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Research Article

PHYSICOCHEMICAL AND PHYTOCHEMICAL STANDARDIZATION OF IRSA (Iris ensata Thunb.)

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ABSTRACT

Unani Medicine possesses a large number of Drugs used in upper respiratory tract infectious (URTI) diseases as mentioned by eminent Unani Physicians based on their own long term experience and good results. A doubt always remains in mind regarding the standardization of Unani drugs and scientific validation for their use in infectious diseases. It provides the analytical characteristics which may prove to be useful in fixing the physicochemical standard for the Unani herbal drugs. Physicochemical and Phytochemical Standardization is considered a prerequisite for the assessment of biological activity or determination of biological standards of the plant material. So, a study was designed to standardize herbal drug –Irsa

Irsa (Iris ensata) belong to the family Iridaceae, its root is used in respiratory diseases such as asthma, cough, diphtheria and pneumonia. An effort has been made to carry out the physicochemical and phytochemical studies of plant.

I. ensata was standardized on physicochemical parameters as Extractive Values: pet. Ether (2.9%), di-ethyl ether (4.58%), chloroform (2.20%), acetone (3.54%), alcoholic (10.03%), aqueous (14.13%); Solubility: Water (9.44 %) & Alcohol (1.16 %); Moisture contents (3.45 %), Total Ash values (6.93%), pH of 1% (6.76) & 10% solution (6.16) and loss on drying (5.3%). Phytochemical Analysis revealed the presence of almost all the phyto-constituents in the test drug sample i.e. alkaloid, flavonoids, glycoside, carbohydrate, tannin, protein, amino acids, starch and resins.

Keywords: Irsa, Physicochemical, Phytochemical, Standardization.

INTRODUCTION

Irsa (Iris ensata) belong to the family Iridaceae, use of the drug 'Irsa' in Unani system of medicine dates back about a couple of thousands of years. It was mentioned first in kitabul Hashaish by Dioscorides (1st C.B.C.) It is also mentioned by Theophrastus and was not disregarded through the Arabic, Persian and Urdu authors in their books. It was particularly mentioned by Razi (926 A.D.) Ibne Sina (1037 A.D.) Al-Harwi (10th C.A.D). Irsa means the Rainbow, it is a Greek name as proved and was named after the especial characteristic of its flowers. About a dozen species occur in India and a few exotics are cultivated for ornament Irsa is used for cure of the respiratory diseases (Iqbal, 1984). The root of Irsa dried, dark brown small pieces of different shapes but usually they are elongated having transverse wrinkles. The inner surface is light brown. The fracture is hard and fibrous. The odour is pungent and the taste is slightly bitter and aromatic.

The transverse section of root shows the single layer of epidermis which consists of a typical and parenchymatous with thickened outer wall. The cortical region usually made-up of several layers of rectangular to oval parenchymatous cells. Most of these cells studied to posses' oil globules with other yellowish, brown contents and they also give the positive test for tannins with aqueous ferric chloride. The endodermis is found to attach with 4-5 layer highly thick walled cells which are polygonal to oval in shape and they are present in somewhat compact masses. There is no cortical

vascular bundle but vascular bundles are numerous and closely scattered in the pith, internal to the endodermis. Vascular bundles are more or less roundish in shape of tapering to one side. Each vascular bundle consist of phloem and scattered xylem elements which are enclosed by lignified fibrous sheath of 1-3 layers of cells. The parenchymatous cells of pith are thin walled compact and polygonal to oval in shape (Anonymous, 1997).

MEDICINAL PROPERTIES

Irsa is the supreme one among its comrade drugs used in respiratory ailments. It has been praised by the physicians of all times as a "panacea' and is broadly used in a huge number of disases.

Dioscorides, cited by Razi, it heals up the chronic ulcers and absess, useful in insect bite, burns, vitiligo, weakness of muscles, itching and dandruff. It is emmenagogue, anti inflammatory, anti orchitis, used in cold cough, in all humours, pneumonia, dyspnoea. The egesta accumulated in the chest are attenuated and resolved by Irsa, it is emetic and purgative.

