Indian Journal of Unani Medicine
Vol. XVII, Issue 2, July-December, 2024, Pp. **115-121**
ISSN No.: **0974-6065**

https://doi.org/10.53390/IJUM.2024.17203

ANTI-OXIDANT ACTIVITY OF AN AQUATIC PLANT *JAR-UL-NAHR* (*POTAMOGETON NATANS* LINN): A UNANI DRUG

Iqra Rifat[1], Mohd. Afsahul Kalam[2], Suheena Khanday*[3] and Huzaifa Salati[4]

[1]JRF (Pharmacovigilance), Regional Research Institute of Unani Medicine, Srinagar, J&K
[2]Research Officer (U), Regional Research Institute of Unani Medicine, Srinagar, J&K
[3]Research Officer (U), Central Council for Research in Unani Medicine, New Delhi
[4]PG Scholar Deptt. Ilmul-Advia, Regional Research Institute of Unani Medicine, Srinagar, J&K

Research Paper

Received: **01.08.2024** Revised: **10.08.2024** Accepted: **16.08.2024**

ABSTRACT

The aim of this study was to evaluate the *in vitro* antioxidant assay of methanolic and hydro alcoholic extracts of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* Linn). Antioxidants, also known as inhibitors of oxidation, are compounds that delay or prevent the oxidation of substances, thereby extending their life span. Oxidative stress, resulting from free radicals impact various enzyme systems causing damage that may contribute to conditions like cancer, ischemia, aging, adult respiratory distress syndrome, rheumatoid arthritis, and more. A plant-based diet offers protection against chronic diseases related to oxidative stress due to the antioxidants found in dietary plants. These plants contain diverse chemical families and varying amounts of antioxidants, which are believed to contribute to the health benefits of plant-based diets. Hence, the present study was carried out to evaluate the antioxidant potential of *Jar-ul-Nahr* (*Potamogeton natans* Linn).

Methods: The anti-oxidant activity of methanolic and hydro alcoholic extract was investigated by DPPH radical scavenging against BHA as standard control. In this study, five concentrations (0.125mg/mL,0.25mg/ml, 0.5 mg/mL, 1mg/mL, 2mg/mL and 4mg/mL)of the methanolic and hydro alcoholic extracts of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* Linn)were tested. The antioxidant activity of each extract increased with increasing concentration. The inhibition of the DPPH free radical was found in concentration dependent manner.

Results: On comparing the solvent wise DPPH radical scavenging activity of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* Linn), the reduction ability was found higher in hydro alcoholic extract followed by methanolic extract with little difference.

Conclusion: In conclusion, *Jar-ul-Nahr* (*Potamogeton natans* Linn) which is reported to have significant activity against several human ailments, could be exploited as potential source of natural antioxidants for plant based pharmaceutical industries.

No. of Pages: 7 **No. of Figs: 2** **No. of Table: 1** **References: 10**

Keywords: Jar-ul-Nahr, Antioxidant.

*Corresponding author: suhinakhanday@gmail.com

Introduction:

Since thousands of years, civilizations throughout the world have used medicinal plants to treat a variety of diseases. The oldest written confirmation of medicinal plant usage was found on a Sumerian clay slab, approximately 5000 years old (Petrovska, 2012) Plants contain numerous antioxidants that are primarily produced as secondary metabolites. Phytochemicals, literally meaning 'plant-chemicals,' are non-nutritive chemical components of plants that offer numerous health benefits and disease prevention properties. While these nutrients are non-essential—meaning they are not required by the body for sustaining life —they confer significant health benefits when consumed by humans. Plants produce these chemicals to sustain their own life processes, which, in turn, provide health benefits to humans upon consumption. There are over a thousand known phytochemicals, classified as primary or secondary constituents based on their roles in plant metabolism. Phytochemicals have significant nutraceutical importance (Lawal 2016). They are bioactive constituents that maintain health and serve as a bridge between the food and pharmaceutical industries. Phytochemicals perform numerous functions and possess unique pharmacological effects such as anti-inflammatory, antispasmodic, anti-allergic, antioxidant, antibacterial, antifungal, chemopreventive, neuroprotective, hypotensive, and anti-aging properties. They stimulate the immune system, block the formation of carcinogens, reduce oxidation, slow the growth rate of cancer cells, reduce inflammation, trigger apoptosis, prevent DNA damage, and regulate hormones such as estrogen and insulin, which, in excess, are linked with an increased risk of breast and colon cancer (Karamac 2019).

