtrates were dried separately at 50°C using a Laborota 4000 rotary evaporator. The residue of each solvent extract was dissolved in water, frozen and lyophilized (CHRIST-ALPHA 1-4 Lyophilizator). Aqueous, aqueous acetonic, aqueous methanol and aqueous ethanol crude extract powder were used for investigate phytochemical compounds, determination of total phenol content and for antioxidant screening.

Phytochemical screening

Phytochemical components of different extracts from *T. aphylla* were screened using the methods of Farnsworth[8], Harbone[9], Rizk[10], Alyahia[11] and Silva and al.[12], the components analyzed were: flavonoids, tannins, alkaloids, anthraquinones, saponins, free quinones, cardiac glycosides, cyanogens glycosides, steroids, terpenoids, reducing sugar and gum.

Total phenol content

Total phenol content (TPC) of various Tamarix leaves extracts, water-methanol extract (WME); water-acetone extract (WAE); water-ethanol extract (WEE) and water extract (WE) were estimated by a colorimetric assay according to the method described by Velioglu and al.[13], using Folin Ciocalteu phenol reagent whose absorbance of developed pigment was determined at 725nm. Briefly, for each extract, 1ml of crude extract dissolved in methanol were mixed with 7.5ml FC reagent (diluted 10 fold), the mixture allowed to stand at 22°C for 5min, then 7.5ml Na_2CO_3 (60g/l) were added. The absorbance was read after 90min in a spectrophotometer UV/Vis. (Shimadzu UV mini 1240). The total phenol compounds were determined using a standard curve prepared with Gallic acid. Results were expressed as mg GAE (Gallic Acid Equivalent) per 100g dry weight of lyophilized crude extract. The phenols content was carried out in triplicate.

Antioxidant activity: DPPH assay

Lyophilized WME, WEE, WAE and WE were dissolved in methanol. The antioxidant assay was determined by $DPPH^{\cdot}$ [14]. $DPPH^{\cdot}$ solution was prepared by dissolving $DPPH^{\cdot}$ in methanol, for each extract 3.9ml of a $6x10^{-5}$M methanol DPPH were added to 0.1ml extract (different concentrations were tested). Absorbance was determined after 30min at 515nm using a spectrophotometer. The percentage inhibition activity was calculated from {[(Ac-At)/Ac] x100}, where Ac is the absorbance of the control and At is the absorbance of the extract. The inhibition curves were prepared and IC50 values defined as the amount of antioxidant necessary to decrease the initial $DPPH^{\cdot}$ concentration by 50% was determined.

RESULTS AND DISCUSSION

The average total phenol content of *Tamarix aphylla* extracts tested for each solvent type were present in table 1. The phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. The use of an alcoholic solution provides satisfactory results for the extraction process[15]. Aqueous alcohol solvents are the best solvents for extraction phenolic compounds from *Tamarix aphylla* leaves. Aqueous acetone and water are inefficient solvents for extraction of total phenols from plant leaves studied. The average total phenols content (mg GAE/g crude extract) of aqueous methanol extract was significantly high (262.26mg/g) than aqueous acetone, water extracts (165.12mg/g, 115.37mg/g) and better than aqueous ethanol extract (199.54mg/g). The use of mixture alcohol and water present the advantage of modulating the polarity of alcohol solvents, also adding that solubility of polyphenols depends mainly on the hydroxyl groups, the molecular size and the length of hydrocarbon.

Table 1
Total phenol content of different extract from T. aphylla leaves

	Yield of crude extract (%)	TPC (mgGAE/g extract) ± SD
Water extract	8.07	115.37 ± 2.97232
Water-Ethanol extract	4.95	199.54 ± 1.60503
Water-Methanol extract	6.75	262.26 ± 1.96642
Water-Acetone extract	3.70	165.12 ± 3.74123

SD: Standard deviation

The phytochemical screening showed a conspicuous absence of alkaloids in all extracts, then a remarkable presence of tannins in particular hydrolysable tannins. Others metabolites and bioactive compounds were identified such as flavonoids, cardiac glycosides, steroids and terpenoids. Also, we did not detect cyanogens glycosides and saponins (Table 2).

