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# Pharmacognostical evaluation of *Terminalia Arjuna* bark on different marketed samples

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**Abstract**

*In this study, three different marketed samples of Terminalia arjuna bark were evaluated for Pharmacognostic parameters and compared with standard data for authentication. All three samples were examined for microscopic characters, ash values, extractive values, Thin Layer Chromatography (TLC) and various chemical tests. Of the samples tested sample no-1 complied with the standard data and considered more authentic than other two samples.*

**Keywords:** *T.arjuna*, Ash values, Extractive values, Ellagic acid.

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## INTRODUCTION

*Terminalia arjuna* is a native Bangladeshi, deciduous and ever green tree, standing 20-30m above ground level. It belongs to Combretaceae family[1]. It is found in Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi and Deccan region near ponds and rivers. Ancient Indian physicians used the powdered tree bark of *Terminalia arjuna* for alleviating "Hritshool" (angina) and other cardiovascular conditions. All the parts of the plant have been used for their therapeutic beneficiary effect from ancient times. *T. arjuna* helps to maintain a healthy heart and decrease the effects of stress and anxiety[2]. Its stem bark possesses glycosides, large quantities of flavonoids, tannins and minerals. Flavonoids have been detected to exert antioxidant, anti inflammatory and lipid lowering effect while glycosides are cardiotonic, thus making *Terminalia arjuna* unique amongst currently used medicinal plants. Bark powder boiled with water and inhaled to cure headache and to kill worms in the teeth[3], juice is used as antacid[4] and fruits are found useful as tonic[5]This tree is cone-shaped with white bark and elliptic leaves placed in opposite directions. The sap of Arjuna tree is milky white in colour. The flowers are yellowish and fruits are fibrous woody and smooth. *Terminalia arjuna* bark is currently available in the market as herbal preparation and has been used for treatment and prevention of various cardiovascular diseases, but quality of those product is a matter of concern. In this study, an attempt has been made to evaluate various Pharmacognostic parameters of few selected samples of *Terminalia arjuna* bark product available in the market and compare with standard data. These standards are of utmost importance not only in finding out the genuinity, but also in the detection of adulterants in marketed drugs as well as in Forensic detection.

## MATERIALS AND METHODS

### Materials

The powder bark of *Terminalia arjuna*, three samples were purchased from local market of Vapi, Gujarat, India. Ellagic acid was procured from Biomax (Thane) bombay.

**Methods**

**Physico-Chemical parameters of *T.Arjuna* stem bark:**

Physico-Chemical parameters were determined for of T.Arjuna stem bark according to the method describes in W.H.O guidelines[6].

**Moisture content:**

The powdered material (10gm) was placed in a moisture disc and dried to a constant weight in a oven in 100-105$^0$C.The loss of weight (in mg/g) of air dried was calculated as follows.

$$\% \text{ of moisture content} = \frac{\text{Weight loss X } 100}{\text{Weight of the sample}}$$

**Total Ash**:

3 gm of drug was weighed and incinerated in a China dish at a temperature not exceeding 450$^o$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.

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

**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 Bark powder:**

**Alcohol-soluble extractive:** 5 gm of accurately weighed powdered drug was taken in a stoppard 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:

$$\text{Alcohol-Soluble Extractive} = \frac{\text{Wt. of extractive}}{\text{Wt. of drug}} \text{ X } 100$$

**Water Soluble extractive:** 5 gm of accurately weighed powdered drug was taken in a stoppard 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:

$$\text{Water-Soluble Extractive} = \frac{\text{Wt. of extractive}}{\text{Wt. of drug}} \text{ X } 100$$

**PRELIMINARY PHYTOCHEMICAL SCREENING:**

Chemical tests were carried out on the methanolic and petroleum ether extract using standard procedures to identify the constituent[7,8]. The plant extract was assayed for the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, steroids, glycosides, carbohydrates and proteins or amino acids.

**Test for alkaloids:**

Each extract (0.5 g) was stirred with 5 mL of 1% HCL on a steam bath. The solution obtained was filtered and one mL of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extract.