Razi quotes Galen (199 A.D.) in his book "the container" (Al-Hawi) that it has emmenagogue, anti tussive, anti epileptic, astringent, regenerative and anti pleuritic actions, it is use ful in pneumonia, pneumothorax, eclampsia, palpitation, chill, liver pain and spermatorrhoes. Ibn-e-Masewaih describes the white sosan as hot phlegmic pains of nerves and uterus are cured, Maseeh reported its efficacy in headache, the poultice for ulcers and wounds, vitiligo itch, baldness, nerve injuries and redness of skin (Kiritikar, 1996; Ghani, 1921; Ibne Baitar, 1985). Ibne Sina recommends its efficacious actions in strengthening the wisdom and intellect, cures head injuries skull bone fractures, phlegmic and hot swelling. Ibne Baitar quoted that it is useful in tooth ache, dyspnoea, spleenomegaly, rigidity of uterus, flatulence, it is abortifacient, anti tussive (Nadkarni, 1989). The fermentation with sosan is benifecial in endometritis, adenitis and hard swelling. Antaki (1597 A.D.) states its efficacy in dyspnoea, asthma, thorasic pain, hydropes, jaundice, haemorrhoids, liver complaints, and sciatica. Its clyster is employed in all aforesaid disorders. Dymock (1890) reported that a century back it is used in asthma, cough, fever, dyspnoea and skin diseases.

No work has been reported regarding standardization of this drug so far. Keeping in mind the medicinal importance of this plant in Indian System of Medicine, a physic-chemical and phytochemical study of Irsa was carried out following various parameters.

MATERIALS AND METHOD

The drug samples of Irsa were collected from Dawakhana Tibbiya College A.M.U. Aligarh and was properly identified by botanical literature survey and then confirmed by Prof. S. H. Afaq (Pharmacognosy section - Dept. of Ilmul Advia). A herbarium sample was prepared and submitted in the museum of the Department of Ilmul Advia Faculty of Unani Medicine, AMU, Aligarh (Voucher No. SC-0144/14) for the future reference.

Irsa (*Iris ensata* Thunb)
Family: Iridaceae

Root of Irsa was grounded to get coarse powder and then subjected to physicochemical and phytochemical studies to determine the physicochemical constants, Unani Pharmacopoeia (Anonymous, 2007) was consulted and for fluorescence study of the extract, the schedule mentioned by Afaq et al (1994) was followed. Physicochemical parameters studies was Ash values (total ash, acid insoluble ash and water soluble ash) and loss on drying at 105oC were determined and estimated in percentage using method as recommended in Unani Pharmacopoeia (UPI, 2007). The moisture content was determined using Toluene distillation

method. The successive extractive value of powder drug in Petroleum ether (60-80 oC), Di-ethyl ether, Acetone, Chloroform, Ethyl alcohol and Aqueous were successively estimated; pH of 1% and 10% aqueous solution was also checked. The water soluble and Ethanol soluble matter were determined. The Fluorescence analysis of the successive extract was studied under day light as well as Ultra Violet (short and long wave length) light. The Preliminary qualitative study of phyto-chemicals was also done.

Evaluation of Organoleptic Characteristics

The organoleptic Characters of Irsa was evaluated based on the method described by Afaq et al. (1994). Organoleptic evaluation refers to evaluation of the powder drug by its colour, odor, taste and texture (Table-1).

Physicochemical Study

The Physicochemical study included the determination of extractive values of the test drug in different solvents alcohol and water soluble contents, moisture content, ash values, loss of weight on drying, pH values (Table-2).

ASH VALUES

Total Ash

3 gm of drug was incinerated in a silica crucible of constant weight at a temperature not exceeding 450o C in a muffle furnace until free from carbon, cooled and weighed the percentage of ash was calculated by subtracting the weight of crucible from the weight of crucible + ash. The percentage of total ash was calculated with reference to the weight of drug taken (Anonymous, 2007).

Water Soluble Ash

The obtained ash was boiled with 25ml of distilled water for 5 min. The insoluble matter was collected in an ashless filter paper; (Whatman No. 42), washed with hot water and ignited in crucible, at a temperature not more than 450o C, the weight of insoluble ash was subtracted from the weight of total ash, giving the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug taken (Anonymous, 2007).

Acid Insoluble Ash

The total ash was boiled with 25 ml of 10% hydrochloric acid for 5 min. The insoluble matter was collected on ash less filter paper (Whatman No. 42), washed with hot water and ignited in crucible at a temperature not exceeding 450o C till constant weight. The percentage of acid-insoluble ash was calculated with reference to the weight of drug taken (Anonymous, 2007).

