Indian Journal of Unani Medicine
Vol. XVII, Issue 2, July-December, 2024, Pp. **97-109**
ISSN No.: **0974-6065**

https://doi.org/10.53390/IJUM.2024.17201

PHARMACOGNOSTICAL STUDY OF *JAR-UL-NAHR* (*POTAMOGETON NATANS* LINN.) (WHOLE PLANT) FOUND IN DAL-LAKE KASHMIR

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Research Paper

Received: **16.08.2024** Revised: **25.08.2024** Accepted: **05.09.2024**

ABSTRACT

Recently, the plant-derived substances have become of great interest due to their versatile applications. Medicinal plants are the richest source of drugs of traditional systems of medicine, modern medicines, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The medicinal value of these plants is due to the presence of bioactive phytochemical constituents that produce specific physiological effects on human body. Since time immemorial plant products have been part of phytomedicines that can be derived from any part of the plant like bark, leaves, flowers, seeds, etc i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is of great importance because such information will be of value for the synthesis of complex chemical substances. This study is aimed to evaluate the physicochemical, macroscopic, microscopic and preliminary phytochemical studies of the whole plant of *Jar-ul-Nahr* (*Potamogeton natans* Linn), belonging to the Potamogetonaceae family. Potamogeton natans, commonly referred to as Floating Pondweed, is a submerged aquatic plant with potential applications in various fields, including medicine. This is a very common pondweed. Large oval floating leaves are softly heart-shaped at base. The plant possesses many important phyto-chemicals like sesquiterpenes, phenolic compounds, essential oils, etc. The plant has anti-inflammatory, antioxidant, antifungal, antitumor, antiseptic, spasmolytic and hemostatic properties. As there is no detailed standardization work reported on whole part of *Potamogeton natans* L., the objective of this study was to work out the physicochemical parameters, macroscopic and microscopic study and preliminary phytochemical constants. The study revealed specific identities for the crude drug which will be useful in identification and to check the adulteration of the raw drug.

No. of Pages: 13 **No. of Fig: 9** **No. of Tables: 10** **References: 23**

Keywords: Styptic, haemostatic, *jar ul nahr,* coagulant.

INTRODUCTION

In todays' era traditional medicines are getting more response and demand day by day, due to the less side effect, cost effectiveness, better efficacy and good faith of society in herbal medicine and their products when compared to the toxicity and

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adverse effects which usually occur due to the allopathic drugs.(Yadav 2008). About 30% of prescribed medicines are prepared directly or indirectly from plants worldwide. (Vimalavady 2012, Anwar 1979,Yadav 2008). Unani system of Medicine, one of the oldest traditional systems has been serving mankind from centuries by alleviating ailments through drugs derived from natural resources like plants, minerals and animals. However, the plant kingdom has been used more often as compared to two other sources. Practically all types of plants have been used for medicinal purposes that include shrubs, herbs, creepers, climbers and even aquatic plants but researchers have given less attention towards aquatic plants (hydrophytes). The pondweed family, Potamogetonaceae, comprises 110 species that are sectioned over six genera with *Potamogeton* as the largest genus (Les D.H. 1990). Several plants from the genus have been utilized in folk medicine. (Les D.H, Shirshova T.I 2012). For instance, in China, *P. natans* L. is utilized in the treatment of the inflammation of the eye lining and in a combination with *P. perfoliatus* L. to cure some skin conditions. Infusions of the leaves of *P. natans* are utilized for stomach cramps and diarrhea, as well as for an antiscorbutic and for wound healing in Arabic medicine (Bhowmik S 2013). In Unani System of Medicine, *Potamogeton natans* Linn which is an aquatic plant, is known by *Jar-ul-Nahr* and is described by various scholars like *Ibn Sīnā* , Ghani, *Ibn Baytār*, Kabiruddin etc. for its haemostatic property, but nowadays this drug is lesser known and under exposed among Unani physicians due to its non-availability or limited accessibility or lack of identification. As this plant is available in various lakes in Kashmir, so its need of an hour to re-introduce the drug and to carry out various pharmacological studies as mentioned in *Unani Tibb*.

Plant material collection

A survey tour to Dal Lake was conducted in December 2021 to collect plant specimen for future reference and making the herbarium for the authentication of plant. The voucher specimen was deposited in the herbarium of survey of Medicinal Plants Unit (SMPU), RRIUM, Srinagar under voucher specimen no.5866-5869. For the collection of flowers another survey tour was conducted in month of august 2022. The collection was done in the same area of Dal Lake where it was done before. The plant was 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.