Table 2
Phytochemical compounds identified in different extracts

Metabolite	W. extract	W-E. extract	W-M extract	W-A extract
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	tr	+	+	+
Cyanogens glycosides	-	-	-	-
Free quinines	-	-	-	-
Anthraquinones	+	-	-	-
Saponins	-	-	-	-
Reducing sugar	+	-	-	-
Gum	tr	-	-	-

tr: trace
W: Water (100%)
W-E: Water-Ethanol (30-70; v-v)
W-M: Water-Methanol (30-70; v-v)
W-A: Water-Acetone (30-70; v-v)

Another remarkable observation is that the higher yield of extract showed with aqueous methanol solvent, followed by water solvent. These yields (Table 1) give details and explain the higher total phenolic compounds when we chosen organics solvent (alcohols) whose polarity is modified with water. These mixtures become ideal and selective to extract a great number of bioactive compounds of which phenolic compounds. Whereas water given more amount of yield, but only is not good to extract polyphenols. Water extracts only the water-soluble bioactive compounds; moreover much other residual substances and impurities are present in the aqueous extracts. It appears from our work that some of phenolic compounds and others compound pharmacologically interesting of the leaves from *Tamarix aphylla* are not extractible with only water is why the hydroalcoolic mixtures are suitable to extract different bioactive compounds. In our work, the mixture of methanol with water was the best solvent than the mixture of ethanol with water for extract phenolic compounds from *Tamarix aphylla* leaves.

The stable radical α, α diphenyl β picrylhydrazyl ($DPPH^{\cdot}$) has been widely used for the screening of substances with potential antioxidant activity[16] measured as a decolorizing effect following the trapping of the impaired electrons of $DPPH^{\cdot}$. Lower value of IC50 indicates higher antioxidant activity. Results are shown in figure 1. All extract presented a good scavenging activity, but maceration using aqueous acetone as solvent, aqueous ethanol and water showed a powerful antioxidant activity. These activities in the following decreasing order were: aqueous acetone extract (0.080mg/ml ± 0.00064) > aqueous ethanol (0.140mg/ml ± 0.00129) > water extract (0.173mg/ml ± 000103) > aqueous methanol extract (0.911mg/ml ± 0.01188). In our work, crude extract of the leaves from *Tamarix aphylla* obtained with aqueous acetone has present a strong and potent scavenging capacity against free radical $DPPH^{\cdot}$, whereas with this same solvent, we recorded the lowest content

polyphenols compared to other solvents obtained with Folin Ciocalteu method. On the other hand, aqueous methanol extract has higher total phenolic content than aqueous ethanol, water and aqueous acetone extracts, but it did not exhibit the highest antioxidant activity than the three other extracts. It is possible in this context phenolic compounds existing in aqueous acetone extract possess an ideal structure for the scavenging of free radicals since they present a number of hydroxyls acting as hydrogen donators which makes them an important and very powerful antioxidant agents[17,18].

