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PHYTOCHEMICAL INVESTIGATIONS OF *TAMARIX INDICA* WILLD. AND *TAMARIX PASSERNIOIDES* DEL. ex DESV. LEAVES FROM PAKISTAN

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Abstract

Plant-derived chemicals play an important role in protection against invading microbes that cause many infectious diseases and also found to be beneficial for therapeutics in prevention of many other noninfectious ailments. This research involves the investigation of such bioactive chemicals using leaves of two common *Tamarix* species. Qualitative and quantitative analysis using four different solvents showed methanol has the best extraction potential followed by acetone, ethanol and chloroform. *Tamarix indica* was found to contain most of the metabolites in higher quantity as compared to *Tamarix passernioides*.

Introduction

Phytochemicals are plant-derived chemicals that are not essential nutrients but known to have protective or disease preventive properties (Ahmed & Urooj, 2010). Plants are abundant in these chemicals that play a substantial role in the prevention from microbial, insecticidal or herbivorous predation (Cowan, 1999). These metabolites present in fruits and herbs may also protect human from a range of diseases (Argal & Pathak, 2006). Secondary metabolites like alkaloids, flavonoids, terpenoids, tannins, phenols and glycosides contain antimicrobial, anthelmintic, antidiarrhoeal activities while saponins and polypeptides are considered to contain anticancer and antiviral activities respectively **(**Cowan, 1999; Kumar *et al*., 2010; Vidyadhar *et al*., 2010). Various screening methods have been used to assess these compounds through extraction in solvents with similar polarity (Ncube *et al*., 2008).

Decoctions from different parts of a plant usually contain diverse mixture of phytochemicals (Cowan, 1999). Variability in the concentrations of these extracted substances depends upon the choice of plant part, the extraction procedure and the extractant used (Taylor *et al.*, 2001; Ncube *et al*., 2008; Sarwat *et al*., 2012). Mainly the solvent employed in such studies accounts for the complexity and diversity of the compounds being extracted (Tiwari *et al*., 2011, Gul *et al*., 2012).

The current investigation involves the use of *Tamarix indica* Wild., and *Tamarix passernioides* Del. ex Desv., the perennial shrubs or small trees, belonging to the genus *Tamarix* of family Tamaricaceae. These plants have feathery foliage and slender branches consisting of small scale like leaves (Qaiser, 1981). The aerial parts of *Tamarix* species are used for treatment of chronic diseases such as diarrhea and dysentery while the bark is used as an astringent tonic (Panhwar & Abro, 2007). A decoction of this plant is observed to possess antinociceptive activity (Sarker & Sarker, 2009) and roots have antinociceptive, cytotoxic and diuretic properties (Rahman *et al*., 2011). This study is designed to assess the phytochemical screening of two *Tamarix* species to investigate the chemical differences between these species and the use of different extractants to find a suitable and efficient solvent system.

Materials and Methods

Collection of plant material: The plant samples were collected from the Botanical Garden, University of Karachi. The species were identified and voucher specimens were deposited in the Herbarium of the University of Karachi. The plant leaves were cleaned washed and shade dried for 15 days. Dried leaves of both specimens were separately ground into fine powder with the help of electrical grinder and the powdered material was kept in airtight bottles to prevent from moisture until further analysis.

Preparation of extracts: Powdered leaf sample (10g) of both species was suspended using 100ml of four different solvents: methanol, ethanol, acetone and chloroform. The mixtures were kept at room temperature for 48 hours with constant shaking then filtered using Whatmann filter paper # 40 (125mm). The filtrate was dried in fume hood overnight at room temperature. The residue was weighed and used for phytocompounds screening.

Phytochemical investigations

a. Qualitative analyses: Preliminary qualitative phytochemical screening for various metabolites such as phenolic compounds, tannins, glycosides, anthraquinone derivatives (Evans, 1997) alkaloids (Wagner, 1993; Evans, 1997), fixed oils and saponins (Kokate, 1999), proteins (Gahan, 1984), amino acids (Yasuma and Ichikawa, 1953), carbohydrates (Ramakrishnan *et al*., 1994) and gums and mucilage (Whistler and BeMiller, 1993) were performed.

b. Quantitative analyses: For quantitative analysis, total proteins (Bradford, 1976), soluble carbohydrates (Yemm & Willis, 1954), phenols (Singleton and Rossi, 1965), alkaloids (Harborne, 1973), tannins, flavanoids (Boham & Kocipai-Abyazan, 1974) and saponins (Obadoni & Ochuko, 2001) were measured.