Extractive Values

The successive extractive values of the test drug in different organic solvents viz. petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, alcohol and distilled water were determined by soxhlet method using a soxhlet's apparatus. The heat was applied for six hours for each solvent on a heating mantle. The extracts were filtered and after evaporation of the solvents; the extractive values were determined with reference to the weight of drug. The procedures was repeated five times and the mean value for each extract was calculated (Anonymous, 2007).

Water and Alcohol Soluble Contents

5 gm of the air dried powdered drug was taken with 100 ml of distilled water, in a glass stoppard conical flask for 24 hours. The mixture was carefully shaken frequently for 6 hours and then allowed standing for 18 hours. It was filtered and 25ml of filtrate was evaporated to dryness on a water bath. The residue was dried at 105o C to constant weight, cooled in desiccator for 30 minutes and weighed. The percentage of water soluble matter was calculated with reference to the amount of air dried drug. The percentage of alcohol soluble matter was determined as above by using alcohol in place of water (Anonymous, 2007).

Moisture Content

The toluene distillation method (Dean and Stark Method) was used for the determination of moisture content. 10 gm of drug was taken in the flask of the apparatus and 75ml of distilled toluene was added to it. Distillation was carried out for 6 hours and the process was repeated five times. The volume of water collected in receiver tube (graduated in ml) was noted and the percentage of moisture calculated with reference to the weight of the air dried drug taken for the process (Jenkins, 1967).

Loss of Weight on Drying

10 gm of drug was taken, spread uniformly and thin layered in a shallow petridish. It was heated at a regulated temperature of 105o C, cooled in a dessicator and weighed. The process was repeated many times till two consecutive weights were found constant. The percentage of loss in weight was calculated with respect to initial weight (Jenkins, 1967).

pH Value

Determination of pH was carried out by a synchronic digital pH meter (model no. 335) equipped with a combined electrode. The instrument was standardized by using buffer solution of 4.0, 7.0, and 9.20 to ascertain the accuracy of the instrument prior to the experiment. The pH value of 1% and 10% aqueous solution of powder drug solution was measured (Anonymous, 2007).

Qualitative Analysis

The qualitative analysis of different chemical constituents, present in test drugs was carried out according to the scheme proposed by Bhattacharjee and Das (1969).

The powder of the test drugs was extracted with petroleum ether (bp.60-80o C). The petroleum ether extract (I) was tested for free phenols, alkaloids and sterols/terpenes. A part of this extract was saponified and portion (II) was tested for fatty acids, whereas, unsaponified portion (III) was tested again, phenols, and sterols/terpenes for confirmation. The defatted marc was divided into two portions. One portion was extracted with hot water and the other with ethanol (70%). The aqueous (IV) and ethanolic (V) extracts were tested for alkaloids, flavonoids, saponins, sugars and tannins. Aqueous extract was extracted with ether and ether soluble portion (VI) was tested again for alkaloids, sterols/terpenes, whereas, water-soluble portion (VII) was tested for glycosides. The water-soluble portion was again hydrolyzed with 5% hydrochloric acid and extracted with chloroform. The aglycone portion (VIII) was tested for insoluble hydrochloride of alkaloid. Chloroform soluble portion (IX) was tested for alkaloids and sterols/terpenes, whereas; water-soluble fraction (X) was tested for alkaloids. One part of this water-soluble portion was basified with any alkali (ammonia) and extracted with immiscible solvent (ether). The solvent soluble part (XI) was again tested for alkaloids (Table-3).

Test for Alkaloids : A drop of Dragendroff's reagent in the extract was added. The brown precipitate shows the presence of alkaloids (Afaq et al, 1994).

Test for Carbohydrate / Sugars

Fehling's Test: In the aqueous extract, a mixture of equal parts of Fehling's solution A and B previously mixed was added and heated. A brick red precipitate of cuprous oxide indicates the presence of reducing sugars.

Molisch test: In an aqueous solution, α-napthol was added. Afterwards, concentrated sulphuric acid was gently poured. A brown colour ring at the junction of the two solutions indicates the presence of the sugar (Afaq et al, 1994).

Test for Flavonoids: A piece of Magnesium ribbon was added to the ethanolic extract of the durg followed by drop wise addition of concentrated HCl. Colour ranging from orange pink to red is a confirmatory test for flavonoid (Afaq et al, 1994).