Physico-Chemical Evaluation

The air dried coarse powdered aerial part of *Jar-ul-nahr* (*Potamogeton Natans* L) was subjected to undergo certain physical and chemical processes to estimate the ash values (total ash, water soluble ash, acid insoluble ash); extractive values (water soluble extractive value, alcohol soluble extractive value) and also moisture content (loss on drying). The procedures were followed as per Indian pharmacopeia, 1996. All the calculated values are presented in Table no.01.

Macroscopical and sensory evaluation:

The detailed macroscopic evaluation is necessary to differentiate between the related species having similar appearance (Anonymous, 1998). The macroscopic evaluation involved the detailed study of the visual appearance and sensory profile of the crude drug sample. The sample of plant was examined by naked eye and other sensory organs for the shape, size, colour, odour, taste, and other external features of the stem, leaves and flowers of *Jar-ul-Nahr* (*Potamogeton natans* L) were evaluated for its size, colour, shape odour, consistency through the naked eyes. (Table 02 Fig 1).

Microscopical evaluation

The whole plant of *Jar-ul-Nahr* (*Potamogeton natans* Linn) were powdered and sliced and then boiled in chloral hydrate solutions for few minutes. A little quantity of powder was taken onto a microscopic slide, evenly spread with the help of brush, stained with phloroglucinol solution and a drop of concentrated HCL, then few drops of glycerine (10%) was added to it. The

slides were covered with a cover slip and observed under microscope for various microscopic characters. (Radhika 2010) (Fig 2).

a) Powder study

The Whole plant of *Jar-ul-nahr* (*Potamogeton Natans* Linn) were powdered separately and both stained and unstained slides were prepared. These slides were then observed under microscope. Fig 3 shows the powder microscopy of *Jar-ul-Nahr* (whole plant) *Potamogeton Natans* L. showing), a. Prismatic calcium oxalate, b. Non lignified isodiametric cells, c. Mesocarp, d. Reticulated vessels (d1), Pitted vessels (d2), e. Thick wall epidermal cells.

b) Transverse section

For microscopic evaluation, free hand sections of the fresh stem, root and leaf of *Jar-ul-Nahr Potamogeton Natans* L. were then cut and cleared with chloral hydrate solution and water. Both stained and unstained sections were prepared. Sections were stained with phloroglucinol and hydrochloric acid in the ratio of 1:1 (Tylor et al, 1981, Brain et al, 1975) Fig 04, 05 and 06 show stained slides of transverse section of stem root and leaf respectively of the plant.

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. After pulverization, the powder was stored in dried and air tight glass containers for the phytochemical investigation. 600g dried coarsely powdered material of whole plant of *Jar-ul-Nahr* was subjected to successive extraction in soxhlet apparatus. Soxhlation was performed at 60^0c using Petroleum ether (40/60) as non-polar solvent at first. Exhausted material was dried and afterward extracted with Ethylacetate, Methanol and Hydro-alcoholic in increasing order of polarity. In each solvent, Soxhlation was continued until no color 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, and their extractive values were calculated. (Chaudhari 1996).

Preliminary Phytochemical investigation of the extracts:

- The petroleum Ether, ethyl acetate, Methanol and Hydro alcoholic extract of whole plant of *Jar-ul-Nahr* were subjected to phytochemical screening to identify constituents like alkaloids, glycosides, tannins, phenols, carbohydrates, flavonoids, Proteins, Saponins and Sterols present in the plant. Table 03. (Gokhale SB 1994, Harborne JB 1995, Shanmugam S 1990, Anonymous. 1998, Pawar 2014).

Thin layer chromatography (TLC)

Jar-ul-nahr (*Potamogeton natans* L) was subjected to TLC profiling. Suitable solvent system was developed to act as mobile phase for these extract solutions. For Pet ether extract of *Jar-ul-nahr (Potamogeton natans* L), the solvent system developed was Petroleum Benzene and Ethylacetate in the ratio of 8:2. For Ethyl acetate extract of the plant, 2 solvent systems were used as Petroleum ether and Ethylacetate in the ratio of4.5:5.5 and Petroleum benzene: Ethylacetae in the ratio of 7:3 that was developed was 25% ethyl acetate in 75% of Pet ether. For Methanolic extracts of *Jar-ul-nahr* (*Potamogeton natans* L) the solvent system developed was Toluene: Ethylacetate: Formicacid: Methanol (1:7:2:1).The retention factor (Rf) values were correspondingly calculated and showed in the Table no. 04.

Results:

Table 1: Physicochemical Standard of different extractive values of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* L.).

