

**Quality Profile of Araq-e-Bedmusk from *Salix caprea* flowers.**Ajaz Ahmed<sup>1</sup>, \*Wajahat A. Shah<sup>1</sup>, Seema Akbar<sup>2</sup>, Shamshad A. Khan<sup>3</sup>.

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**ABSTRACT**

**Aim:** The present contribution was aimed at the quantitative analysis of Araq-e-Bedmusk (floral aromatic water obtained from *Salix caprea*). Furthermore, this study was also aimed to determine its antioxidant and anti-inflammatory activities. **Ethanopharmacological relevance:** Unani physicians prescribe it in nervousness and palpitation, also as a supporting medicine in psychological stress and cardiac affections.

**Methods:** The floral water (Araq-e-Bedmusk) was extracted with *n*-hexane and petroleum ether separately. Antioxidant activity was assessed as free radical scavenging capacity (RSC) towards 2, 2-diphenyl-1-picrylhydrazil (DPPH) radicals. Anti-inflammatory activity was examined by HRBC membrane stabilization method and Quantitative analysis of the aromatic water was evaluated by GLC. **Results:**

Quantitative analysis of petroleum ether extract of aromatic water was performed by GLC which revealed the presence of five constituents. On the other hand GLC analysis of hexane extract of aromatic water showed the presence of 7 constituents. The percentage scavenging effects of aromatic water of *Salix caprea* on DPPH radical were 25.50 %, 32.98 % and 39.83 % at 1 ml, 2 ml and 3 ml respectively. Furthermore, percentage stabilization of HRBC membrane of this aromatic water was 2.68, 14.23 and 35.34 at 1 ml, 2 ml and 3 ml, respectively. **Conclusion:** The percentage of these constituents in the aromatic water plays an important part in determining its quality. The potent antioxidant activity and significant anti-inflammatory potential of aromatic water may justify the Unani claim. It should be noted that the GLC analysis and pharmacological evaluation of the aromatic water (Araq-e-Bedmusk) from Unani Dawakhanna are the first time report from the *Salix caprea*.

**Keywords:** Araq-e-Bedmusk, *Salix caprea*, antioxidant, anti-inflammatory, GLC.

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**INTRODUCTION**

Active oxygen species and free radicals that are generated due to oxidative stress have shown to be actively involved in the pathogenesis of age-related diseases [1, 2]. Such as cancer and coronary heart disease and neurodegenerative disorders such as Alzheimer's disease [3]. Compounds that can scavenge free radicals have great potential in ameliorating these disease processes [4]. Natural antioxidants occur in all parts of plants. Plants may contain many different antioxidant components such as phenolic compounds, nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other

endogenous metabolites, which are rich in antioxidant activity [5-10]. There is currently immense interest in natural antioxidants and their role in human health and nutrition [11]. The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several examples have revealed the fact that the plants which contain antioxidant potential demonstrate the beneficial effects in inflammatory diseases. It has been suggested that many anti-inflammatory drugs may exert some of their effects by scavenging oxidants

and decreasing formation of reactive oxygen species (ROS) by activated phagocytes [12]. Most of the anti-inflammatory drugs are steroidal or nonsteroidal anti-inflammatory drugs. Though they are very useful, they have a number of severe adverse effects such as gastrointestinal disturbances and body fat redistribution. Hence, there is a need to develop safe and new anti-inflammatory agents with minimum side effects. In this scenario, use of plant derived products to treat inflammation and related condition becomes a viable and valid approach. Aromatic water is water enriched with both the essential oil and the water-soluble volatile components of a plant, which modify and balance the action of the pure oil. The water-soluble volatile components are actually in solution and give the aromatic water additional properties not possessed by the essential oil alone. Many related products on the market are produced by adding an essential oil to water (*floral waters*) or are by-products of the steam distillation of essential oils (*hydrosols*). *Anethum graveolens* (Dill water) possess Anti-spasmodic, mild diuretic, gentle expectorant, galactagogue properties [13]. *Salix caprea* (Goat willow) is common species of willow and is native to Europe western and Central Asia [14]. *Salix caprea* belonging to family Salicaceae is a deciduous tree growing 8-10m at a fast rate. The plant is widely used in folk medicine for rheumatoid arthritis, malaria, various hemorrhages, gout, neuralgia and intestinal diseases as an antipyretic, analgesic, anti-inflammatory, antibacterial, hemostatic, sedative, and antihelminthic agent [15]. The distilled water from the flowers is a prodiase, cordial and stimulant that is highly valued as a medicine and used externally in the treatment of headache and ophthalmia [16]. In Unani medicine a distillate of flowers of *Salix caprea* is also known as Araq-e-Bedmusk. Unani physicians prescribe it in nervousness and palpitation, also as a supporting medicine in psychological stress and cardiac affections [17]. Four flavonoid glycosides namely astragalins, quercimeritin, isorhamnetin-7-O-D-glucoside and isoquercitin has been reported from flowers [18,19]. 1, 4-Dimethoxybenzene a floral scent compound has been identified from the pollen [20].