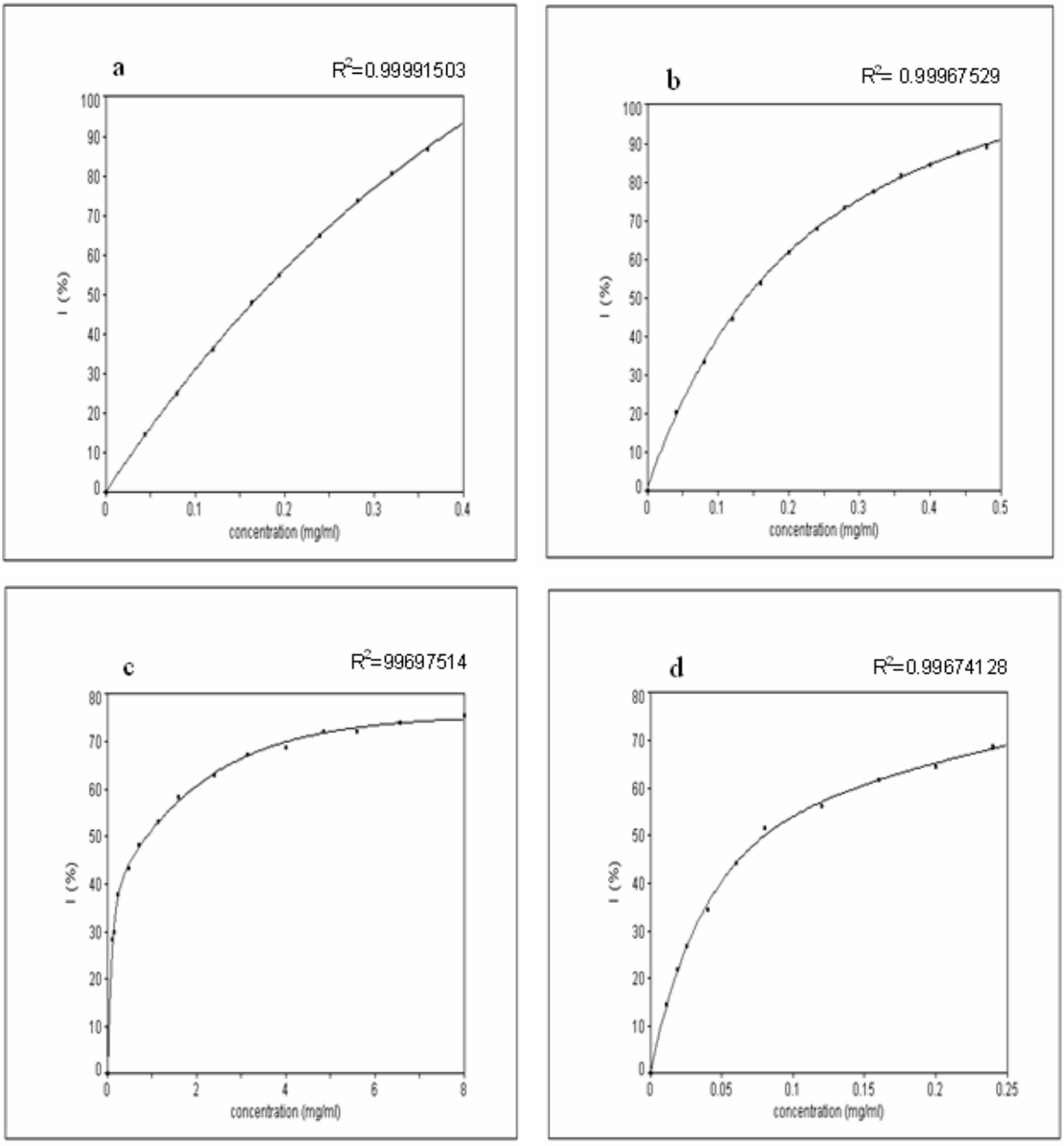

Figure 1

Scavenging capacity of four T. aphylla leaves extracts expressed in percentage at different concentrations (a: aqueous extract; b: aqueous ethanol extract; c: aqueous methanol extract; d: aqueous acetone extract)

Our work explains clearly why for each solvent taken individually, total phenol content determined by FC assay present a good correlation with antioxidant activity, but it is not the case when we compare between extracts obtained by various solvents. Different reports are found in the literature, whereas some authors found correlation between the total phenolic content and the antioxidant activity[13,19-22], others found no such relationship[23-27] .Antioxidant activity of extracts is strongly dependent on the solvent due to the different antioxidant potentials of compounds with different polarity[28,29] and FC assay gives a crude estimate of the total phenolic compounds present in an extract .it is not specific to polyphenols, but many interfering compounds may react with the reagent giving elevated apparent phenolic concentrations[30]. In addition, various phenolic compounds respond differently in this assay, depending on the number of phenolics groups they have and total phenolic content does not incorporate necessarily all the antioxidants that may be present in an extract[31]. In this study if methanol appears ideal for extract a high amount of phenolic compounds, acetone was the ideal solvent for extract bioactive compounds from *Tamarix aphylla* leaves with potential antioxidant activity.

IN CONCLUSION

In any research in phytotherapy, it is necessary to choose solvent according to biological activity required and not that which gives a high amount on bioactive compounds. From there crude extract or fraction expressing good biological capacity indicates that the substance with powerful biological effect exists in this extract and must be isolated and purified to confirm its pharmacological and medical use.

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