Sl. No	PARAMETERS	VALUE
1.	**Ash value**	
	Total ash value	9.4%
	Water soluble ash value	1.6%
	Acid insoluble ash value	5%
2.	**Loss on drying**	8.8%
3.	**Extractive values**	
	Pet Ether	0.64%
	Ethyl acetate	0.68%
	Methanol	1.86%
	Hydro alcohol	4.2%
4.	**Hot extractive value**	
	Ethanol	5
	Aqueous	15.2
5.	**Cold Extractive value**	
	Ethanol	4.2
	Aqueous	9.6

Table 2: Macroscopic characteristics of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* Linn).

Plant Part	Odour	Colour	Taste	Shape
Stem	Fishy when fresh. On drying smells characteristic	Dark green, leathery opaque with translucent longitudinal veins.	Tasteless in beginning and astringent later	Cylindrical, without many branches, and grows from 1 to 2 meters
Leaves	Fishy when fresh. On drying smells characteristic.	Green coloured in early stage and later dark brown coloured.	Tasteless	4-11 x 2- 4.5 (Length x width) Both submerged and floating more or less spirally arranged. Floating leaves are firm, oval-elliptic to egg shaped with 17-37 veins flanking mid-rib.
Flower	Fishy when fresh. On drying smells characteristic.	Green colour in early stage and dull yellow in later stage.	Tasteless	Flower spikes are dense and cylindrical, 5 – 10 cm long pointed at tip rounded at base. Flowers from May to September.

Fig 1: A Image of Potamogeton natans in natural source, b. When taken out of water, c. After multiple washing and d. Herbarium.

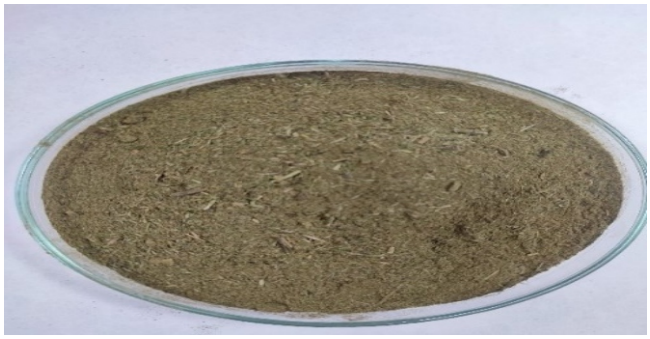

Fig 2: Powder form of Jar-ul-Nahr (Whole Plant)

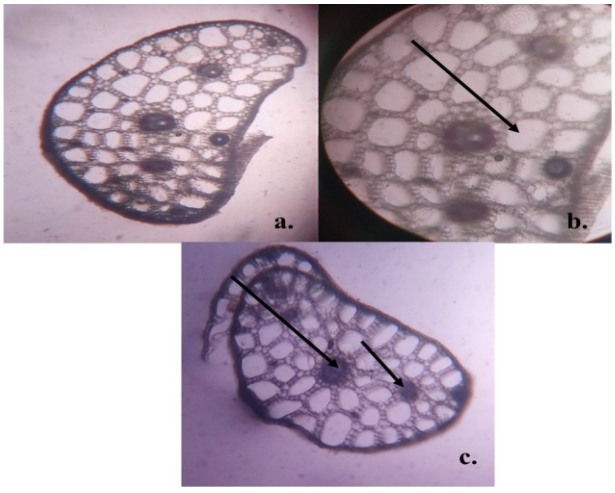

Fig 2: Transverse section of stem a. Showing epidermis with chloroplast, b. Showing aerenchyma and and c. Shows vascular bundles.

