Comparative Physico-chemical and Phyto-chemical Study of Different Samples of a Unani Pharmacopoeial Preparation Itrifal Ustukhuddus

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Abstract

In the present study, three pharmacopoeial compound formulations of Itrifal Ustukhuddus have been selected for physicochemical and phytochemical study. Itrifal Ustukhuddus is a reputed poly-herbal preparation of Unani system of medicine. It is commonly used in the treatment of chronic sinusitis and related conditions. The parameters studied for quality assurance of Itrifal Ustukhuddus included physicochemical parameters and qualitative and quantitative analysis of various phytochemicals. The TLC profile of the test drug was also prepared. Three samples of Itrifal Ustukhuddus prepared by three different pharmaceutical units were taken up for the study with an aim to compare the physicochemical and phytochemical parameters in order to check the quality of different samples in in market. It was concluded that all three samples were of pharmacopoeial standard.

Keywords: Itrifal Ustukhuddus, Standardization, Physicochemical, Phytochemical

Introduction

For thousands of years, natural products have been used in traditional medicine all over the world. It is believed that plant derived drugs are safe and more dependable and have little side effects than the costly synthetic drugs. 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. The plant drugs have been accepted due to their safety, efficacy, cultural acceptability and lesser side effects (Kamboj, 2000).

Unani medicine uses hundreds of polyherbal and other compound preparations both pharmacopoeial and non-pharmacopoeial. One important polyherbal preparation Itrifal Ustukhuddus is commonly used by Unani physicians to manage especially the sinusitis. A number of pharmaceutical companies prepare this pharmacopoeial preparation. Although, a number of single and compound drugs are standardized on routine basis but unfortunately, the different samples of the same drug prepared by different manufacturing units are usually not undertaken to ensure the quality and to establish their bio-equivalance.

In view of the above, the present study was designed to study the three samples of Itrifal Ustukhuddus prepared by Dawakhana Tibbiya College, A.M.U., Aligarh, Sadar Dawakhana, Delhi and Indian Medicine Pharmaceutical Corporation Limited (IMPCL), Almora, on physicochemical parameters to establish their quality

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standards. The findings will help to know the status of the supply of pharmacopoeial drugs by the drug industry and will also help to know whether the guidelines set by the Govt. are being followed or not in terms of using genuine crude drugs and the methodology recommended for the manufacturing.

Materials and Methods

Market samples of Itrifal Ustukhuddus manufactured by Dawakhana Tibbiya College, AMU, Aligarh (Batch No. 01, Mfg. date 09/2014, Exp. Date 08/2017), Sadar Laboratories, Delhi (Batch No. 117, Mfg. Date 04/2014, Exp. Date 03/2017) and Indian Medicine Pharmaceutical Corporation Limited, Almora (Batch No. UTI 05, Mfg. Date 08/2014, Exp. Date 07/2017) were purchased from Local market of Aligarh. (These samples henceforth will be known as DKTCS, SLS and IMPCLS, respectively).

The Physicochemical parameters included the organoleptic characters of all three test drugs, alcohol and water soluble matter, specific gravity, moisture content, ash values, loss of weight on drying and pH values (Afaq *et al*., 1994; Jenkins *et al*., 1967; Anonymous, 2009). The phytochemical analysis included 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 (Afaq *et al.,* 1994, Anonymous, 1968; 1970; 2009; 1982). The Physico-chemical and Phytochemical standardization of all three samples of Itrifal Ustukhuddus was undertaken in Ayush Section, Delhi Test House (A Unani and Ayurvedic Medicines Testing Laboratory), Azadpur, Delhi, India.

(i) Physico-Chemical Analysis

Organoleptic characters of all three samples such as appearance, physical state, colour, smell and taste were observed.

Specific Gravity

The specific gravity of all three samples was determined at 25°C by using a specific gravity bottle.