Several plants possess natural antioxidants that counteract oxidizing species and free radicals produced by the body. 65 Antioxidants can be sourced both internally and externally. Internally, antioxidants are generated through the activity of body enzymes, including superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase. Externally, antioxidants are obtained from dietary sources such as vitamins A, E (alpha-tocopherol), C (ascorbic acid), minerals, and polyphenols, which are predominantly plant-based (Nwozo 2023). Natural antioxidants that are of plant origin, especially oral herbs, have fewer side effects than chemical antioxidants and helps in protecting the body against free radicals. Flavonoids act against the detrimental effects of reactive oxygen species (ROS) such as superoxide radicals, by acting as antioxidant. Excessive production of free radicals may lead to tissue injury (Nargesi S 2018). Cell membranes that are composed of unsaturated lipids are most susceptible to free radicals. High concentrations of free radicals in cells and tissues induce oxidative stress, which can be produced by a variety of detrimental factors such as X-ray radiation, UV, gamma, strenuous physical exertion, smoking, drug addiction, alcoholism, stress and drug addiction. Chronic oxidative stress has been reported to produce a variety of diseases that includes cancer, heart related diseases, and increases the pace of aging. Several secondary metabolites of lipid oxidation, such as 4-hydroxynonenal and malondialdehyde, can react with biological components such as proteins, amino acids, and DNA. Malondialdehyde can be formed both enzymatically and non-enzymatically, and has been linked to health issues such as mutation and carcinoma (Yashin A 2017). There is growing recognition that oxidative stress, which arises from an imbalance between reactive molecule production and neutralisation, is the primary cause of many diseases prevalent in today's world (Hazra 2008). Because Unani treatments are safe and have a proven track record of effectiveness, they are also considered to be reasonably priced. Along with a range of naturally occurring antioxidants, they also contain vitamins, minerals, active steroids, alkaloids, glycosides, and tannins. In this study, whole plant of *Jar-ul-Nahr* (*Potamogeton natans* Linn), regularly prescribed by local practitioners

to cure various ailments, is subjected to screening for antioxidant activity. Till date, data about antioxidant property of this plant were lacking, therefore, this study was reported for evaluation of its *in vitro* antioxidant potential including the scavenging of DPPH.

Mechanisms of action

Free radicals are highly reactive species with short lifespans that cause damage to macromolecules such as proteins, DNA, and lipids. These reactive oxygen species (ROS) tend to react with electrons from other molecules in the body, impacting various enzyme systems and causing damage that may contribute to conditions like cancer, ischemia, aging, adult respiratory distress syndrome, rheumatoid arthritis, and more. It is assumed that antioxidantsact by two modes. The first one involve process of breaking a chain, in which the primary antioxidant donates an electron to a free radical in the system (e.g., lipid radical), that results in the formation of a new and more stable radical. Flavonoids, ascorbic acid and tocopherols are examples of primary antioxidants. The second mechanism includes removal of ROS initiators (secondary antioxidants) by quenching chain-initiating catalyst. This mechanism is capableof deactivating high energy species like absorption of UV light, O_2, chelations of metal catalyzing free radical reactions or by inhibition of peroxidases, such as lipoxygenases or xanthine oxidase. A secondary is any chemical that can react with the initiating radical and suppresses the initiating enzyme or reduces the O_2 level without creating ROS (Diplock 1998).

In non-dividing cell, cancer rarely occurs. Mutation can occur when a cell multiplies and its DNA is damaged. ROS damage to cells has been shown in vitro and in vivo studies and has indicated that it may play the role of carcinogenesis in different ways (Diplock 1998).

They may cause:

- ✓ Structural change of DNA such as gene sequence amplification.
- ✓ Mutations in Base pair (the oxidized form of guanine has altered base-pairing properties) and translocations.
- ✓ Activation or suppression of signal transduction pathways – in the majority of squamous cell carcinomas of the lung, over expression of a growth factor receptor is commonly involved.
- ✓ Abnormal cell-to-cell communication that permits unrestricted cell proliferation.
- ✓ Interference with genes that control cell growth, inhibiting apoptosis or necrosis.

