Physico-chemical and phytochemical standardization of Itrifal Ustukhuddus: A pharmacopoeial unani compound formulation

Abdul Razique, Abdul Latif

Abstract
Physico-chemical standardization is an important part in quality control of Unani drugs both single as well as compound formulation as the therapeutic efficacy of the drugs mainly depends upon their chemical constituents. Therefore, the determination of physico-chemical characters for the authenticity of a drug is necessary before studying it for pharmacological activity. In the present study a pharmacopoeial compound formulation Itrifal Ustukhuddus has been selected to confirm its parameter according to the guidelines given in Unani Pharmacopoeia of India. The parameters studied for quality assurance of Itrifal Ustukhuddus includes physicochemical studies and qualitative analysis of various phytochemicals was estimated that revealed the presence of carbohydrates, glycosides, phenols, proteins, steroids and resins. The TLC profile of this formulation was also performed. This study will help in setting down pharmacopoeial standards in determining the quality and purity of compound drug formulation which is in use since time immemorial in chronic catarrh, sinusitis, as a Nervine tonic.

Keywords: Itrifal Ustukhuddus, Standardization, Physico-chemical, Phytochemical, Sinusitis

1. Introduction
For thousands of years, natural products have been used in traditional medicine all over the world. It is also believed that plant derived drugs are safe and more dependable and have little side effects than the costly synthetic drugs and many of which have adverse effects and beyond the reach of poor patients. These medicinal plants are being used by about 80% of the world population primarily in the developing countries for treating different diseases. The medicinal value of a crude drug depends on the presence of one or more chemical constituents of physiological importance. They may be glycosides, alkaloids, resins, enzymes etc. They have been accepted due to their safety, efficacy, cultural acceptability and lesser side effects [1]. Thus emphasis was laid on the plants based drugs as they are cheap, easily available, rich in alkaloids, and possess significant biological activity. It is necessary to provide pure, quality control and authentic medicines to the world population. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial contamination, etc. Hence it has become necessary to standardize the efficacy and safety measures of the traditional drugs so as to ensure supply of medicinal plant materials with good quality. The determination of physico-chemical characters for the authenticity and purity of a drug is necessary before studying it for pharmacological activity. The pharmacological activity of drug is due to the presence of chemical constituents. Therefore the qualitative analysis of various phytochemicals was estimated that revealed the presence of glycosides, flavonoids, phenols, steroids and resins. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines and its products and therefore be used for authentication and identification of the herbal products. No enough work has been reported regarding standardization of this drug so far. Keeping in view the beneficial effects of Itrifal Ustukhuddus in the nervous and respiratory disorders and its frequency of usage, an attempt has been made to find out the physico-chemical and phytochemicals characteristics of this Unani preparation which may prove prelude to fix the chemical standards for this preparation.

3. Materials and Methods
The marketed sample of Itrifal Ustukhuddus (Batch No. 01, Mfg Date 09/2014, Expiry date 08/2017 manufactured by Dawakhana Tibbiya College Muslim University, Aligarh-202001,
Itrifal Ustukhuddus has subsequently detected by the following methods.

#### 3.2.3 Moisture Content

The percentage of water soluble matter was calculated with reference to the weight of drug. The procedures were repeated five times and the mean value was calculated.

#### 3.2.4 Water Soluble Ash

The obtained ash was boiled with 25 ml of distilled water for 5 min. The water soluble ash was calculated with reference to the weight of drug taken.

#### 3.2.5 Loss of Weight on Drying

5 gm of drug was taken into flat petridish, spread uniformly and thin layered in a shallow petridish. It was heated at a regulated temperature of 105 °C, cooled in a desiccator 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.

#### 3.2.6 pH Value

Determination of pH was carried out by a digital pH meter (model no. DB 1011, Make Decibel) 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% solution and 10% of powder drug solution was measured.

#### 3.3 Phytochemical Evaluation

#### 3.3.1 Test for Alkaloids

- A drop of Dragendorff’s reagent was added in the sample taken in a test tube. The brown precipitate shows the presence of alkaloids.
- 1 ml aqueous extract of the sample was taken in a test tube and a drop of Mayer’s reagent was added. The white precipitate indicated the presence of alkaloids in the test solution.

#### 3.3.2 Test for Flavonoids

Magnesium ribbon was added to the ethanolic extract of the material followed by drop wise addition of concentrated HCl. colour change from orange to red is a confirmatory test for flavonoids [8].