Extractive Values

The extractive values of the all test drugs in different organic solvents viz. petroleum ether, diethyl ether, chloroform, alcohol and distilled water were determined by the soxhlet apparatus. The heat was applied for six hours on a water bath for each solvent except water, which was heated directly 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 crude drug. The procedures were repeated five times and the mean value was calculated.

Water and Alcohol Soluble Contents

5 gm of all samples of Itrifal Ustukhuddus were taken separately into 250 ml glass stoppard conical flask. 100 ml of distilled water were added and kept for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Samples were filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat bottom dish, and dried at 105°C to constant weight. 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.

Moisture Content

The toluene distillation method was used for the determination of moisture content. 10 gm of each drug was taken in a flask and 75 ml of toluene was added to it. Distillation was carried out for 6 hours and the process was repeated for 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.

Ash Values

Total Ash

2 gm of each sample was incinerated in a silica crucible of a constant weight at a temperature not exceeding 450°C in a muffle furnace until carbon free ash obtained, cooled and weighed and the percentage of ash was calculated by subtracting the weight of crucible from the weight of crucible with ash. The percentage of total ash was calculated with reference to the weight of drug taken.

Acid Insoluble Ash

The total ash of each sample 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.

Water Soluble Ash

The obtained ash of each sample 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.

Loss of Weight on Drying

5 gm of each sample was taken into a flat petridish and spread uniformly into a thin layer. 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.

pH Value

Determination of pH of each sample 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.

(ii) Quantitative estimation of sugar, protein and crude fibre content

The quantitative estimation of total and reducing sugar of each sample was carried out as per the method described in Unani Pharmacopoeia of India (Anonymous, 2009). The quantitative estimation of protein of each sample was carried out as per the method described in Pharmacopoeia of India (Anonymous, 2014). The quantitative estimation of crude fibre content of each sample was also carried out as per the method described in IS: 10226, 1982.

(iii) Phytochemical Evaluation

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.

Test for Flavonoids

Magnesium ribbon was added to the ethanolic extract of the material followed by drop wise addition of conc. Hcl. Colour change from orange to red is a confirmatory test for flavonoids (Fransworth, 1966).

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.

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.

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.

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 (Brewster and Mc Even, 1971).

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.

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 (Brewster and Mc Even, 1971).

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.

(iv) Chromatographic Studies

Thin Layer Chromatography (TLC)

It was carried out on TLC pre-coated aluminum plates with silica gel 60 of F_{254} (layer thickness 0.25 mm) (E Merck) of alcoholic and methanolic extract. Taking Toluene: Ethyl acetate: Formic acid in ratio (2: 5: 1.5) as the mobile phases. The R_f values of the spots were calculated by the following formula (Anonymous, 1968):

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

Results and Discussion

(i) Physico-chemical Studies

The colour of each test samples was dark brown in colour, semisolid preparation with specific odour and sweetish bitter in taste.

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

Specific Gravity

The specific gravity of Dawakhana sample (DKTCS), Sadar Laboratories sample (SLS) and IMPCL sample (IMPCLS) was determined at 25°C by using a specific gravity bottle and was found 1.320±0.01, 1.312±0.02 and 1.314±0.02 respectively.

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 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 (Jenkins *et al.*, 1967) . The mean percentages of extractive values of each sample of Itrifal Ustukhuddus in different organic solvents are given in Table 1.

Water and Alcohol Soluble Matter

Percentage of solubility is also considered 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 of each samples of Itrifal Ustukhuddus are given in Table 1.

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 types of micro-organisms such as 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 of each sample is given in Table 1.

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 a drug, because the sand, earthy matters are generally added for increasing the weight of the drug resulting in higher ash percentage. Therefore, the ash value determination serves as the basis of judging the identity and cleanliness of a drug and give information related to its adulteration in inorganic matter (Jenkins *et al.*, 1967). The mean of percentage of each samples are given in Table 1.