Statistics: Duncan's multiple range test was used to find out the significant difference between the extraction potential of different solvents and independent sample t-test was performed at α=0.05 to determine the significant difference between the phytochemicals of both species by using SPSS 17 software.

Results

The results of qualitative analyses revealed that out of twelve metabolites tested, only fixed oils and fats were absent in both species with all solvents while alkaloids, flavonoids, carbohydrates, anthraquinones and terpenoids were uniformly present in both species. Polypeptides/amino acids, saponins and polyphenols were absent in chloroform extract of both species, however these three metabolites were present with other solvents. Gums and mucilage were present only in ethanol extract of both species. Glycosides were absent in *Tamarix passernioides* with all solvents and also in chloroform fraction of both species. Acetone and chloroform were inefficient in extracting tannins as compared to ethanol and methanol in both species (Table 1).

On quantitative basis, the amount of phenols, carbohydrates and proteins were significantly ($p \square 0.05$) high in the methanol fraction followed by acetone, ethanol and chloroform in both *Tamarix* species. Methanol showed a significantly ($p \square 0.05$) high extraction potential for flavonoids, alkaloids and saponins. Ethanol was the second best extracting solvent in case of flavonoids followed by acetone and chloroform. For alkaloids extraction, methanol, acetone and ethanol appeared to be significantly ($p \square 0.05$) efficient in *Tamarix indica* while in case of *Tamarix passernioides* efficiency of solvent methanol fraction was high followed by acetone, ethanol and chloroform. The total content of saponins in *Tamarix indica* with ethanol, acetone and chloroform was found to be equally effective ($p \square 0.05$) after methanol while in *Tamarix passerinoides* ethanol and acetone were as effective as methanol (Table 2). Between the two species examined, apart from phenolics, there was a significant difference ($p<0.05$) in the amount of all other extracted metabolites with the methanol decoction. *Tamarix indica* observed to have significantly greater ($p<0.05$) amount of carbohydrates, proteins, alkaloids and saponins than *Tamarix passernioides* that have significantly higher ($p<0.05$) amount of flavonoids (Table 3).

Table 1. Qualitative phytochemical screening of *Tamarix indica* and *Tamarix passernioides*.

Phytochemical test	Ethanol		Methanol		Acetone		Chloroform	
	Tamarix indica	*Tamarix passernioides*	*Tamarix indica*	*Tamarix passernioides*	*Tamarix indica*	*Tamarix passernioides*	*Tamarix indica*	*Tamarix passernioides*
Alkaloids								
Mayer's test	+	+	+	+	+	+	+	+
Wagner's test	+	+	+	+	+	+	+	+
Carbohydrates								
Benedict's test	+	+	+	+	+	+	+	+
Polypeptides/Amino acid								
Biuret test	+	+	+	+	+	+	-	-
Ninhydrin test	+	+	+	+	+	+	-	-
Gums & Mucilages								
Alcohol 95% test	+	+	-	-	-	-	-	-
Saponins								
Foam test	+	+	+	+	+	+	-	-
Fixed oils & fats								
Spot test	-	-	-	-	-	-	-	-
Polyphenols								
Gelatin test	+	+	+	+	+	+	-	-
Lead acetate test	+	+	+	+	+	+	-	-
Alkaline reagent test	+	+	+	+	+	+	-	-
Glycosides								
Borntrager's test	+	-	+	-	+	-	-	-
Anthraquinones	+	+	+	+	+	+	+	+
Terpenoids Salkowski test	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	-	-	-	-

Key: + (Present), - (Absent)

Table 2: Quantitative phytochemical analysis of *Tamarix indica* and *Tamarix passernioides*.