Test for Glycosides: The test solution is to be filtered and sugar is removed by fermentation with baker's yeast. The acid is removed by precipitation with magnesium oxide or barium hydroxide. The remaining ethanolic extract contains the glycosides which are subsequently detected by the following Methods:

The hydrolysis of the solution is to be done with concentrated sulphuric acid and after the hydrolysis sugar is determined with the help of Fehling's solutions. The Molisch's test is done for sugar using α-napthol and concentrated sulphuric acid (Afaq et al, 1994).

Test for Tannin: Ferric chloride solution was added in the aqueous extract of the drug. A bluish-black colour, which disappeared on addition of dilute sulphuric acid followed by a yellowish brown precipitate, shows the presence of tannin.

Test for Proteins

Millon's reaction: To the test solution, Millon's reagent was mixed and white coloured precipitate showed the presence of proteins.

Test for Starch: 0.015 gm of Iodine and 0.015 gm of Potassium Iodide was added in 5 ml of distilled water, 2 ml of iodine solution formed was added to 2 ml of aqueous test solution, the presence of blue colour indicates the presence of starch.

Test for Phenol: 5– 8 drops of 1% aqueous solution of Lead acetate was added to aqueous or ethanolic test solution. The presence of yellow coloured precipitate indicates the presence of phenols (Afaq et al, 1994)

Test for Sterol/Terpenes:

Salkowski reaction- In the test solution of chloroform 2 ml sulphuric acid (concentrated) was mixed from the side of the test tube. The colour of the ring at the junction of the two layers was observed. A red colour ring indicates the presence of the sterols/terpenes (Afaq et al, 1994).

Table 1: Physicochemical study of Powder of Irsa

S. No.	Parameters	Percentage (w/w)*
1	**Ash value**	
	Total ash	6.93
	Acid insoluble ash	1.03
	Water soluble ash	5.90
2	**Soluble Part**	
	Ethanol soluble	6.29
	Aqueous soluble	4.80
3	**Successive Extractive Values**	
	Pet. Ether	2.9
	Di-ethyl ether	4.58
	Chloroform	2.2
	Acetone	3.45
	Alcohol	10.13
	Aqueous	14.13
4	**Moisture content**	3.46
5	**Loss on Drying**	4.34
6	**pH values**	
	1% water solution	5.16
	10% water solution	5.67
7	**Bulk density**	0.67

*Note: Values are average of three experiments.

Table 2: Preliminary Screening of major Phyto-chemicals

S. No	Chemical Constituent	Tests/Reagent	Inference
1	Alkaloids	Dragendorff's reagent	+
		Wagner's reagent	+
		Mayer's reagent	+
2	Carbohydrate	Molisch's Test	+
		Fehling's Test	+
		Benedict Test	+
3	Flavonoids	Mg ribbon and Dil.Hcl	+
4	Glycosides	NaOH Test	+
5	Tannins/Phenols	Ferric Chloride Test	+
		Liebermann's Test	+
		Lead Acetate Test	+
6	Proteins	Xanthoproteic Test	+
		Biuret Test	+
7	Starch	Iodine Test	+
8	Saponins	Frothing with $NaHCO_3$	+
9	Steroid/Terpenes	Salkowski Reaction	+
10	Amino Acids	Ninhydrin Solution	-
11	Resin	Acetic Anhydride test	+

Indications: '–' Absence and '+' presence of constituent.

Table: 3 FTAR Analysis of Irsa

	Extract	Day Light	UV Long	UV Short
1.	**Pet. ether**	Brown	Black	Greenish
2.	**Di-ethyl ether**	Brownish	Dark Brown	Greenish
3.	**Chloroform**	Light Green	Blackish	Light Green
4.	**Acetone**	Brown	Black	Blackish
5.	**Alcohol**	Greenish	Black	Green
6.	**Aqueous**	Brownish	Black	Black