Table 1: Physico-chemical analysis of Itrifal Ustukhuddus

S. No.	Physicochemical Parameter	DKTCS Mean±S.E.M.	SLS Mean±S.E.M.	IMPCLS Mean±S.E.M.
1	Specific gravity	1.320 ± 0.01	1.312 ± 0.02	1.314±0.02
2	Moisture content (%)	14.80 ± 0.02	13.73 ± 0.03	10.43 ± 0.02
3	Loss of weight on drying at 105°C (%)	24.53 ± 0.02	24.28±0.04	19.36±0.02
4	Ash value in (%)			
	Total Ash	1.10 ± 0.00	1.50 ± 0.00	1.16 ± 0.00
	Acid Insoluble Ash	0.23 ± 0.01	0.49 ± 0.00	0.06 ± 0.00
	Water Soluble Ash	0.44 ± 0.00	0.35 ± 0.00	0.32 ± 0.00
5	pH value			
	pH at 1% aqueous solution	3.81 ± 0.00	3.89 ± 0.00	5.88 ± 0.00
	pH at 10% aqueous solution	3.72 ± 0.00	3.86 ± 0.00	5.70 ± 0.00
6	Solubility (%)			
	Alcohol Soluble extractive	62.65 ± 0.88	45.25 ± 0.40	23.10 ± 0.20
	Water Soluble extractive	58.56 ± 1.20	55.60 ± 0.20	64.80 ± 0.10
7	Extractive values in different organic solvent (%)			
	Petroleum Ether	1.27 ± 0.05	0.60 ± 0.02	0.40 ± 0.02
	Diethyl Ether	0.11 ± 0.01	0.09 ± 0.02	0.07 ± 0.02
	Chloroform	0.64 ± 0.05	0.20 ± 0.04	0.14 ± 0.04
	Ethanol	45.88 ± 0.85	16.60 ± 0.45	10.60 ± 0.04
	Aqueous	20.70 ± 0.95	47.97 ± 0.20	38.47 ± 0.20
8	Sugar Contents (%)			
	Total Sugar	61.59	60.75	62.03
	Reducing Sugar	47.02	41.02	52.90
	Non-reducing sugar	14.57	19.73	9.13
9	Protein (%)	1.27±0.02	1.38±0.04	1.12±0.02
10	Crude fibre content (%)	0.20±0.04	0.22±0.02	0.16±0.02

Loss of Weight on Drying at 105°C

Percentage of loss of weight on drying at 105° C 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 each samples of Itrifal Ustukhuddus are given in Table 1.

pH of 1% and 10% Solution

pH value of the drug is also an important parameter to determine its quality and standard. Further, it also helps in determining the pharmaco-dynamic and pharmaco-kinetic character of a drug (Gilman *et al*., 2001). The mean of pH value of 1% and 10% solution are given in Table 1.

(ii) Quantitative analysis for sugar, proteins and crude fibre content

Quantitative estimation of each sample of Itrifal Ustukhuddus was carried out for total and reducing sugar. The quantitative determination of protein and crude fibre content was also carried out in each test sample; the results are given in table 1.

(iii) Qualitative phytochemical analysis for various chemical constituents

Qualitative phyto-chemical analysis of each samples of Itrifal Ustukhuddus was also carried out for the determination of the presence of alkaloids, flavonoids, glycosides, tannins, phenols, starch, steroids/terpenes, amino acids and resins. The results are given in table 2. The biological activity of medicinal plants and crude drugs depends mainly on the physiologically active constituents present in the drug. The presence of a number of constituents in the test drugs indicated that their medicinal value is mainly indicated to these chemical constituents.

