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# Antioxidant Study of Unani Formulation ARQ Mundi

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## Abstract

Arq mundi (a clear aqueous distillate of flowers of the plant *Sphaerenthus indicus*) a Unani product recommended by the Unani practitioners in a variety of cardiovascular, neurodegenerative, and other disorders was evaluated for antioxidant property in various *in vitro* models. The hydrogen donating ability was measured in the presence of 1,1 diphenyl-2-picryl hydroxyl (DPPH) radical, reducing power was studied according to the reaction of $Fe^{3+}$ to $Fe^{2+}$, and inhibition of nitric oxide radical generation was also studied. The $IC_{50}$ values for all the above studied in vitro models were encouraging and found to be 1 ml (undiluted preparation). Based on our result, it is revealed that arq mundi possesses free radical scavenging ability. Free radicals have been reported to be one of the causes of the above organ disorders. Thus, our study justifies application of the arq in the above disorders.

**Keywords**

Arq, Mundi, DPPH, Unani medicine

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**INTRODUCTION:**

A wide variety of health care systems such as allopathy, homeopathy, ayurveda, Unani, siddha etc. are in practice across the world with the unanimous aim of benefiting the patient by getting rid of his/her ailments. Unani is one such system, where the preparations known as arqs are perhaps widely preferred in addition to others. Arqs are liquid preparations, obtained by distillation of macerated crude drugs in aqueous medium[1]. Different arqs like arq gulab, arq mundi, arq gauzaban etc. have been used by the traditional practitioners for the successful treatment / management of a wide variety of organ related disorders. However, these systems still lack sufficient scientific background. Hence, in the current study an effort has been made to evaluate the antioxidant efficacy of arq mundi using different *in vitro* models.

Arq mundi is a clear, non-viscous liquid preparation, obtained by the distillation of flowers of the plant *Sphaeranthus indicus*., Linn. (Family: Compositae) duly macerated in water. In Unani system of medicine, it has been advocated for a variety of cardiovascular, neurodegenerative, ocular diseases and specifically indicated for diseases arising from putrefaction of blood. Observation reveals that most of the afore mentioned organ diseases arise due to free radical involvement. As the arq has been yielding satisfactory results in these disease conditions, it produced a ray of hope for possible antioxidant activity in the preparation. This is one of the reasons for undertaking the current study.

Further, oxidative stress is produced by free radicals, predominantly reactive oxygen species (ROS) and reactive nitrogen species (RNS) and toxins, which cause tissue damage from oxidative stress. Free radicals generated as a consequence of a variety of biological reactions are largely responsible for diverse diseases and disorders viz. cardiovascular, cerebral dysfunction (Parkinsonism, epilepsy,

Alzheimer's disease), diabetes, inflammatory disorders, gastrointestinal disorders, immune deficiency syndromes (cancer and AIDS) and ageing. Medicinal plants and their traditional formulations rich in antioxidants can prevent oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent diseases.

Phytochemical literature reveals the presence of tannins, glycosides, alkaloids, reducing sugars and volatile oils and diterpenoids in *Sphaeranthus indicus*[2]. At the same time a number of biological activities such as antibacterial[2], antifungal[3], macrofilaricidal[4], wound healing[5], immunomodulatory[6,7], *in vitro* antioxidant property[8] and anti-inflammatory activity[9] of this plant have been reported. These reports further augmented the earlier interest in undertaking the evaluation of antioxidant property of arq mundi in different *in vitro* models.

**Materials and Methods:**

*Drugs:* Arq mundi (M/s. Hamdard Laboratories, Ghaziabad, India) was purchased from the retail Unani pharmacy. All chemicals and materials required to carry out this research were of analytical grade and were obtained from the regular store supplies.

**Experimental procedure:**

**DPPH radical scavenging activity[10]*:***

This was measured by a spectrophotometric method. To a methanolic solution of DPPH (200µm), 1ml of the test sample (undiluted preparation) was added. An equal amount of ethanol was added to the control. After 20 minutes the decrease in the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and percentage inhibition calculated by using the formula

$$\text{Inhibition \%} = \frac{\text{Control- Test}}{\text{Control}} \times 100$$

**Nitric oxide radical inhibition activity[11]:**

The sodium nitroprusside method was adopted to generate nitric oxide in aqueous solution at physiological pH., which interacts with oxygen to produce nitrite ions. This measures Greis' reaction. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the test solution (undiluted preparation 1 ml) was incubated at $25^0$ C for 150 minutes. After incubation 0.5 ml of reaction mixture was removed and 0.5 ml of Gries reagent (1% sulphanilamide, 2% $H_3PO_4$ and 1% Naphthalene diamine dihydrochloride) was added. The absorbance of the chromophore form was evaluated at 546 nm.

The percentage of nitric oxide inhibition can be determined using this formula:

$$\text{Inhibition \%} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

In this equation, $A_0$ represents the absorbance of the control sample, while $A_1$ indicates the absorbance of the test sample.

**Determination of reducing power[12]:**

The reducing power of test sample was determined according to the method of Oyaizes (1985). The test sample was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at $50^0$C for 20 minutes. Aliquot of trichloroacetic acid (2.5 ml) was added to the mixture, which was then centrifuged at 1000 rpm for 10 minutes. The upper layer of solution (2.5ml) was mixed with distilled water (2.5 ml) and freshly prepared $FeCl_3$ solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The percentage reducing power was calculated using the formula

$$\text{Inhibition \%} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

**RESULT AND DISCUSSION:**

**Table 1. Antioxidant activity of arq mundi using different *in vitro* models**

| Sl No. | Type of the model | Volume of undiluted arq used | Percentage inhibition |
|---|---|---|---|
| 1 | DPPH method | 1 ml | 83.21±0.91 |
| 2 | Nitric oxide | 1 ml | 72.07±1.34 |
| 3 | Reducing power | 1ml | 100.16±0.93 |

It is quite apparent from the results compiled in Table-1 that the test preparation arq mundi has satisfactory free radical scavenging activity. The $IC_{50}$ values for all the *in vitro* models used viz., DPPH, Nitric oxide and reducing power was encouraging and found to be 1 ml (undiluted preparation).

In practice, arqs are usually administered in large doses (approximately 60ml) and the frequency of administration is either two or three times a day, based on the need.

It is well known fact that it is very difficult to determine the exact chemical composition and

strength of the arqs, so that their concentration can be represented in terms of any unit per volume. Hence, in the present study, the ability of minimum volume of undiluted arq (1ml) to scavenge the free radicals in different *in vitro* models was determined, which can be further extrapolated to any volume that is used in the therapeutic practice.

**CONCLUSION:**

In conclusion, the free radical scavenging activity of arq mundi was found to be satisfactory and may be attributed to the presence of tannins and volatile oil contained in the flowers of the plant used for its preparation. Since volatile oils and tannins have been reported to possess free radical scavenging activity. Thus, at this juncture, our preliminary study supports the current application of arq mundi in organ disorders. However, the detailed *in vitro* and *in vivo* studies are more rewarding for further substantiation of our results.

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