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Research Article

IN -VITRO ANTI OXIDANT PROPERTIES OF TRADITIONAL POLY HERBAL HEPATOPROTECTIVE FORMULATION *Jawarish E Utraj*

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ABSTRACT

Since ages humans have been dependent on plants as one of their primary source of food for survival. Plants are not only responsible for providing food but also they have many unexplored medicinal properties, humans have been able to harness only a very less proportion of its potentials to cure many deadly disease.

Aim: In the present research work an attempt has been made to establish the Physicochemical, Phytochemical and Anti oxidant property of JEU which may be help full for its standardization
Methods: Standardized operating procedure for the preparation of JEU was developed in accordance with National Formulary of Unani Medicine. The formulation was also subjected to preliminary phytochemical, physicochemical evaluation and was tested for its antioxidant activity by using DPPH method and NO Scavenging activity

Results: The IC₅₀ Value of the extract in DPPH assay was found to be 49.60 and 45.44 for NO assay. Furthermore to characterize and identify the major constituents preliminary phyto chemical tests were carried out

Conclusion: The present results demonstrated that JEU could be a promising candidate for developing hepatoprotective agent which helps further to combat Liver disorders with an effective treatment strategy

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INTRODUCTION

The Unani System of Medicine is a medical system that deals with the management of health and diseases. It provides preventive, promotive, curative and rehabilitative healthcare with holistic approach (1). The fundamental framework of this system is based on deep philosophical insights and scientific principles, including the Empedoclean theory of four Elements i.e. Air, Water, Fire and Earth; four proximate Qualities (Kayfiyāt) i.e. Hot, Cold, Wet and Dry described by Pythagoras, and the Hippocratic theory of four Humours (Akhlā) - Blood (Dam), Phlegm (Balgham), Yellow Bile (Ṣafṛā') and Black Bile (Sawdā'). Any disturbance in the equilibrium of humours causes disease, and therefore the treatment aims at restoring the equilibrium by giving factors (including drugs) of opposite temperament. Liver (Kabid) is also an organ for production of humours (Akhlāt) for nourishment, growth and development of the human body. Each of the four humours named dam (sanguine), balgam (Phlegm), safra (Yellow bile) and sauda (Black bile) carries their own normal temperaments (2). The derangement in quality and quantity of humours, leads to liver pathologies. The

liver is an organ possessing an amazing restorative capacity and huge internal reserves, enough to maintain life is 1/7 of its mass. The liver can work for a long time with increased strain, as hepatocytes are restored, but everything has a limit. And when a person (especially from an early age) loads the liver with excess of fats, carbohydrates, flavor enhancers, preservatives, food additives, vegetable fats (palm, coconut oil) - the liver does not withstand colossal toxic load and its diseases occur (3). The main cause of this disease in men (70%) is alcohol, such hepatosis is called alcoholic fatty hepatosis. Jawarish is a polyherbal or single drug formulation which comes under the class of majoon. Majoon is a semi solid medicinal preparation where one or more single drugs of plant, animal or mineral origin are mixed in power or liquid forms in the base (Qiwam) made of purified honey, sugar, candy or jaggery (4). These include preparations like jawarish, Itrifal, Barshasha, Dawa-ul-misk, Mufarrehat, Luboob, Khamira and Laooq. etc. Now coming to jawarish, It is an Arabic word derived from Gawarish means digestive. jawarish is a type of majoon which somehow tastes better than majoon, It is semisolid preparation whose Powder of is coarse than powder of Majun. Persians introduced it. Its consistency is more solid

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than Majun as it may break if dried.. In this formulation, 10 ingredients are present which are as follows: Muraba-e-turanj - Citrus medica ,Zanja beel-Zingiber officinale, Filfil daraz-Piper longum, Darchini-Cinamomum zeylanicum, Mastagi-Pistacia lentiscus, Bisbasa-Myristica fragrans, Barg-e-tambol-Piper bettle, Qaranful-Syzgium aromaticum, Ood hindi-Aquilaria agallocha, Sumbal-ut-teeb- Nardostachys jatamansi(5)

MATERIAL AND METHODS

Plant Collection and Identification

The raw materials for the formulation were collected from the local markets and herbal drug dealers of Bangalore Karnataka, India. The various plant parts i.e. leaves; seeds were thoroughly washed in cold water to remove the earthy material. in the month of December 2018. The collected plant material were authenticated by Dr. N.Ayyappan, Researcher, French Institute of Pondicherry

Preparation of Jawarish e utraj

In National Formulary of Unani Medicine (NFUM) part II Vol 1 the general method of preparation for jawarish has been mentioned In-house Preparation of *Jawarish e utraj* consists of following steps.

For making majoon or any of its allied preparations, Qiwan (base) of different consistencies (tar) is generally made, depending on the nature of ingredient drugs to be used in a particular formula. The ingredient drugs in a Qiwan may be used either in powder or liquid form. The Qiwan (base) is generally made by adding Aab (water), Araq (distillate) or Aab-e-Samar (fruit juice), etc., in any of the bases of purified Honey with Sugar, Candy or Jaggery etc., and boiled over a low fire till it acquires a required consistency. The bases are generally purified by adding Aabe-Leemu (Lemon juice), Satt-e-Leemu (Lemon extract) or Shubb-e-Yamani (Alum) etc., before making the Qiwan. Afterwards, the ingredient drugs are mixed in Qiwan to prepare Jawarish, Majoon, Itrifal, Halwa and Dawa. For making Majoon or any of its preparations the consistency of Qiwan of Majoon is Three Tar Indication Its consistency is more solid than Majun as it may break if dried. Dose 5 to 10 gms Storage: Should be stored in air tight containers and should be kept away for sunlight (6).