Damage to proteins e.g. DNA repair enzymes, makes it more difficult to correct a mutation, once it has occurred. The immune system is able to provide protection and to prevent the body from invading organisms and to remove damaged, aged or modified cells that that can lead to cancer. All cell membranes, including white blood cell membranes are made up of lipids, which are vulnerable to free radical damage. It has now been revealed that there are number of connections between free radical reactions and immune cell dysfunction. White blood cells membrane fluidity can be reduced by reactive oxygen species (ROS), lowering their function dramatically. The loss of membrane fluidity has been linked to lymphocytes diminished ability to respond the immune system stimuli. Immune cells DNA can also be damaged that result in mutations and impairs normal functioning of cell. Ironically, certain chemotheraphy medications and radiation used in treatment of cancer are based on damage of free radical. The barrage of free radicals that indiscriminately damage good cells as well as malignant ones results in well documented side effects such as hair loss, lowered immunity and gastro-intestinal disorders. (Pendry BA 2005).

The term '*Harārat Gharība*' (external heat) is commonly used in the Unani System of Medicine and corresponds to excessive or unnatural heat; it is assumed to be the main predisposing factor for responsible for changing normal humours as it produces *Ihtirāq*. Similarly *Ihtirāq* in Unani medicine, modern medicine

defines oxidative stress (OS) as a critical tissue-destructive mechanism and a major factor in the development of chronic diseases. Harman proposed the free radicals theory in 1950, which states that,"free radical damage to cellular macromolecules in aerobic organisms affects their lifespans". OS is a disproportion between systemic generation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates. Reactive oxygen metabolites (ROM) also known as free radicals are oxygen-containing molecules that have one or more unpaired electrons which react and damage other cellular content including proteins, lipids, and DNA. Similary, *Ihtirāq*damages the normal humoral composition (Kausar F 2020). Sylvie *et al*., found that increased rates of resting energy expenditure are linked to greater levels of inflammation and OS is associated with higher rates of resting energy expenditure (REE). Itillustrates that higher REE corresponds to raised heat production and it is linked to OS. This raisedheat is clearly linked with '*Harārat Gharība*' as stated in classical literature. Furthermore, oxidation is typically an exothermic process that produces heat. As a result of the undesired oxidation, unwanted heat is produced, which burns the normal cellular constituents. By the related denominators of combustion and excess heat, the OS of bio molecules links to the *Ihtirāq*of Unani pathology. However, along with holding excess heat co-relatable with OS as a basic pathology, an even more significant point is that Unani Medicine shows combustion and excess heat to be more than a massive and simple increase in heat; instead depending on different cause and associations; it comes in a variety of forms with each particular forms of excess heat requiring very different types of treatments rather than simple increasers of coldness (Kausar F 2020).

MATERIALS AND METHODS

Source of data collection

The data were collected from Regional Research Institute of Unani Medicine (RRIUM), Srinagar Jammu and Kashmir.

Plant material collection

A survey tour to Dal Lake in Srinagar, J&K was conducted on 13 August 2021, to collect plant specimen during its flowering stages for future reference and preparation of herbarium for authentication of identification. Another survey tour to Dal Lake was conducted in December 2021 to collect plant specimen without flowers for making herbarium and for conduction of research work. The collection was done in the same area of Dal Lake where it was done before. The voucher specimen was deposited in the herbarium of survey of Medicinal Plants Unit (SMPU), RRIUM, Srinagar under voucher specimen no.5866-5869.

Identification and authentication of plant material

The plant was also authenticated by Dr. Akhter H. Malik KASH, and submitted in the museum of Centre for Biodiversity and Taxonomy (CBT), University of Kashmir under specimen voucher No.8040. A sample specimen of collected material of the plant was deposited in herbarium, Department of Botany, Kashmir University J&K for the future references.

Chemicals and Reagents

All the solvents, chemicals and reagent used in the present research work were of analytical grade (Merck Specialties Private Limited, Mumbai, Himedia laboratories Pvt Ltd, Mumbai). The solvents used for extraction were Methanol, and Hydro alcohol.

Extraction of crude drug material:

The whole part of the plant of Jar-ul-Nahr (*Potamogeton natans* L.) was collected, cleaned and dried under shade at room temperature. The dried whole plant was pulverized using stainless steel mixer grinder.600g dried coarsely powdered material of whole plant of Jar-ul-Nahr was subjected to extraction in soxhlet apparatus. Soxhlation was performed at 600c using Methanol and Hydro-alcohol. In each solvent, Soxhlation was continued until no colour was observed in siphon tube and evaporated for residue. Absence of residual confirmed the completion of extraction. The extracts were

evaporated until they were completely dry.