However there are no critical reports on Quantitative analysis as well as on biological activities of Araq-e-Bedmusk. Therefore, the present contribution was aimed at the investigation of the Quantitative analysis and biological activities of the floral aromatic water (Araq-e-Bedmusk) obtained from Unani Dawakhanna.

#### **MATERIALS AND METHODS**

The Aromatic water (Araq-e-Bedmusk) of *Salix caprea* flowers was purchased from Unani Dawakhanna, (Hamdard), Srinagar, Jammu and Kashmir, India. The identity of the Sample has been authenticated by Dr. Ifat, Regional, Research, Institute of Unani, Medicine, Srinagar.

**Method.** The obtained Araq-e-Bedmusk was extracted with *n*-hexane and petroleum ether separately. The obtained extracts of aromatic water was then concentrated, at 35°C using roto evaporator and subsequently analyzed.

#### **Gas liquid chromatography/flame ionization detection (GLC/FID).**

The quantitative analysis was carried out by high-resolution GLC using a Varian 3400 gas chromatograph equipped with flame ionization detector (FID) and OV-1 column 30 m × 0.25 mm × 0.25 μm (Instrumentation division Shri Mata Vashno Devi University, Katra, Jammu) . The operating conditions were as follows: carrier gas was helium with a flow rate of 2 ml/min, split ratio 1:20. The oven temperature was programmed with an initial temperature of 40 °C, 2 min isothermal, 300 °C, 4 °C/min, then 10 min isothermal. Injector and detector temperatures were set at 250 and 300 °C, respectively. Areas under peaks of chromatogram were used for calculation of abundance of each component.

#### **In vitro Anti-inflammatory activity of Aromatic water of *Salix caprea* flowers.**

**Human Red Blood Cell (HRBC) membrane stabilization method:** The blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. In various concentration of Aromatic waters (1 ml, 2 ml and 3 ml) added 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC

suspension. It was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm and a

$$\text{Percentage Stabilization} = \frac{100 - (\text{optical density of drug})}{(\text{optical density of control})} \times 100$$

#### STATISTICAL ANALYSIS:

All observations were presented as Mean  $\pm$  SEM.

#### Determination of antioxidant activity

The free radical scavenging activity of the Aromatic water was measured in terms of hydrogen donating or radical- scavenging ability using the stable radical DPPH. 0.1 mM solution of DPPH in Methanol was prepared and 1.0 ml of this solution was added in various concentration (1 ml, 2 ml and 3 ml) of Aromatic water. Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula.

$$\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where  $A_0$  was the absorbance of the control (blank, without Aromatic water) and  $A_t$  was the absorbance in the presence of the Aromatic water. All the tests were performed in triplicate and the graph was plotted with the mean values.