Solvents	Phenols (µg/ml)		Carbohydrate (µg/ml)		Protein (µg/ml)		Flavonoids (%)		Alkaloids (%)		Saponins (%)	
	Tamarix indica	*Tamarix passernioides*	*Tamarix indica*	*Tamarix passernioides*	*Tamarix indica*	*Tamarix passernioides*	*Tamarix indica*	*Tamarix passernioides*	*Tamarix indica*	*Tamarix passernioides*	*Tamarix indica*	*Tamarix passernioides*
Methanol	13.4^{a} ± 2.1	11.6^{a} ± 1.3	14.15^{a} ± 0.3	10.49^{a} ± 0.13	548^{a} ± 42	415^{a} ± 35	0.66^{a} ± 0.06	0.81^{a} ± 0.04	0.16^{a} ± 0.01	0.13^{a} ± 0.02	0.35^{a} ± 0.04	0.26^{a} ± 0.02
Acetone	6.17^{b} ± 0.5	9.29^{b} ± 0.21	9.05^{b} ± 0.2	7.04^{b} ± 0.19	327^{b} ± 37	412^{a} ± 39	0.024^{c} ± 0.004	0.04^{c} ± 0.02	0.10^{a} ± 0.01	0.079^{b} ± 0.004	0.27^{b} ± 0.03	0.25^{a} ± 0.01
Ethanol	3.36^{c} ± 0.2	4.55^{c} ± 0.25	6.94^{c} ± 0.98	5.68^{c} ± 0.08	296^{b} ± 24	328^{b} ± 29	0.30^{b} ± 0.05	0.41^{b} ± 0.03	0.19^{a} ± 0.3	0.056^{c} ± 0.004	0.23^{b} ± 0.02	0.23^{a} ± 0.02
Chloroform	1.25^{d} ± 0.05	2.1^{d} ± 0.15	4.49^{d} ± 0.8	4.48^{d} ± 1.45	145^{c} ± 36	330^{b} ± 10	0.011^{c} ± 0.01	0.018^{c} ± 0.002	0.013^{a} ± 0.003	0.013^{d} ± 0.001	0.22^{b} ± 0.02	0.20^{b} ± 0.01

The data is mean ± SD (n = 3). Means followed by the same letter within the same column were not significantly different using Duncan's Multiple Range Test at 5% level (SPSS, Windows 17)

Table 3. Difference between the phytochemical constituents of two *Tamarix* species.

Phytochemicals	*Tamarix indica*	*Tamarix passerinioides*	*p*-value
Phenols (µg/ml)	13.4	11.6	0.275
Carbohydrates (µg/ml)	14.1	10.4	0.001
Proteins (µg/ml)	548	415	0.014
Flavanoids (%)	0.66	0.81	0.023
Alakaloids (%)	0.16	0.12	0.028
Saponin (%)	0.35	0.26	0.016

Independent sample t-test was performed using SPSS 17 at α=0.05

Discussion

The procedures for extraction and the nature of the solvent including their polarity affect the rate, composition, diversity in quality and quantity of metabolites being extracted (Elof, 1998, Ncube *et al.,* 2008). Among the four solvents used, methanol was found to be most efficient in qualitative and quantitative extraction of primary and secondary metabolites. It was observed that chloroform is comparatively an inefficient solvent for extraction of all phytochemicals under investigation.

It is well established that extracted organic compounds of plants are either aromatic or saturated in nature; hence their extraction is favored initially by alcoholic solvents such as methanol and ethanol (Cowan, 1999). The higher content of metabolites in methanol extract may reflect the polarity of this solvent.

The presence of phytochemicals in the leaves of *Tamarix* species justifies the local use of this plant for the treatment of various disorders. The leaves are rich in flavonoids, saponins, polyphenols and proteins with considerable amount of carbohydrates and alkaloids. Qualitative tests also indicate the presence of gums, glycosides, anthraquinones, terpenoids and tannins.

Phytoconstituents or their derivatives are plant specific contributing to the taxonomic distinctness and significance of plants (Bate-Smith, 1962), for instance hydrolysable tannins, tamarixinins are particularly found in certain species of family Tamaricaceae such as *Tamarix pakistanica* Qaiser (Yoshida *et al*., 1993).

According to the phytochemical components found in these plants, it can be proposed that *Tamarix* species may possess antibacterial, anti-inflammatory, anti-viral, anti-allergic, antioxidant, anti-diabetic and anti-carcinogenic properties as observed in different studies (Djurdjevic *et al.,* 2006; Mohammedi & Atik, 2011; Drabu *et al*., 2012).

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