Antioxidant activity

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of extracts was determined by method described by Pellegrini *et al.* (1999). The DPPH reagent was DPPH (8 mg) dissolved in MeOH (100 mL) for a solution concentration of 80μL/mL. To determine the scavenging activity, 100μL DPPH reagent was mixed with 100 μL of stock solution in a 96 well microplate and was incubated at room temperature for 30 min. After incubation, the absorbance was measured 514 nm using an Elisa reader (Erba Chem-7 Semi automated analyzer). Stocksolution of the Methanolic and hydroalcoholic extract was prepared to the concentration of 4mg/mL, 2mg/mL, 1mg/mL, 0.5mg/mL, 0.25mg/mL. The experiment was repeated three times. BHA was used as standard controls. The radical scavenging activity was determined by using below equation:

$$\% \text{ Inhibition} = (A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}} * 100$$

Where, A_{Control} represents absorbance of control at t=0 min and A_{Sample} represents absorbance of sample at t=30 mins. BHA was used as reference standard and IC_{50} values were calculated for each test solution i.e conc. required to inhibit formulation of DPPH radical by 50%.[120]

Statistical analysis

Graph Pad Prism 7.0 (Graph Pad software, Inc.) and MS(Microsoft) Excel 2007 were used for statistical analysis and graph composition. The measured data is expressed as the mean ± Standard deviation (SEM) of a minimum of three independent experiments. The statistical significance between control and treatment groups was determined by unpaired t–test and between multiple groups by one way analysis of variance (ANOVA). The significance level was set at $p<0.001$.

Result

The DPPH radical scavenging activity of methanolic and hydro alcoholic extracts ofwhole plant of *Jar-ul-Nahr*(*Potamogeton natans* Linn) were determined by the decrease in absorbance induced by plant antioxidants. Five concentrations (0.125mg/mL,0.25mg/ml, 0.5 mg/mL, 1mg/mL, 2mg/mL and 4mg/mL)of the extracts were tested. The antioxidant activity of each extract increased with increasing concentration. The inhibition of the DPPH free radical was found in concentration dependent manner. On comparing the solvent wise DPPH radical scavenging activity ofwhole plant of *Jar-ul-Nahr* (*Potamogeton natans* Linn), the reduction ability was found higher in hydro alcoholic extract followed by methanolic extract with little difference. Percentage inhibition for hydro alcoholic and methanolic extract was calculated to be 29.698% and 29.44% respectively and IC_{50} were 3.044 and 2.832 respectively and reduction ability of BHA was 52.34 and IC $_{50}$value was 3.044 as shown in Table1; Fig.1& Fig.2.

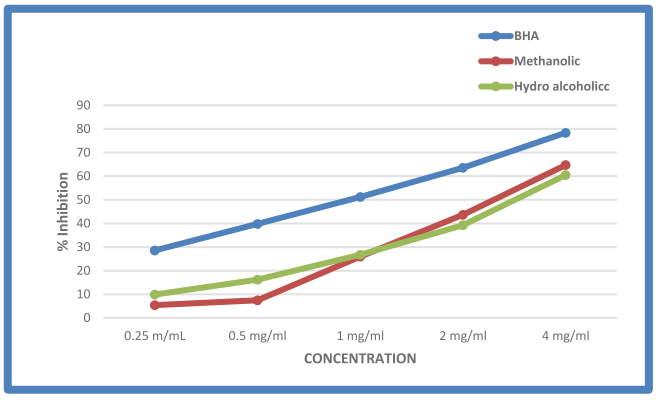

Fig.1: Graphical representation of DPPH radical scavenging activity of BHA, methanolic and hydro alcoholic extracts of whole plant of *Jar-ul-Nahr* (*Potamogeton natans L*).

