

Pharmacognostical Evaluation of *Terminalia Chebula* fruits on different market samples

Manish Pal Singh^{1*} and Chandra Shekhar Sharma²

¹*Shrinathji Institute of Pharmacy, Nathdwara, Rajsamand, India*

²*B. N. College of Pharmacy, Udaipur, Rajasthan, India*

*Corres. author: manish_bn@yahoo.co.in
Phone No. : +91-98287-83211

Abstract: The different market samples of *Terminalia chebula* fruits evaluated by Pharmacognostic parameters compare with standard data. All three samples carried out microscopic characters, ash values, extractive values, T.L.C., & chemical tests. All the data of three samples were compared with standard data, the sample no-1 was more authentic than among all the three samples.

Keywords: *T. Chebula*, Ash values, Extractive values, Gallic acid

Introduction

Terminalia Chebula has been extensively used in ayurveda, unani & homoeopathic medicine and has become cynosure of modern medicine. The Sanskrit name 'Haritaki' is rich with meaning, referring to the yellowish dye (harita) that contains the god Siva (Hari, i.e. the Himalayas) and that it cures (harayet) all the diseases¹. Its other commonly used Sanskrit name, Abhaya, refers to the 'fearlessness' it provides in the face of the disease. According to Indian mythology, this plant originated from the drops of ambrosia (Amrita) which fell on the earth when Indra was drinking it².

T. Chebula possesses a wide variety of activities like antimicrobial³, antioxidant⁴, antiviral⁵, anticarcinogenic⁶, hypocholesterolemic⁷, radioprotective⁸, antispasmodic & antipurgative⁹.

The present study has been undertaken three samples of fruits were evaluated for their pharmacognostic parameters comparison w.r.t. standard data.

Materials and Methods

Sample source:- The fruits of *Terminalia chebula* three samples were purchased from local market of Udaipur.

Microscopic characters-¹⁰

Colour:- The untreated part of the drug was taken and

colour of the drug was examined under sunlight.

Odor and Taste:- A small portion of the drug was taken, slowly and repeatedly inhaled the air over the material and examined the odor. And taste, a small portion of drug was taken on the tongue and find out the taste of drug.

Size and Shape:- Width and length of fruit was measured with the help of scale. Shape of fruit was confirmed by comparing with literature.

Surface characteristic:- Longitudinally wrinkled and ridges were confirmed by comparing with literature.

Ash values of fruit powder:-

Total Ash:- 3 gm of drug was weighed and incinerated in a China dish at a temperature not exceeding 450°C until free from carbon, cooled and weighed, until a constant weight was obtained for three successive readings. Percentage of ash was calculated with reference to air dried drug.

$$\text{Total Ash} = \frac{\text{Wt. of ash} \times 100}{\text{Wt. of drug}}$$

Acid-Insoluble Ash:- The total ash was obtained by boiling for 5 min with 25 ml of dilute hydrochloric acid; the insoluble matter was collected in a Gooch crucible, the insoluble matter was wash with hot water and ignite to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated.

Extractive values of fruit powder:

Alcohol-soluble extractive:- 5 gm of accurately weighed powdered drug was taken in a stoppered conical flask and add 100 ml of 90% alcohol, and shake constantly for 6hr in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated by:-

Alcohol-Soluble

$$\text{Extractive} = \frac{\text{Wt. of extractive} \times 100}{\text{Wt. of drug}}$$

Water Soluble extractive:- 5 gm of accurately weighed powdered drug was taken in a stoppered conical flask and add 100 ml of chloroform water, and shake constantly for 6hr in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated by:-

Water-Soluble

$$\text{Extractive} = \frac{\text{Wt. of extractive} \times 100}{\text{Wt. of drug}}$$

Chromatographic Studies (T.L.C.):¹¹**TLC plate dimension- 7X3 cm****Adsorbent used- Silica gel for TLC**

Preparation of plates: - Silica gel with a mean pore width of preferably 6 to 10 nm is used as a base material. As smaller the particles better the separation efficiency. Silica gel plates of 0.2 mm thickness were prepared by spreading method. And final spot taken on a precoated silica gel 60 F₂₅₄ TLC plate (E.Merck) of uniform thickness (0.2 mm) and develop it in the solvent system to a distance of 0.8 cm.