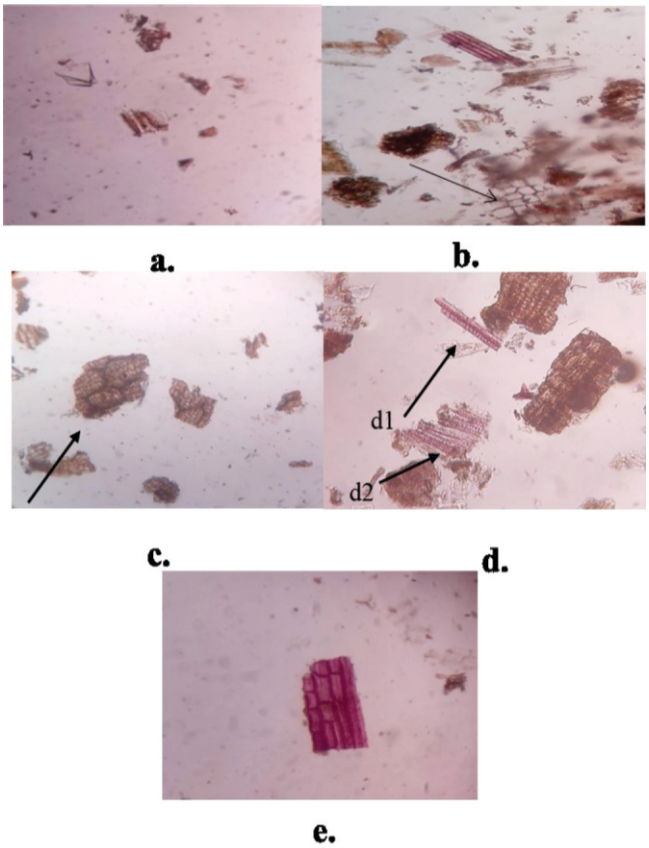

Fig.3: Powder microscopy of Jar-ul-Nahr (whole plant), a. Prismatic calcium oxalate, b. Non lignified isodiametric cells, c. Mesocarp, d. Reticulated vessels (d1), Pitted vessels (d2), e. Thick wall epidermal cells.

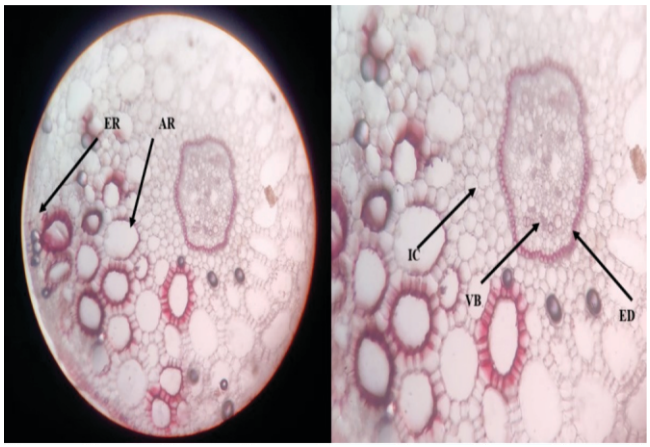

Fig. 5: Transverses section of root showing epidermis (ER), Aerenchyma (AR), Inner cortex (IC), Single layer endodermis (ED) and vascular bundle (VB).

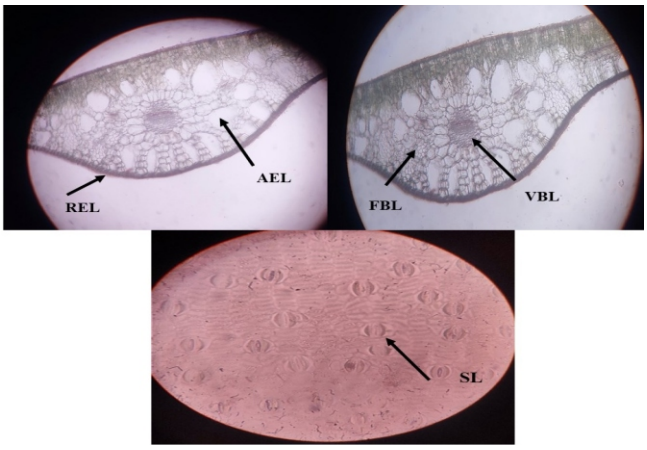

Fig. 6: Transverse section of leaf showing rectangular epidermis (REL), Aerenchyma (AEL), Vascular bundle (VBL), Fibre bundle (FBL) and SL showing stomata that are present on upper surface of leaf and are absent on lower surface.

Table 3: Phytochemical screening of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* L.).

S.No	TESTS	Petroleum ether	Ethyl acetate	Methanolic	Hydro alcoholic
1.	**CARBOHYDRATES**				
A	Molisich's test	-	-	+	+
b.	Fehling's test	-	-	-	+
c.	Benedict's test	-	-	-	+
2.	**TANNINS**				
a.	5%FeCl3	-	+	-	+
b.	Lead acetate	-	-	-	+
3.	**FLAVONOIDS**				
a.	Shinoda test	-	-	+	+
4	**PHENOLICS**				
a.	1%FeCl3	_	_	+	+
5.	**ANTHRAQUINONE GLYCOSIDES**				
a.	Borntrager's test	-	-	+	+
6.	**CARDIAC GLYCOSIDES**				
a.	Keller killiani Test	-	+	+	+
b.	Legal test	-	-	-	-
7.	**TERPENOIDS**				
a.	Salkowski's test	+	-	-	-
8.	**PHYTOSTEROLS**				
a.	Libermann's test	+	+	-	-
b.	Salkowski test	+	-	-	-
9.	**ALKALOIDS**				
a.	Dragendroff's reagent	-	-	-	-
b.	Mayer's reagent	-	+	-	-
C	Wagers test	-	+	-	+
d.	Hagers test	-	-	-	-
10.	**PROTEINS**				
a.	Millon's test	-	+	+	-
b.	Biuret test	-	-	-	-

Table 04: TLC profile of different extracts of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* L.) along with R_f values.