#### 3.3.3 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-naphthol and concentrated sulphuric acid.

#### 3.3.4 Test for Tannins

Ferric chloride solution was added in the aqueous extract of the drug. A bluish-black colour, which disappeared on addition

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Uttar Pradesh, India) was procured from local market of Okhla, New Delhi. The Physicochemical parameters includes the organoleptic characters of test drug, alcohol and water soluble matter, specific gravity, moisture content, ash values, loss of weight on drying and pH values [2, 3, 4]. The phytochemical analysis includes determination of successive extractive values of the test drug in different organic solvents using soxhlet apparatus, qualitative and quantitative estimation of the chemical constituents present in the drug sample and thin layer chromatography [2, 4, 5, 7]. The Physico-chemical and Phytochemical Standardization of Itrifal Ustukhuddus has been carried out at Ayush Section, Delhi Test House (A Unani and Ayurvedic Drugs Testing Laboratory), Azadpur, Delhi for the development of pharmacopeial standards.

#### 3.1 Organoleptic characters

Organoleptic characters of test sample such as appearance, physical state, colour, smell and taste were observed.

#### 3.2 Physico-Chemical Analysis

#### 3.2.1 Extractive Values

The extractive values of the test drug in different organic solvents viz. petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, alcohol and distilled water were carried out by a soxhlet apparatus. The heat was applied for six hours 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 were repeated five times and the mean value was calculated.

#### 3.2.2 Water and Alcohol Soluble Contents

5 gm of Itrifal Ustukhuddus was taken into 250 ml glass stoppard conical flask and add 100 ml of distilled water and alcohol and kept for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105 °C to constant weight and weigh. The percentage of water soluble matter was calculated with reference to the drug. The percentage of alcohol soluble matter was determined as above by using alcohol in place of water.

#### 3.2.3 Moisture Content

The toluene distillation method was used for the determination of moisture content. 10 gm of drug was taken into flat petridish, spread uniformly and thin layered in a shallow petridish. It was heated at a temperature not exceeding 450 °C and weighed after cooling in desiccator. The percentage of acid-insoluble ash was calculated with reference to the weight of drug taken.

#### 3.2.4.2 Acid Insoluble Ash

The total ash was boiled with 25 ml of 5N hydrochloric acid for 5 min. The insoluble matter was collected on ash less filter paper (Whatman No. 41), washed with hot water and ignited in crucible at a temperature not exceeding 450 °C and weighed after cooling in desiccator. The percentage of acid-insoluble ash was calculated with reference to the weight of drug taken.

#### 3.2.4.3 Water Soluble Ash

The obtained ash was boiled with 25 ml of distilled water for 5 min. The insoluble matter was collected in an ashless filter paper, (Whatman No. 41) washed with hot water and ignited in crucible, at a temperature not exceeding 450 °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 drug taken.

#### 3.2.5 Loss of Weight on Drying

5 gm of drug was taken into flat petridish, spread uniformly and thin layered in a shallow petridish. It was heated at a regulated temperature of 105 °C, cooled in a desiccator 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.

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- 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-naphthol and concentrated sulphuric acid.

#### 3.3.4 Test for Tannins

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.

3.3.5 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 this solution formed was added to 2 ml of aqueous test solution, the presence of blue colour indicates the presence of starch.

3.3.6 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 colour precipitate indicates the presence of phenols [9].

3.3.7 Test for Steroid/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 steroids/terpenes.

3.3.8 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 [9].

3.3.9 Test for Resins
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.

3.4 Quantitative estimation of Sugar, protein and crude fibre content
The quantitative estimation of total and reducing sugar of sample was carried out as per the method described [4]. The quantitative estimation of protein of sample was carried out as per the method described [11]. The quantitative estimation of crude fibre content of sample was also carried out as per the method described [5].

3.5 Chromatographic Studies
Thin Layer Chromatography (TLC)
It was carried out on TLC pre-coated aluminum plates with silica gel 60 of F254 (layer thickness 0.25 mm) (E Merck) of alcoholic, methanolic and aqueous extract. Taking Toluene: Ethyl acetate: Formic acid in ratio (2: 5: 1.5) and Butanol: Acetic acid: Water: Ethyl acetate (3:1:1:5) as the mobile phases. The Rf values of the spots were calculated by the following formula.

\[ R_f \text{ Value} = \frac{\text{Distance traveled by the spot}}{\text{Distance traveled by solvent system}} \]

4. Results and Discussion
4.1 Organoleptic characters
The colour of test sample is dark brown in colour, semisolid preparation with specific odour and sweetish bitter in taste.

4.2 Physico-Chemical Studies
Physico-chemical study is also important, because it helps in characterization of constituent or group constituents that frequently lead to establish the structure-activity relationship and likely mechanism of action of the drug. Phytochemical constituent 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 condition. Thus, keeping in view the above considerations, both the physico-chemical & Phytochemical studies were carried out and the results found are given in table 1 & 2 respectively.