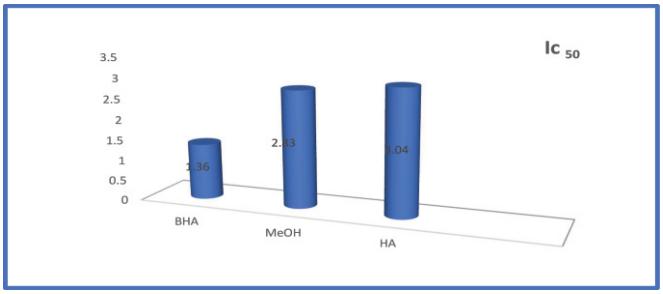

Fig. 2: Graph representation of $IC_{50}$of BHA, MeOH and HA extracts of whole plant of *Jar-ul-Nahr* (*Potamogeton natams* L.).

Table 1: DPPH based % inhibition and IC_{50} value of whole plant of *Jar-ul-Nahr*.

Concentration (mg/ml) / Solvent type	Percentage Inhibition					Mean	IC 50 (mg/mL) M
	0.25 mg/mL	0.5 mg/mL	1 mg/mL	2 mg/mL	4 mg/mL		
BHA (standard control)	28.56	39.8	51.2	63.56	78.4	52.34	1.361
Methanolic extract	5.41	7.49	25.94	43.66	64.72	29.44	2.832
Hydroalcoholic extract	9.89	16.26	26.76	39.23	60.49	29.69	3.044

Conclusion

Unani plant *Jar-ul-Nahr* (*Potamogeton natans* Linn) that is reported to have significant activity against several human ailments showed antioxidant activity as evidenced by the scavenging of the free radicalDPPH. It was concluded that antioxidant activity of two extracts (MeOH and HA) of *Jar-ul-Nahr* increased with increasing concentration (0.125 mg/mL,0.25mg/ml, 0.5 mg/mL, 1mg/mL, 2mg/mL and 4mg/mL) with higher antioxidant activity in hydro alcoholic extract followed by methanolic extract with little difference.

References

1. **Petrovska, B.B.** (2012) Historical review of medicinal plants' usage. *Pharmacognosy Review*, 6,1–5.
2. **Karamac, M.; Gal, F.; Longato, E.; Meineri, G.; Janiak, M. A.; Peiretti, P. G.; Peiretti,** P. G. Antioxidant Activity and Phenolic Composition of Amaranth (Amaranth Spp.) during Plant Growth. *Antioxidants*. 2019, 8, 173. DOI: 10.3390/antiox8060173.
3. **Lawal, B.; Shittu, O. K.; Obiokpa, F. I.; Berinyuy, E. B.; Mohammed, H.** African Natural Products with Potential Antioxidants and Hepatoprotectives Properties: A Review. *Clinical Phytosci.* 2016, 2–23. DOI: 10.1186/s40816-016-0037-0.
4. **Nwozo, O. S.** *et al.* (2023) 'Antioxidant, phytochemical, and therapeutic properties of medicinal plants: a review', *International Journal of Food Properties*, 26(1), pp. 359–388. doi: 10.1080/10942912.2022.2157425.
5. **Hazra B., Biswas S., Mandal N.**: Antioxidant and free radical scavenging activity of Spondias pinnata. BMC Complement Altern Med 2008, 8:63-72
6. **Nargesi S, Moayeri A, Ghorbani A, Seifinejad Y, Shirzadpour E, Amraei M.** The effects of *Matricaria chamomilla* L. hydroalcoholic extract on the atherosclerotic plaques, antioxidant activity, lipid profile and inflammatory indicators in rats. *Biomedical Research and Theraphy*. 2018; 5(10): 2752 – 2761.
7. **Yashin A, Yashin Y, Xia X and Nemzer B.** Antioxidant activity of spices and their impact on human health: A review. MDPI 2017; 6(70): 1-18.
8. **Diplock AT, Charleux JL, Willi GC, Kok FJ, Evan C, Roberfroid M** *et al* . Functional Food Science and Defence against Reactive Oxidative Species. *British Journal of Nutrition*. 1998; 80(1): S77-S112.
9. **Pendry BA**. Analysis of selected Medicinal as Antioxidants with therapeutic Potential for

treating Diseases related to Free Radical Damage. Thesis submitted to school of Health and Social Science Middle Sex University. 2005: p 6, 17- 18, 26.

10. **Kausar F, Amin KMY, Bashir S, Parvez A, Ahmad P.** Concept of *Ihtirāq*in Unani Medicine – A correlation with oxidative stress, and future prospects. *Journal of Ethno Pharmacology*. 2020:Pp 1-12.