#### RESULTS AND DISCUSSION

GLC analysis of petroleum ether fraction of aromatic water from Unani Dawakhanna (UD) revealed the presence of five constituents (**Table 1**). On the other hand GLC analysis of hexane extract of aromatic water from Unani Dawakhanna revealed the presence of 7 constituents: (**Table 2**). The percentage of these constituents in the aromatic water plays an important part in determining its quality. The quality and price of aromatic water is based on the percentage content of a single chemical component, so separation and measurement of individual components is very important. Aromatic waters were also evaluated for anti-inflammatory activity by HRBC membrane

control was prepared by omitting the extracts. The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

stabilization method. The percentage protection for aromatic water were 2.68, 14.23 and 35.34 at 1 ml, 2 ml and 3 ml, respectively whereas the standard Diclofenac sodium holds the percentage protection (71.29 at 100 ug/ml) (**Table 3**). Aromatic water was also evaluated for anti-oxidant activity by free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical method and we found that the percentage scavenging activity of aromatic water from the Unani Dawakhanna were 25.50 % ,32.98 % and 39.83 % at 1 ml , 2 ml and 3 ml respectively. Whereas the standard Ascorbic acid holds the percentage scavenging (82.42) (**Table 4**).

It has been suggested that many anti-inflammatory drugs may exert some of their effects by scavenging oxidants and decreasing formation of reactive oxygen species (ROS) by activated phagocytes [22]. Since oxidative stress inside the living organism is due to generation of active oxygen species. Free radicals have shown to be actively involved in the pathogenesis of age-related diseases such as cancer and coronary heart disease and neurodegenerative disorders such as Alzheimer's disease. In Unani medicine a distillate of flowers (Aromatic water) of *Salix caprea* is also known as Araq-e-Bedmusk. Unani physicians prescribe it in nervousness and palpitation, also as a supporting medicine in psychological stress and cardiac affections. Thus the aim of present study was also to determine the antioxidant, anti-inflammatory as well as Quantitative analysis of aromatic water (Araq-e-Bedmusk) of *Salix caprea* flowers. The results were found very promising. The potent antioxidant activity and significant anti-inflammatory potential of aromatic water may justify the Unani claim. It should be noted that the GLC analysis and pharmacological evaluation of the aromatic water (Araq-e-Bedmusk) from Unani Dawakhanna are the first time report from the *Salix caprea*.

**Table 1: GLC analysis of petroleum ether extract of aromatic water (Unani Dawakhanna).**

Sr. No.	R.T.	Area %	Height %	Peak width
1	0.88	75.1768	51.5777	0.238
5	1.26	0.2131	0.7202	0.052
3	1.39	23.5091	45.6941	0.074
4	1.71	1.0278	1.8947	0.087
5	2.15	0.0739	0.1136	0.112

**Table 2: GLC analysis of Hexane extract of aromatic water (Unani Dawakhanna).**

Sr. No.	R.T.	Area %	Height %	Peak width
1	0.28	0.6614	2.0811	0.071
5	0.41	0.0000	1.1406	0.063
3	0.77	0.0156	0.0542	0.060
4	0.86	97.2195	92.0339	0.191
5	1.34	1.0118	2.5137	0.150
6	1.48	0.8101	1.8200	0.123
7	1.87	0.2816	0.3566	0.142

**Table 3: Anti-inflammatory activity of aromatic water from Unani Dawakhanna.**

Sr. No.	Sample	Conc.	Percentage protection
1	Aromatic water from Unani Dawakhanna	1 ml	2.68 $\pm$ 0.24**
		2 ml	14.23 $\pm$ 1.22**
		3 ml	35.34 $\pm$ 0.12**
2	Diclofenac (standard)	100 ug/ml	71.29 $\pm$ 1.40**

The results were expressed as mean  $\pm$  S.E.M\*\* [N=3].

**Table 4: Anti-oxidant activity of aromatic water from Unani Dawakhanna.**

Sr. No.	Sample	Conc.	Percentage protection
1	Aromatic water from Unani Dawakhanna	1 ml	25.50 $\pm$ 0.3**
		2 ml	32.98 $\pm$ 0.2**
		3 ml	39.83 $\pm$ 0.4**
2	Ascorbic acid (standard)	100 ug/ml	80.42 $\pm$ 1.42**

The results were expressed as mean  $\pm$  S.E.M\*\* [N=3].

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