4.2.1 Specific Gravity
The specific gravity of (Itrifal Ustukhuddus) Unani compound formulation was determined at 25 °C by using a specific gravity bottle and was found 1.320±0.011.

4.2.2 Ash Values
The ash value is useful in determining authenticity and purity of drugs. Ash value is the residue that remains after complete incineration of the drug, which consists chiefly of silica, partly derived from the constituents of the cells and their walls and partly from foreign mineral matters, mainly soil. Ash value plays an important role in ascertaining the standard of the drug, because the sand, earthy matters are generally added for increasing the weight of a drug resulting in the higher ash percentage. Therefore, the ash value determined for the basis of judging the identity and cleanliness of a drug and give information related to its adulteration in inorganic matter [3]. The mean of percentage of total ash, acid insoluble ash and water soluble ash was found as 1.12 ± 0.00, 0.23 ± 0.01 & 0.44±0.00 respectively.

4.2.3 Extractive Value
The Extractive value is a parameter for detecting the adulteration in any drug. The amount of the extract that the drug yields in a solvent is often an approximate measure of the amount of a certain constituents that the drug contains. Therefore, for establishing the standards of any drug these extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one [3]. The percentage of extractive values of Itrifal Ustukhuddus in different organic solvents was found as: 1.27 ± 0.05, 0.11 ± 0.01, 0.64 ± 0.05, 45.88 ± 0.85, 24.98 ± 0.95 with petroleum ether, diethyl ether, chloroform, ethanol and water respectively.

4.2.4 Water and Alcohol Soluble Matter
Percentage of solubility is also considered as an index of purity, as alcohol can dissolve almost all substances including glycosides, resins, alkaloids etc. The Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value. The mean percentage of alcohol and water soluble matters was found to be 62.65±0.88 & 58.56±1.20 respectively.

4.2.5 Moisture Content
The moisture content of the drugs is variable because mostly herbal drugs are hygroscopic and excessive moisture content becomes an ideal medium for the growth of different type of micro-organisms like bacteria and fungi. They subsequently spoil the purity of drug. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability
of drugs. The percentage of moisture content by Toluene distillation method was found to be 14.80 ± 0.02.

4.2.6 pH value
pH value of the drug is also an important parameter. The drugs in the opposite pH are unionized and absorbed rapidly from stomach. On account of having high acidic pH, the drugs get ionized in stomach because pH of stomach is reported to be about 3.5 [10]. The mean of pH value of 1% and 10% solution, were 3.81 ± 0.00 & 3.72 ±0.00 respectively.

4.2.7 Loss of Weight on Drying at 105 ºC
Percentage of loss of weight on drying at 105 ºC indicates towards the loss of volatile substance along with the water, which is determined by subtracting the moisture contents of the drug from the loss of weight in drying. So the percentage of loss of weight determined for Itrifal Ustukhuddus was found to be 24.53 ± 0.02.

4.3 Qualitative Analysis for Various Chemical constituents
Qualitative phyto-chemical analysis of Itrifal Ustukhuddus was also carried out for the presence of alkaloids, flavonoids, glycosides, tannins, phenols, starch, steroids/Terpenes, amino acids and resins. The results are given in table 2.

4.4 Quantitative Analysis for Sugar, Proteins and crude fibre content
Quantitative estimation of the Itrifal Ustukhuddus was also carried out for total & reducing sugar. The quantitative determination of protein and crude fibre content was also carried out in the test sample; the results are given in table 1. As the therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents may cause lesser therapeutic values of the drugs and therefore, they are considered as low standard drugs. Our findings will be helpful in predicting the biological activity of the drug.