Activation plates: - Plates were activated at 105⁰ C for 45 min in an electric oven.

Sample Application: - 2gm of sample powdered drug was taken in to a conical flask, add 20 ml 2M hydrochloric acid and heat on a water bath for 30 min at 100⁰ C. Cool and filter. The filtrate was transfer into a separating funnel and extract with diethyl ether (3x 15 ml). Concentrate the combined diethyl ether extract to 10 ml.

Standard Solution: - prepare 1mg/ml solution of gallic acid in water.

Solvent System: - Chloroform: Ethyl acetate: Formic acid (2.0: 2.0: 0.8).

Chamber Preparation: - A clean and dry chamber was taken. The chamber was lined with the filter paper. The strips of filter paper should be cut in such a way that a window remains allowing observation of the development process. 53 ml of the solvent was

introduced to a height of 0.5 to 1 cm in the chamber in order to moisten the filter paper and to equilibrate the chamber with solvent vapor. The closed chamber was allowed to saturate with solvent vapor. The TLC was then introduced in the chamber in such way that the system just wet the lower edge of the plate sorbet. The solvent system should not wet the part of the plate where the spots were applied, any contact between the side of the plate and the filter paper should avoid.

Development of Chromatogram:-

The solvent migrates up the plate through the sorbet by capillary action. The substance was separated as a result of interaction between the samples, mobile and stationary phase into individual component. Migration behavior of the separated substance is given in the form of R_f value (relative to front).

$$\text{RF} = \frac{\text{Distance traveled by solute (solute front)}}{\text{Distance traveled by solvent (solvent front)}}$$

Ascending development of chromatogram was done. The plate was removed from the chamber, when the solvent front had reached the predetermined height and the solvent front was marked precisely with pencil. Then the plate was dried and observed under UV light.

Visualization:-

Scan the plate under UV at 254 nm and 366 nm and finger print profile. Spray the plate with 5% ferric chloride in methanol. Note the R_f of the band separated.

Phytochemical analysis:¹² Phytochemical screening procedures carried out were adapted from the previous work on plant analysis. Determination of alkaloids, 0.5g of the sample was weighed accurately and defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 min with 5.0 mL of aqueous HCL in a water bath. The resulting mixture was centrifuged at 3000rpm for 10min to remove filtrate (supernatant) 1.0 mL of the filtrate two determinations was treated with a few drops of Mayer's reagent and a second 1.0 mL portion was treated similarly with Dragendorff' reagent. Turbidity or precipitations with either of these reagents were taken as evidence for the presence of alkaloids.

Test for Saponin: Ability of Saponins to produce frothing in aqueous solution was used as screening test for the sample 0.5g of dried extract was shaken with water in a test tube, frothing which persist on warming was taken as evidence for the presence of Saponins.

Test for tannins: 5.0g of dried extract was stirred with 10.0 mL of distilled water. This was filtered and ferric chloride reagent was added to the filtrate. A blue, black precipitate was taken as evidence for the presence of tannins.

Test for anthraquinones: 5.0g of dried extract was shaken with 10.0 mL of benzene, this was filtered

and 5.0 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammonia cal (lower) phase indicated

the presence of free hydroxyanthraquinones.

Test for Cardiac glycosides: 0.5g of dried extract was dissolved in 2.0 mL of glacial acetic acid containing one drop of ferric chloride solution. This was then under laid with 1.0 mL of concentrated H₂SO₄. A brown ring obtained at the interface indicated the presence of cardenolides.

Discussion

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there has been an emphasis standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identifications evaluation of plant drugs by Pharmacognostic studies is still more reliable, accurate and in expensive means. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its

Identity and purity and should be carried out before any tests are undertaken. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. The organoleptic or macroscopic studies yielded important characteristics, such as the fractured surfaces of fresh and dried fruits, typical tongue sensitizing aromatic taste and aromatic and characteristic odour of the fruits which are useful diagnostic characters.