Extracts	Solvent system	No. of spots	Rf values
Ethyl acetate	Petroleum ether: Ethylacetate (4.5:5.5)	4	0.71; 0.76; 0.82;0.91,
	Petroleum Benzene: Ethyl acetate (7:3)	4	0.15; 0.49; 0.62; 0.90
Methanol	Toluene: Ethylacetate: Formicacid: Methanol (1:7:2:1)	3	0.14; 0.31;0.75
Petroleum ether	Petroleum Benzene: Ethyl acetate (8:2)	8	0.09; 0.19; 0.24; 0.29; 0.31; 0.41; 0.58; 0.92

Table 05 : pH values of whole plant of Jar-ul-Nahr (*Potamogeton natans* L.)

pH values		
Sample	**pH**	**Temp**
pH of 1 % solution	5.5	20^0C
pH of 10 % solution	5.6	20^0C

Table 06 :: Powdered drug reaction with different chemical reagents of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* L.)

Treatments	Observations
Picric acid	Light Green
Conc. Hcl	Green
Conc.H_2SO_4	Dark Green
Conc.HNO_3	Light Brown
Iodine solution	Brown
Ferric chloride	Green

Table 07: Swelling index of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* L.)

Swelling index		
Part used		**Swelling index**
Whole plant		Nil

Table 08: Foaming index of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* L.)

Foaming index		
Part used		**Foaming index**
Whole plant		Nil

Table 9: Determination of Heavy metals of whole plant of *Jar-ul-Nahr*.

S. No.	Parameters	Results	Permissible limits as per ASU Pharmacopoeia's	Inference
1.	Lead	0.0000 mg/L	10 ppm	Within the permissible limits
2.	Cadmium	0.0137 mg/L	0.3 ppm	Within the permissible limits
3.	Mercury	0.8535 mg/L	1.0 ppm	Within the permissible limits

Table 10: Fluorescence Analysis of whole plant of Jar-ul-Nahr (*Potamogeton natans* L.)

Sl. No.	Treatment	Day Light	UV(254nm)	UV(366n)
1.	Powder as such	Green	Green	Green
2.	Powder treated with distilled water	Green	Dark Green	Black
3.	Powder treated with GAA	Golden	Dark Green	Light Red
4.	Powder treated with conc. HCl	Green	Dark Green	Dark Brown
5.	Powder treated with conc. HCl + H_20	Golden	Light Green	Black
6.	Powder treated with Pet. Ether	Light Green	Light Green	Light Green
7.	Powder treated with 5% Iodine	Brown	Green	Black
8.	Powder treated with methanol	Greenish	Greenish	Dark Orange
9.	Powder treated with ethylacetate	Light Green	Green	Orange
10.	Powder treated with conc. H^2SO^4	Dark Green	Dark Green	Parrot Green
11.	Powder treated with conc. H_2SO_4 +H_2O	Brown	Green	Black
12.	Powder treated with picric acid	Light Green	Dark Green	Black
13.	Powder treated with 5% $FeCl_3$	Green	Dark Green	Black
14.	Powder treated with chloroform	Brownish	Light Green	Black
15.	Powder treated with HNO_3	Light Brown	Olive Green	Black
16.	Powder treated with HNO_3+H_2O	Brown	Green	Black
17.	Powder treated with Acetone	Greenish	Greenish	Orange
18.	Powder treated with Benzene	Light Green	Light Green	Light Green
19.	Powder treated with ccl4	Green	Dark Green	Black