Table 1: Physico-chemical analysis of Itrifal Ustukhuddus

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Physicochemical Parameter</th>
<th>Mean±S. E. M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss of weight on drying at 105 ºC (%)</td>
<td>24.53 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>Specific Gravity</td>
<td>1.320 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>Moisture content (%)</td>
<td>14.80 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>Ash value in (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Ash</td>
<td>1.12 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Acid Insoluble Ash</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Water Soluble Ash</td>
<td>0.44 ± 0.00</td>
</tr>
<tr>
<td>5</td>
<td>pH value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH at 1%</td>
<td>3.81 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>pH at 10%</td>
<td>3.72 ± 0.00</td>
</tr>
<tr>
<td>6</td>
<td>Solubility (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alcohol Soluble extractive</td>
<td>62.65 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>Water Soluble extractive</td>
<td>58.56 ± 0.20</td>
</tr>
<tr>
<td>7</td>
<td>Extractive values in different organic solvent (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pet Ether</td>
<td>1.27 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Diethyl Ether</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>0.64 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>45.88 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>24.98 ± 0.95</td>
</tr>
<tr>
<td>8</td>
<td>Sugar Contents (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Sugar</td>
<td>61.59</td>
</tr>
<tr>
<td></td>
<td>Reducing Sugar</td>
<td>47.02</td>
</tr>
<tr>
<td></td>
<td>Non-reducing sugar</td>
<td>14.57</td>
</tr>
<tr>
<td>9</td>
<td>Protein (%)</td>
<td>1.27±0.02</td>
</tr>
<tr>
<td>10</td>
<td>Crude fibre content (%)</td>
<td>0.20±0.04</td>
</tr>
</tbody>
</table>

4.5 Thin Layer Chromatography (TLC)
Thin Layer Chromatography (TLC) is one of the important parameters used for detecting the adulteration for judging the quality of the drug, the resolution of different kinds of chemical components are separated by using TLC and calculating the Rf values after detecting the spots in order to standardize the drug for its identity, purity and strength. The TLC studies of different extracts obtained in different organic solvents of the test drug have been conducted and Rf values of various spots appeared in different solvents system have been noted (table-3&4 figure 1, 2 & 3).

Table 2: Qualitative analysis of the phyto-constituents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical Constituent</th>
<th>Tests/Reagent</th>
<th>Test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendorff’s reagent</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s reagent</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s reagent</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>Mg ribbon and Dil. Hel</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>NaOH Test</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>Ferric Chloride Test</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Starch</td>
<td>Iodine Test</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Phenols</td>
<td>Lead Acetate Test</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Steroid/Terpenes</td>
<td>Salkowski Reaction</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Amino Acids</td>
<td>Ninhydrin Solution</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Resin</td>
<td>Acetic Anhydride test</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Thin layer chromatography of Itrifal Ustukhuddus

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent System</th>
<th>Visible in</th>
<th>No. of Spots</th>
<th>RF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Toluene : Ethyl acetate: Formic acid (2: 5: 1.5)</td>
<td>Daylight</td>
<td>3</td>
<td>0.06, 0.7, 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV short</td>
<td>4</td>
<td>0.3, 0.4, 0.7, 0.8</td>
</tr>
<tr>
<td>Methanol</td>
<td>Toluene : Ethyl acetate: Formic acid (2: 5: 1.5)</td>
<td>Daylight</td>
<td>2</td>
<td>0.7, 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV short</td>
<td>3</td>
<td>0.5, 0.7, 0.8</td>
</tr>
</tbody>
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<th>RF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Butanol: Acetic acid: Water: Ethyl acetate (3:1:1:5)</td>
<td>Spray (by Anisaldehyde Sulphuric acid)</td>
<td>5</td>
<td>0.07, 0.2, 0.4, 0.5, 0.6</td>
</tr>
<tr>
<td>Aqueous</td>
<td>Butanol: Acetic acid: Water: Ethyl acetate (3:1:1:5)</td>
<td>Spray (by Anisaldehyde Sulphuric acid)</td>
<td>5</td>
<td>0.08, 0.1, 0.2, 0.4, 0.8</td>
</tr>
<tr>
<td>Methanol</td>
<td>Butanol: Acetic acid: Water: Ethyl acetate (3:1:1:5)</td>
<td>Spray (by Anisaldehyde Sulphuric acid)</td>
<td>2</td>
<td>0.07, 0.2</td>
</tr>
</tbody>
</table>
5. Conclusion
The compound Unani Formulation Itrifal Ustukhuddus used in the present study is of the standard parameter as given in Unani Pharmacopoeia. Besides the qualitative estimation, quantitative analysis of the test sample was also carried out. The results obtained from the study could be utilized as a reference for setting limits for the reference standards for the quality control and quality assurance of Itrifal Ustukhuddus.

6. Acknowledgement
We are thankful to Delhi Test House (A Unani and Ayurvedic Drugs Testing Laboratory), Azadpur, Delhi for providing necessary facilities during this research work.

7. References

Fig 1: TLC of Alcoholic Extract of Itrifal Ustukhuddus
Solvent system- Toluene: Ethyl acetate: Formic acid (2: 5:1.5)

Fig 2: TLC of Methanolic Extract of Itrifal Ustukhuddus
Solvent system- Toluene: Ethyl acetate: Formic acid (2: 5:1.5)

Fig 3: TLC of Itrifal Ustukhuddus after derivatisation