**Testing for tannins and phenolics:**

Each extract (0.5 g) was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5% $Fecl_3$ reagent were added to the filtrate. Blue-black or blue green colouration or precipitation was taken as an indication of the presence of phenolics and tannins.

**Test for saponins:**

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered; 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and observed for the formation of emulsion.

**Test for steroids:**
Two ml of acetic anhydride was added to 0.5 ethanolic extract of each sample with 2 ml $H_2S0_4$. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

**Test for terpenoids (Salkowski test):**
Five ml of each extract was mixed in 2 ml of chloroform, and concentrated $H_2S0_4$ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face formed to show positive results for the presence of terpenoids.

**Test for Steroid (Liebermann Burchard reaction):**
To th extract Chloroform, 2ml acetic anhydride and 2 drops conc.$H_2SO_4$was added from the side of the test tube.No red or blue colour were appeared indicating the absence of steroids.

**Legal's test for Glycosides:**
To the hydrolysate 1ml of pyridine and few drops of sodium nitropruside solution was added and then it was made alkaline with sodium hydroxide solution.Apperance of pink to red colour shows the presence of glycosides.

**Keller-Killiani test for glycosides:**
A total of 1 mL of glacial acetic acid, few drops of ferric chloride solution and conc. $H_2SO_4$ (Slowly through the sides of the test tube) were add to the extract. Appearance of reddish brown ring at the junction of the liquids indicated the presence of de-oxysugars.

**Borntrager's test:**
Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added.Ammonical layer turned pink colour shows the presence of anthraquinone glycosides.

**Test for Carbohydrates (Molisch's test):**
A small quantity of the extract were dissolved separately in 4ml of distilled water and filtered.The filtrate was treated with 2-3 drops of 1% alcoholic alpha nephthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube.Appearence of brown ring at the junction of the two liquids showed the presence of Carbohydrates.

**Test for Amino acids (Million's test):**
The above prepared mixture was treated with Million's reagent. No red colour formed due to absence of proteins or amino acids.

**Table 1: Details of samples of *Terminalia arjuna bark***

| Sample No. | Source | Price (Rs /Kg) |
|---|---|---|
| Sample-1 | Aspha ,Surat,Gujarat | 300/Kg |
| Sample-2 | Jamna,Mandip,M.P | 450 /Kg |
| Sample-3 | Shree Narayan ayurvedic pharmacy, Ahmedabad | 400/Kg |

**Table No.2- Determination of Proximate Analysis for bark of *Terminalia arjuna***

| Test | | Result (in %) | | | Inference (in %) |
|---|---|---|---|---|---|
| | | S1 | S2 | S3 | |
| Tests for extraneous material | Foreign matters | 1.023 | 1.022 | 1.044 | Not more than 2.0 |
| | Sand and Silica | Absent | Absent | Absent | Should be absent |
| | Insect Infestation | Absent | Absent | Absent | Should be absent |
| | Rodent Contamination | Absent | Absent | Absent | Should be absent |
| Physico-chemical analysis | Total Ash Content | 15.034 | 15.432 | 16.302 | Not more than 27.0 |
| | Acid insoluble ash | 1.452 | 0.962 | 0.944 | Not more than 2.0 |
| | Moisture Content | 5.021 | 6.332 | 6.651 | Not more than 8.0 |
| Tests for extractive value | Water soluble extractive | 44.03 | 45.88 | 46.02 | Not less than 16.0 |
| | Acid soluble extractive | 42.05 | 44.99 | 44.95 | Not less than 17.0 |

**Identification of marker constituents in the crude drugs by TLC:**
Test Solution: 0.5g of powdered drug was extracted with methanol (3 x 15 ml) under reflux on a water bath. Methanolic extract was filtered and concentrated and made up the volume to 25ml with methanol.

Solvent System: Toluene: Ethyl Format: Formic Acid (5:5:2)

Procedure: Applied 10ml each of test solution and standard solution on precoated Silica Gel 60 F254 plate of uniform thickness of 0.5mm. The plates were developed in the solvent system.