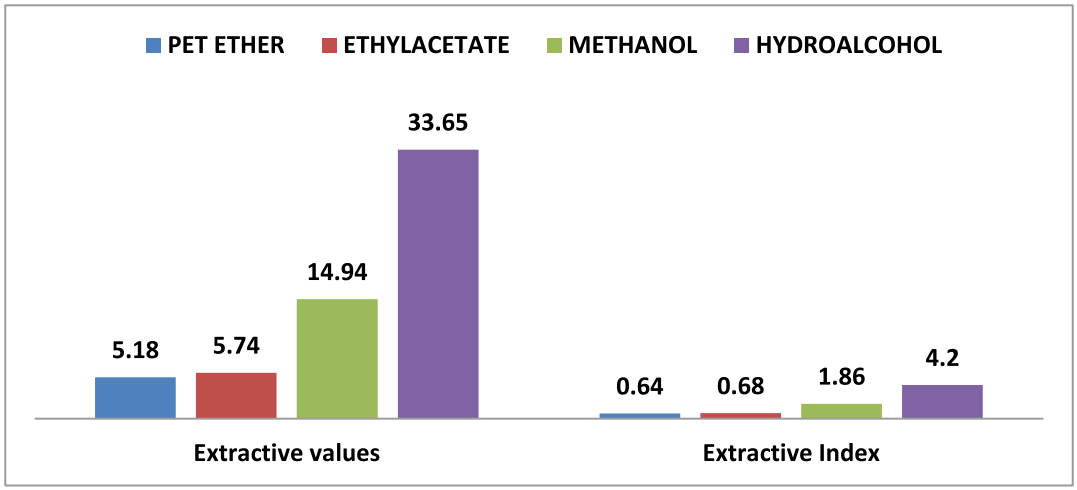

Fig. 7: Comparative extractive values of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* L.)

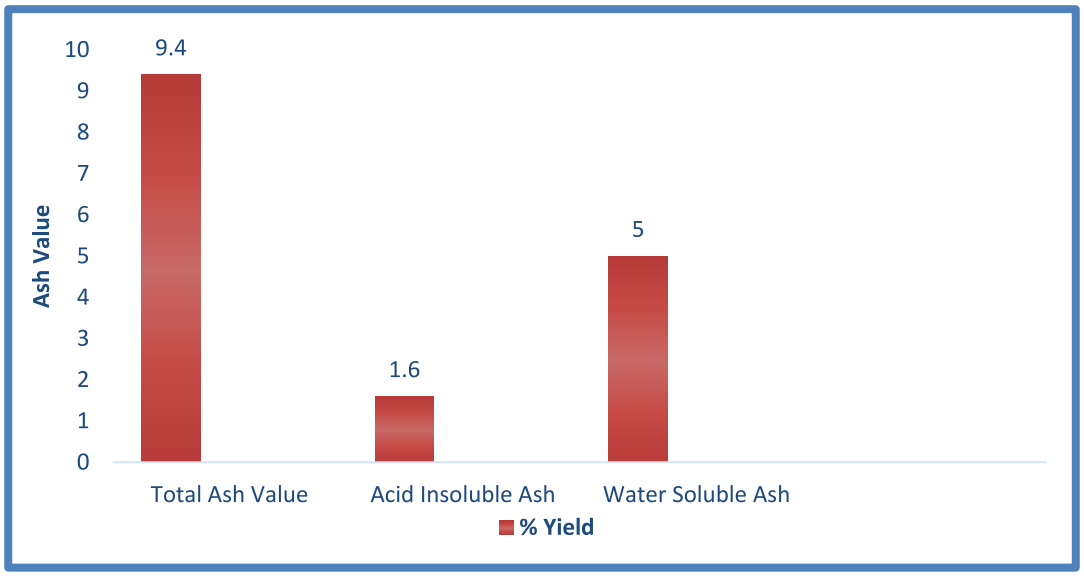

Fig. 8: Graphical representation of whole plant of ash values of *Jar-ul-Nahr* (*Potamogeton natans* L.)