Table: 4 Fluorescence Analysis of Irsa with different chemical reagents

S. No.	Powdered drug + Chemical Reagent	Day light	UV short	UV long
1.	Powdered drug + Conc. HNO_3	Reddish	Green	Black
2.	Powdered drug + Conc. Hcl	Brown	Green	Black
3.	Powdered drug + Conc. H_2SO_4	Dark Brown	Green	Black
4.	Powdered drug + 2% Iodine solution	Dark Brown	Green	Black
5.	Powdered drug + Glacial Acetic acid + HNO_3	Orange	Dark Green	Black
6.	Powdered drug + Glacial acetic acid	Dark Brown	Dark Green	Black
7.	Powdered drug + NaOH (10%)	Brown	Green	Black
8.	Powdered drug +Dil. HNO_3	Brown	Light Green	Green
9.	Powdered drug + Dil. H_2SO_4	Brown	Light Green	Black
10.	Powdered drug +Dil. Hcl	Brown	Bluish Green	Black
11.	Powdered drug + Dragendorff's	Golden	Bright Green	Black
12.	Powdered drug + Wagner's Reagent	Brown	Bright Green	Black
13	Powdered drug + Benedict's reagent	Greenish Blue	Light Green	Green
14	Powdered drug + Fehling reagent	Brown	Green	Black
15	Powdered drug + KOH (10%) Methanolic	Brown	Green	Black
16	Powdered drug + $CuSO_4$ (5%)	Bluish	Light Green	Black
17	Powdered drug + Ninhydrin (2%) in Acetone	Brown	Green	Black
18	Powdered drug + Picric Acid	Yellow	Bright Green	Dark Green
19	Powdered drug + Lead Acetate (5%)	Brown	Light Green	Black

Table: 5 Thin Layer Chromatography

Treatment	Mobile phase:	No of spots	Rf value and colour of spots
		Petroleum Ether Extract	
Day Light	Petroleum ether : Di-ethyl ether (4:1)	1	0.27 (Brown)
UV Short		1	0.27 (Bluish)
Iodine Vapour		1	0.27 (Yellowish)
		Chloroform Extract	
Day Light	Chloroform : Methanol (1:1)	1	0.68 (Brown)
UV Short		2	0.68 (Green), 0.54(Green)
Iodine Vapour		1	0. 68 (Yellowish), 0.75 (Pale Yellow)
		Acetone Extract	
Day Light	Butanol: Acetic acid: Water (5:4:1)	1	0.75 (Brown)
UV Short		1	0.75 (Bluish)
Iodine Vapour		1	0.75 (Pale Yellow)
		Alcoholic Extract	
Day Light	Butanol: Acetic acid: Water (5:4:1)	1	0.74 (Pale Yellow)
UV Short		2	0.80 (Greenish) , 0.75(Light Green)
Iodine Vapour		2	0.75(Pale Yellow), 0.54 (Yellow)

Test for Amino Acids: The ethanolic extract was mixed with ninhydrin solution (0.1% in acetone). After heating gently on water bath for few minutes it gives a blue to red-violet colour that indicates the presence of amino acids (Anonymous, 2007).

Test for Resin: The test solution was gently heated and acetic anhydride was added in it. After cooling, one drop of sulphuric acid was mixed. A purplish red colour that rapidly changed to violet indicates the presence of the resins (Afaq et al, 1994).

Fluorescence Analysis

Fluorescence Analysis of powdered drugs

Fluorescence analyses of the powdered drugs were done for identification. The powdered drugs were treated with different chemicals and observed in daylight and under ultra violet light. The changes in colors were noted (Anonymous, 2007) (Table-3).

Fluorescence Analysis of the successive extracts of drug sample: Successive extracts of all the drug samples viz. Petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol and aqueous were observed in day light and UV light (Anonymous,2007) (Table-4).

Thin Layer Chromatography

Thin Layer Chromatography was carried out on aluminium plates precoated with silica gel 60 F 254(Layer thickness 0.25 mm)for all extract in various phases later sprayed by different spraying reagents. The Rf value of spots were calculated by the following formulae (Anonymous, 2007).

$$\text{Rf value} = \frac{\text{Distance travelled by the Spot}}{\text{Distance travelled by the Solvent}}$$

RESULTS AND DISCUSSION

The present study is an attempt to ascertain the pharmacopoeial standards for the standardization of Irsa the quality, identity , purity and strength of the powder has been undertaken as a tool to bring out several features like ash standards, solubility in alcohol and water, successive extractive values, and qualitative screening of physicochemicals, total alkaloids, total flavonoids, phenol, nitrogen, fatty matter, Sterol/Terpenes, Protien and Carbhydrates. Characterization of an herbal drug is essential for the quality control to check the presence of adultrants as a single drug remedy or its polyherbal Unani formulation.

CONCLUSION

Physico-chemical standardization is of prime importance in quality control of Unani drugs. As the efficacy of many drugs mainly depends upon its physical and chemical properties therefore, the determination of physico-chemical characters for the authenticity of a drug is necessary before studying any medicinal property. Phyto-chemical constituents present in the drug vary, not only from plant to plant but also among different samples of same species, depending upon various atmospheric factors, storage and drying conditions.

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