Visualization: The plates were examined under ultraviolet light at 254nm.
Evaluation: A band (Rf. 0.42) corresponding to ellagic acid is visible in standard and test solution tracks.

**Table No. 3:- TLC Screening of *Terminalia arjuna***

| Samples | Solvent systems | Wavelength(nm) | Rf Values |
|---|---|---|---|
| Sample1 | Toluene: Ethyl Format : Formic acid (5 : 5 : 2) | 254nm | 0.42 |
| Sample2 | | | 0.52 |
| Sample3 | | | 0.48 |
| Ellagic acid(Standard) | | | 0.42 |

**Table 4: Effect of different chemical reagents on *Terminalia arjuna* bark powder samples**

| SL No. | Chemical reagent | Sample-1 | Sample-2 | Sample-3 |
|---|---|---|---|---|
| 1 | Iodine($I_2$) | Greenish brown | Greenish brown | Greenish brown |
| 2 | Glacial acetic acid | Light brown | Light brown | Brown |
| 3 | $Fecl_3$ solution (5%) | Light green | Green | Light green |
| 4 | Lead acetate Solution (10%) | Yellow | Yellow | Yellow |
| 5 | Picric acid | Yellowish brown | Yellowish brown | Yellowish brown |
| 6 | KOH (1%) | Yellowish brown | Yellowish brown | Yellow |
| 7 | NaOH(5%) | Light brown | Brown | Light brown |
| 8 | $HNO_3$ (50%) | Yellowish red | Yellowish red | Yellowish red |

**Table 5: Results of photochemical screenings of successive extracts of barks of *Terminalia arjuna***

| Constituents | Sample-1 | Sample-2 | Sample-3 |
|---|---|---|---|
| Alkaloids | + | + | + |
| Carbohydrates | + | + | − |
| Phytosteroids | − | − | − |
| Glycosides | + | + | − |
| Fixed oils & fats | − | − | − |
| Saponins | + | + | + |
| Phenolic compounds tannins | + | + | + |
| Proteins & amino acids | + | − | + |
| Gums & mucilage's | − | − | − |
| Volatile oils | − | − | − |
| Flavonoids | + | + | + |

*+ ve = Present, − ve = Absent*

## DISCUSSION

The standardization of a crude drug is an integral part for establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. Despite the modern techniques, identifications evaluation of plant drugs by Pharmacognostic studies is still more reliable, accurate and inexpensive 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 crude drugs. The organoleptic or macroscopic studies yielded important characteristics, such, typical tongue sensitizing aromatic taste and characteristic odour of the barks which are useful diagnostic characters.

Ash values and extractive values can be used as reliable aid for detecting adulteration. Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents.[3,4] 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 ass and siliceous earth. Water-soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards.In our present investigation,all the three samples were found within the official limit.

The information obtained from preliminary phytochemical screening will be useful in finding out the quality of the drug. 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 chemical analysis of the bark confirms the presence of Alkaloids,Carbohydrates,saponins,Glycosides, phenolic compounds tannins,Proteins and amino acids and flavanoids.

Thin layer chromatographic studies showed the presence of active principles like Ellagic acid on 0.42 Rf value for sample1 and was similar to the standard 0.42 Rf with prominent blue coloration in both. This further needed to be subject to HPTLC for exact quantification of Ellagic acid. These studies will be carried out in future because this facility is not available in our laboratory at present. Phytochemical chemical studies were carried out for identification of Ellagic acid from Arjuna bark powder using various chemical tests available for tannins in the reference books also confirm that give bluish black / greenish black with $FeCl_3$ is found positive indicating that confirm presence of Ellagic acid in the sample no1.

Of the samples tested sample no-1 complied with the standard data and considered more authentic than other two samples with respect to Pharmacognostical parameters. The present evaluation of various phytochemical parameters will be helpful to check the adulteration in natural valuable drug at the time of consumption for desire pharmacological effect. This study also highlights the need for constant market monitoring of new herbal products to ascertain their quality to Pharmacopoeial standards.

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