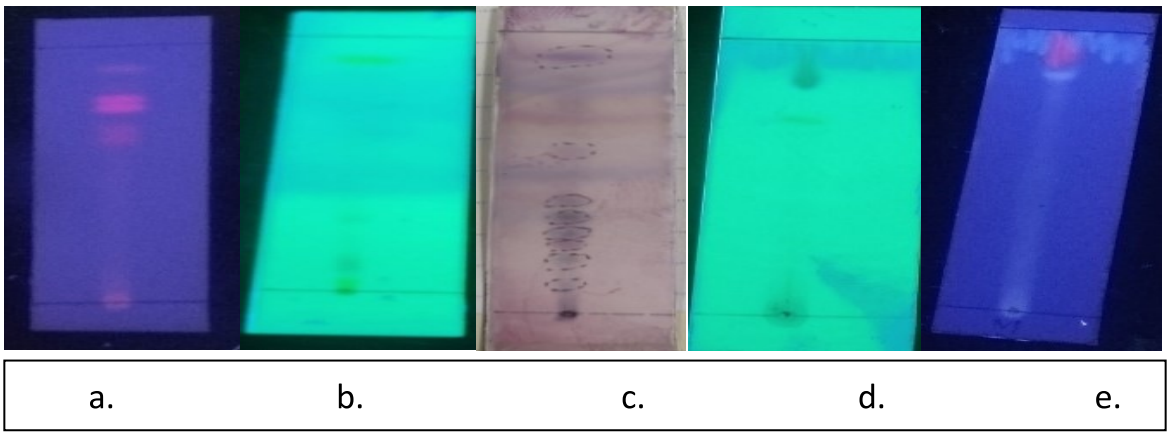

Fig. 9: Showing some plates of TLC a) Petroleum Benzene: Ethyl acetate (7:3) of ethyl acetate b & c) Petroleum Benzene: .Ethyl acetate (8:2) of petroleum ether. d) & e) Toluene: Ethyl acetate: Formic acid: Methanol (1:7:2:1) of Methanol.

Discussion and Conclusion:

Due to the long historical practice and less toxicity of Unani medicines they are gaining more and more attention all over the world for maintaining human heath quality. To satisfy this necessity, the herbal plant materials are interchanged by some unauthentic substituents or adulterants material and also the proper guidelines are not followed for the preparation of formulation etc. So, the safety and quality of crude medicinal drugs as well as of finished herbal products have become a chief concern for health authorities, pharmaceuticals and the public. To stop this adulteration, unethical practice, some sort of uniformity in the manufacture of Unani medicine, the proper standardization process to be needed. (Jitubhai 2011) The present work demonstrates the pharmacognostical evaluation of whole parts of *Jar-ul-Nahr* which will help in proper identification of this plant for future investigations.

The macroscopic examination of drugs refers to evaluation of drugs by color, odor, taste, size, shape and special features, like touch, texture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Organoleptic evaluation means conclusions drawn from studies resulting due to impression on organs of senses. All these parameters were recorded for the whole parts of *Jar-ul-Nahr* (*Potamogeton natans* Linn).

The physicochemical study of the drugs includes ash value, extractive values, loss of weight on drying, thin layer chromatography (TLC), pH values, swelling index, foaming index and fluorescence analysis. The results are mentioned in tables as 01, 04, 05, 07, 08, & 10.The extractive values are the parameter for detecting the adulteration in any drug. For establishing the standard of any kind of drug the extractive value plays a major role. To obtain accurate results, the dug should be extracted with various solvents in order of increasing polarity. In this study, the weight of successive extracts in various solvent of whole plant of *Jar-ul-Nahr* were found to be 5.18; 5.47; 14.94 and 33.65 in petroleum ether; ethyl acetate; methanol and hydro alcoholic extract respectively as shown in Fig. 7. Fixed oils, fats, resin and volatile substances are present in petroleum ether extract. The percentage of successive extracts were 0.64%; 0.68%; 1.86% and 4.2% for petroleum ether; ethyl acetate; methanol and hydro alcoholic extract respectively as shown in Table 01. Clearly, the extractive values are on higher side in hydro alcoholic solvent followed by methanolic solvent and are almost equal in petroleum ether and ethyl acetate solvents as shown in Table 01 & Fig. 07. Percentage yield of petroleum ether was found to be least and maximum for hydro alcoholic. This suggests presence of polar constituents out number presence of non-polar constituents from whole plant of *Jar-ul-Nahr.*

Ash value is a residue that is left over after complete incineration of the drugs. Ash value plays a significant role in ascertaining the standard of a drug, because the dust, earthy and unrequired matter are generally added to increase the weight of drug ultimately increasing ash percentage. Therefore, determination of ash value provides the basis for judging the identity and cleanliness of a drug and provides details with regards to its adulteration with inorganic matter. The percentage of total ash, acid insoluble ash and water soluble ash of whole plant of *Jar-ul-Nahr* were found to be 9.4%, 1.6% and 5% respectively (Table 01, Fig. 08). The percentage of solubility of powder drug is also considered as an index of purity. Almost all substances including resin, glycosides and alkaloids etc. can dissolve in alcohol. With respect to soluble extractives percentage of alcohol varies, whereas the drugs obtained from the sources may produce different extractive values, extracted with the same concentration of alcohol (Afaq 1994). Water-soluble constituents like glycosides, mucilage, tannins etc., are determined by water soluble extractive whereas drugs containing tannins, glycosides, resins, etc., are determined by alcohol-soluble extractive and drugs containing volatile constituents and fats are determined by ether soluble extractives.

(Parasuraman S 2014). Percentage of water and alcohol soluble matters in whole plant of *Jar-ul-Nahr* was found to be 5% w/w and 15.2% w/w by hot method; 4.2% w/w and 9.6% w/w by cold method respectively. (Table 01).