Table 2: Qualitative analysis of the phyto-constituents

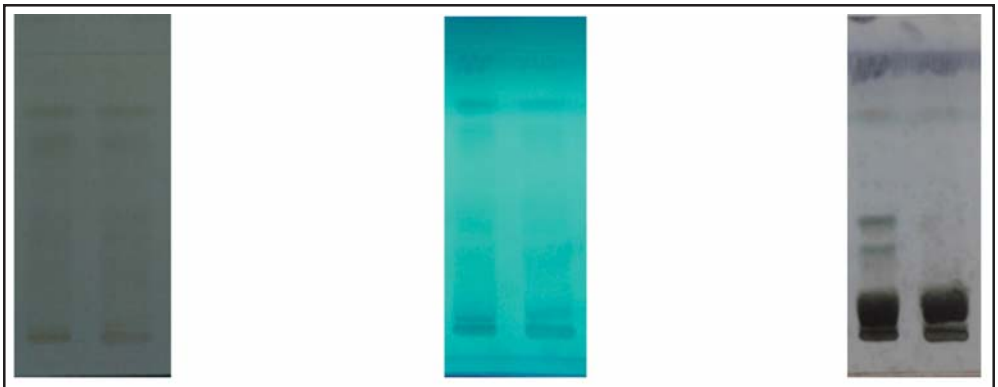
S. No.	Chemical Constituent	Tests/Reagent	DKTCS	SLS	IMPCLS
1	Alkaloids	Dragendorff's reagent Mayer's reagent	–	–	–
2	Flavonoids	Mg ribbon and Dil. HCl	–	–	–
3	Glycosides	NaOH Test	+	+	+
4	Tannins	Ferric Chloride Test	+	+	+
5	Starch	Iodine Test	–	–	–
6	Phenols	Lead Acetate Test	+	+	+
7	Steroid/Terpenes	Salkowski Reaction	+	+	+
8	Amino Acids	Ninhydrin Solution	+	+	+
9	Resin	Acetic Anhydride test	–	–	–

(iv) Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is one of the important parameters used for detecting the adulteration and judging the quality of the drug. The resolution of different kinds of chemical components are separated by using TLC and calculating the R_f values after detecting the spots. If the drug is adulterated, there might be appearance of the other components present as adulterants; in turn the number of spots may increase. On the other hand, the extracted or deteriorated drugs may lose the components and the number of spots appeared might be less. The findings summarized in Table 3 and Fig. 1,2 3 indicated the Rf value of all three samples are almost similar. It atleast partially indicated that genuine samples of crude drug were used to prepare in compound drugs (Table 3 Figure 1, 2 & 3).

Table 3: Thin layer chromatography of Itrifal Ustukhuddus

Extract	Solvent System	Visible in	DKTCS		SLS		IMPCLS	
			No.of Spots	Rf value	No.of Spots	Rf value	No.of Spots	Rf value
Alcoholic	Toluene: Ethyl acetate: Formic acid (2: 5:1.5)	Daylight UV Spray (by Ani-saldehyde Sulphuric acid)	3 4 4	0.06, 0.7, 0.80.3, 0.4, 0.7, 0.8 0.1, 0.3, 0.4, 0.8	2 4 4	0.7, 0.8 0.27, 0.3, 0.7, 0.8 0.06, 0.26, 0.37, 0.8	3 4 4	0.06, 0.7, 0.8, 0.3, 0.41, 0.71, 0.8, 0.06, 0.3, 0.41, 0.78
Metha-nolic	Toluene: Ethyl acetate: Formic acid (2: 5:1.5)	Daylight UV Spray (by Ani-saldehyde Sulphuric acid)	2 3 2	0.7, 0.8 0.3, 0.7, 0.8, 0.1, 0.8	2 3 2	0.7, 0.8 0.3, 0.7, 0.8, 0.1, 0.8	2 3 2	0.7, 0.8 0.05, 0.71, 0.8, 0.07, 0.8



(A) (M) Day light | (A) (M) UV Short | (A) (M) After derivatisation

Fig. 1: TLC of Alcoholic (A) and Methanolic (M) Extract of Itrifal Ustukhuddus (DKTCS)