Qualitative Evaluation of Phytochemicals

The morphological and microscopical examinations have been performed using reference book. The ash value, extractive value and preliminary phytochemical screenings were done using standard protocol to assess its quality and efficacy (7-8).

Investigation of Antioxidant Activity

Determination of antioxidant activity (Scavenging Activity of DPPH Radical) (9)

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple colour typical for free DPPH radical decays, and the absorbance change at $\lambda = 517$ nm is measured. This test provides information on the ability of a compound to donate a

hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. The method was carried out as previously described by Brand *et al* 1995. The methanolic extracts were redissolved in methanol and 5% ethanol, respectively, and various concentrations (10, 50, 100, 500 and 1000 $\mu\text{g/ml}$) of each extract were used. Similar concentrations of ascorbic acid were used as positive control. The assay mixture contained in a total volume of 1 ml, 500 μl of the extract, 125 μl prepared DPPH (1 mM in methanol) and 375 μl solvent (methanol or 5% ethanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at $\lambda = 517$ nm. The radical scavenging activity was calculated from the equation:

$$\% \text{ of radical scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Nitric Oxide Scavenging Activity (10)

The method of Garratt was adopted to determine the nitric oxide radical scavenging activity. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide which interacts with oxygen to produce nitrite ions determined by the use of Griess reagents. Two millilitre of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of plant extract at various concentrations (0.1-1.0 mg/ml). The mixture was incubated at 25°C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm. The amount of nitric oxide radical was calculated following this equation:

$$\% \text{ inhibition of NO} = \frac{[A_0 - A_1]}{A_0} \times 100$$

Where A_0 is the absorbance before reaction and A_1 is the absorbance after reaction has taken place.

RESULTS AND DISCUSSION

Total polyphenolic content and flavonoid content The mean total polyphenolic content of the decoction was 80.45 ± 0.60 mg gallic acid equivalents/g extract respectively. The mean flavonoid content present in the decoction was 58.60 ± 0.70 mg quercetin acid equivalents/g extract respectively.

Physicochemical parameters Physicochemical parameters like foreign matter, percentage of moisture content, total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive and water soluble extractive were determined and depicted in Table no 1 and Table no 2.

Anti Oxidant activity of Jawarish e utraj

The results obtained for antioxidant study for Jawarish e utraj by 1.DPPH scavenging activity and 2.Nitric oxide scavenging activity are reported in Table no 3 and 4 respectively

DISCUSSION

Reactive oxidants produced in biological systems, either by normal metabolic pathways or as a consequence of exposure to external agents have been associated with many different disease conditions such as cancer Alzheimer's disease diabetes

and Liver damage. In the present study, an attempt has been made to evaluate the antioxidant activity of the decoction by use of in vitro methods. The overall results of this investigation demonstrate that the decoction can exert significant antioxidant activity as evident from their ability to (a) scavenge free radicals such as DPPH and Nitric Oxide (b) Presence of high content of poly phenols and flavonoids. Phytochemical screening of Jawarish e utraj revealed the presence of alkaloids, saponins, tannins, flavonoids and other phenolic compounds. The quantitative determination of phytochemicals also revealed that comparatively high amount of polyphenols and flavonoids are present in the decoction. Polyphenols are considered to be the major antioxidant compounds in plants, although they are not the only ones. The antioxidant activity of phenolic acids and flavanoids are reported to be mainly due to their redox properties which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Therefore, phenolic acids and flavanoids contained in this traditional decoction may play a major role in the observed antioxidant activities. Recently, much attention has also been focused on the role of oxidative stress in diabetes, and it has been suggested that oxidative stress may mediate the key and common events in the pathogenesis of different Liver complications. Therefore, ability of the Jawarish e utraj to inhibit generation of free radicals may also assist this plant to mediate its hepatoprotective activity.

CONCLUSION

The present research work involved in the preliminary phytochemical evaluation and in vitro antioxidants activity of a, traditional, polyherbal hepatoprotective Unani formulation-Jawarish e utraj. The data obtained from the present findings clearly demonstrates that the Jawarish e utraj has excellent anti-oxidant properties which may be due to the presence of phytochemicals present in the formulation such as polyphenols and flavonoids. And the current research also paves a pathway to conclude the hepatoprotective potential of Jawarish e utraj may act through free radical scavenging properties.

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Conflict of Interest

None

Table 1 Phytochemical evaluation of extract of Jawarish e Utraj

Constituents	Presence or absence
Tannins	+
Steroidal glycosides	+
Flavanoids	+
Alkaloids	+
Saponin	+
Anthraquinone glycosides	+
Terpenoids	+
Proteins	-
Carbohydrates	+
Volatile oils	+
Fixed oils	+

(+) indicates presences (-) indicates absence

Table 2 Physicochemical Properties of *Jawaris e utraj*

Parameters	Values obtained %w/w on dry weight basis
Foreign matter	NIL
Loss on drying	2.26
Total ash	12.08
Acid soluble ash	7.03
Alcohol soluble extractive value	15.34
Water soluble extractive value	2.5

Table 3 (1). DPPH Scavenging activity of alcohol extract *JeU*

Sample	IC ₅₀ Value
<i>Jawarish e Utraj</i>	49.60±0.01
Standard (Ascorbic acid)	49.58±0.08

Values are expressed as mean ± S.E.M., n=3

IC50 value of the decoction was comparable (P > 0.02) to that of Ascorbic Acid

Table 4 (2). Nitric oxide Scavenging activity of alcohol extract *JeU*

Sample	IC ₅₀ Value
<i>Jawarish e Utraj</i>	45.44±0.12
Standard (Griess reagent)	45.41±0.22

Values are expressed as mean ± S.E.M., n=3

IC50 value of the decoction was comparable (P > 0.05) to that of Griess reagent

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