The percentage of weight loss on drying at 105^{0}C indicates the loss of water along with volatile substance, which is determined by subtracting weight of drug on drying from weight of the original drug. The loss on drying of whole plant of *Jar-ul-Nahr* was found to be 8.8 %.That is within acceptable limit. The percentage of moisture content ranging from 10-20% indicates a suitable range for minimum bacteria well as fungal growth. (Table 01).

The pH values of 1% and 10% solution of whole plant of *Jar-ul-Nahr* (*Potamogeton natans* L) was found to be 5.5 and 5.6 respectively and indicates the presence of slight acidic nature as shown in Table 05.Since the pH of the drug is acidic, it suggests that the drug will show better absorption in the stomach. (Ali W 2016) The foaming and swelling ability in an aqueous decoction of herb is measured in terms of foaming index and swelling index respectively. In this study, the foaming and swelling index was found to be zero as mentioned in Table 08 and 07.

Sometimes the fluorescence analysis of drug under UV light is very characteristic. When drugs are exposed to ultraviolet radiations many drugs and their constituents emit specific colour because the radiant energy excites the solution which emits that particular colour, known as fluorescence. Fluorescence analysis can provide identification marker for identification of the particular herb. Therefore, fluorescence analysis of the powdered drugs treated with different chemical reagents was done and changes in the color observed can be seen in Table 10. The preliminary phytochemical tests for qualitative assessment for carbohydrates, alkaloids, flavonoids, tannins, phenolic compounds, and terpenoids in the petroleum ether, ethyl acetate, methanolic and hydro alcoholic extracts of whole plant of *Jar-ul-Nahr* was carried out. In results, alkaloids and tannins were present in ethyl acetate and hydro alcoholic extract. Anthraquinone glycosides and flavonoids were present in methanolic and hydro alcoholic extract and cardiac glycosides were present in 3 extracts viz; ethyl acetate, methanolic and hydro alcoholic extract. Tannins and anthraquinones are the major phytoconstituent present in this plant which may be responsible for wound healing action. (Abu-Al-Basal M.2001).Steroids were present in petroleum ether and ethyl acetate extract proteins were present in ethyl acetate and methanolic extracts. Tannins and flavonoids are secondary metabolites to which hemostatic activity can be attributed.

Further an attempt has been made to separate the individual chemical constituents of petroleum ether, ethyl acetate, methanolic and hydro alcoholic extracts by TLC profiling as shown in Fig. 09. A no. of solvent systems of low to high polarity was tried on TLC profiling.The extracts were dissolved in corresponding solvents and were then applied on TLC plates by using suitable capillary tubes. Appropriate solvent system was developed to act as mobile phase for these extracts solutions. The petroleum ether extract of *Potamogetonnatans* L. was applied on TLC plate by using suitable capillary tube and developed TLC plate which swas then separately placed in TLC chamber for development using solvent system as petroleum benzene: ethyl acetate. The developed TLC plate was air dried and then viewed in UV chamber. 8 spots were visible on TLC plate at 365 nm and day light. Similarly, the ethyl acetate extract of *Potamogeton natans* L. was treated in different solvent system. The result was obtained when extract applied on TLC plate, was placed separately in solvent system made of i), pet ether: ethyl acetate (4.5:5.5) and ii) petroleum benzene: ethyl acetate (7:3). The developed TLC plate was air dried and then viewed in UV chamber. 4 spots were visible on TLC plate at 265nm and 365 nm respectively, in solvent system. In case of methanolic extract 3 spots were visible on TLC plate at 365 nm and 254 nm using solvent system made of toluene: ethyl acetate: formic acid: methanol (1:7:2:1). Spots on

above mentioned TLC plates were identified and Retention factor (R_f) was calculated by following method. (Pawar 2014)

$$R_f = \frac{\text{Distance travelled by Solute}}{\text{Distance travelled by Solvent}}$$

Contamination of medicinal plants with heavy metals is a big concern for health. It is vital to analyze heavy metals in both source materials and end products in order to assure that the levels of heavy metals do not exceed the essential limits as established by regulations(Yuan 2011) Heavy metal contaminations are usually caused by environmental pollutants and use of pesticides. The analysis for heavy metals in the drug sample showed that the values for Lead, Cadmuim, and Mercury were found to be mg/ L, 0.0000 mg/ L, 0.0137 mg/L, and 0.8535 mg/L, respectively (Table 9), and were under the permissible limits as per ASU Pharmacopoeias (Bijauliya 2017)

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