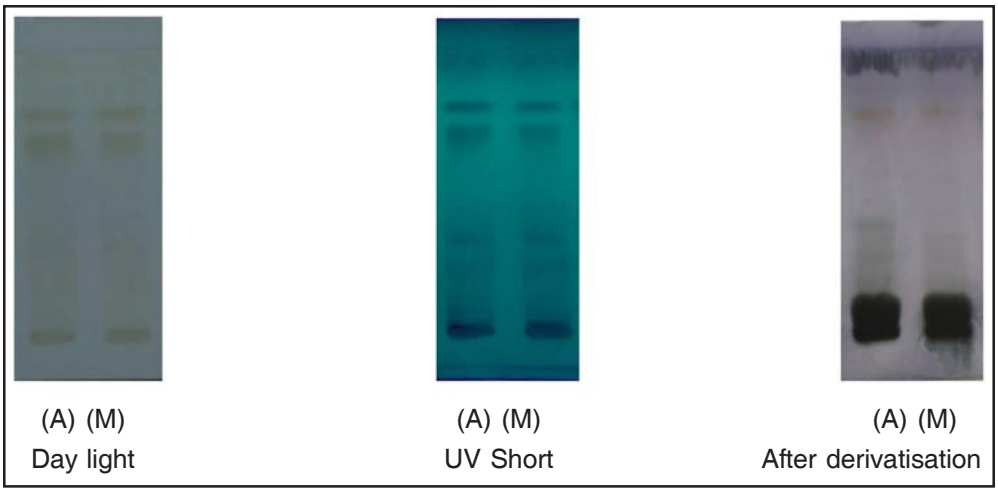


Fig. 2: TLC of Alcoholic (A) and Methanolic (M) Extract of Itrifal Ustukhuddus (SLS)

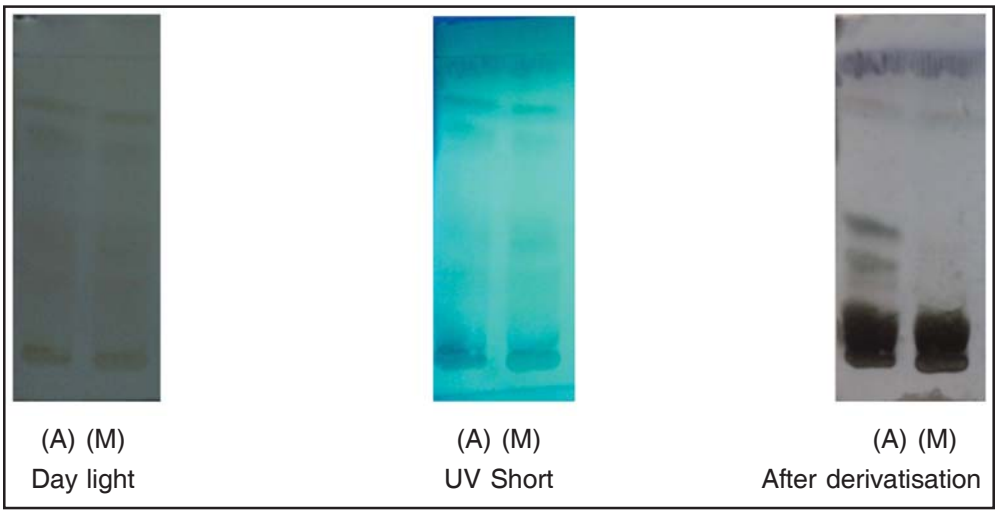


Fig.3: TLC of Alcoholic (A) and Methanolic (M) Extract of Itrifal Ustukhuddus (IMPCLS)

Conclusion

It can be concluded that the market samples of Itrifal Ustokhuddus represented by three major pharmaceutical companies are genuine as they satisfy by and large, the pharmacopoeial standards set by the Unani Pharmacopoeia of India and other legal documents.

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