The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash, and water-soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both

physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially ash and siliceous earth. Water-soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards.

The plant material was subjected to preliminary photochemical screening involving successive solvent extraction by different solvents in order of increasing polarity to obtain diverse polar and non polar phytoconstituents possessing different solubility pattern, followed by various chemical tests for qualitative detection of various chemical constituents. The percent extractives in different solvents indicate the quantity and nature of constituents in the extract. The colour of the extract sometimes may roughly indicate the physical and chemical features of constituents present.

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. As per photochemical screening the fruit of *Terminalia Chebula* contains mainly glycosides. Experimental conditions of TLC and hence, the obtained R_f value differed to some extent from that of literature. The chromatographic profile may serve as a characteristic finger print for qualitative of fruits. After present investigation it can be concluded that the pharmacognostical study of fruit of *Terminalia Chebula* yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. As previously mentioned, *T.Chebula* being morphologically variable species, these information will also be helpful to differentiate *T.Chebula* from the closely related other species and varieties of *Terminalia Chebula* fruit. The all three samples were compared with standard data the sample no-1 was more same pharmacognostical parameters.

Table 1: Details of samples of *Terminalia Chebula* fruit

| Sample No. | Source | Price (Rs /Kg) |
|------------|------------------------------|----------------|
| Sample-1 | S.A. Batliwala(udaipur) | 200 /kg |
| Sample-2 | S.S. Batliwala(udaipur) | 100/kg |
| Sample-3 | Jawariya Brother's (udaipur) | 100/kg |

Table- 2: Macroscopic evaluation of *Terminalia Chebula* fruit

| Parameters | Standard Value | Sample-(1) | Sample-(2) | Sample-(3) |
|-------------------------|--|--------------------------------------|--------------------------------------|--------------------------------------|
| Odour | Odourless | Odourless | Odourless | Odourless |
| Colour | Yellowish brown | Yellowish green | Yellow | Light yellowish green |
| Shape | Ovoid | Ovoid | Ovoid | Ovoid |
| Size | 3.5 - 4.0 cm length, 1.5 -2.0 cm wide | 4.2 cm length, 1.5 cm wide | 4.0 cm length, 2.0 cm wide | 3.3 cm length, 1.9 wide |
| Taste | Astringent | Slightly bitter and sour | Bitter | Slightly bitter |
| Surface Characteristics | Longitudina-lly, Wrinkled, (5-6 ridges on surface) | Longitudina-lly, Wrinkled (5-ridges) | Longitudina-lly, Wrinkled (6 ridges) | Longitudina-lly, Wrinkled (5 ridges) |

Table- 3: Standardization of *Terminalia Chebula* fruit powder

| Parameters | Standard Value | Sample-(1) | Sample-(2) | Sample-(3) |
|----------------------------|----------------|------------|------------|------------|
| Total ash | #NMT 5 % | 2.67% | 3.67 % | 3.33 % |
| Acid insoluble ash | #NMT 5 % | 2.45% | 2.85% | 2.40% |
| Alcohol soluble extractive | ##NLT40% | 43.8% | 39.0 % | 41.6 % |
| Water soluble extract | ##NLT60% | 62.8 % | 43.8 % | 32.8 % |

#NMT-Not more than, ##NLT-Not less than

Table- 4: T.L.C. Identification

| Sample No. | R _f Values of Spot of Sample |
|-----------------------|---|
| Sample-1 | 0.76 |
| Sample-2 | 0.72 |
| Sample-3 | 0.70 |
| Gallic acid(standard) | 0.82 |

Table- 5: Results of photochemical screenings of successive extracts of fruits of *Terminalia chebula*

| Constituents | Sample-1 | Sample-2 | Sample-3 |
|--------------|----------|----------|----------|
| Alkaloids | -ve | - ve | - ve |
| Glycosides | + ve | + ve | + ve |
| Tannins | + ve | + ve | - ve |
| Flavonoids | - ve | - ve | - ve |

+ ve = Present, - ve = Absent

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