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A Comparative Pharmacognostic Evaluation of Different Extracts of *Terminalia bellerica Roxb.* Fruit

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ABSTRACT

Terminalia bellerica is an important traditional Indian medicinal plant used in various ailments and rituals. The use of different parts of this plant like leaves and fruits as a medicament for treatment of various conditions is well documented in literature. However, the studies on phytochemical constituents and medicinal properties in the fruit of this plant are scanty. All three plant fruit extracts viz. chloroform ethanol and methanol carried out physiochemical analysis, ash values, extractive values, T.L.C., and chemical tests. Phytochemical analysis of the extracts of Terminalia bellerica revealed the presence of mainly gallic acid, alkaloids, flavonoids, triterpenoids and amino acids. The presence of gallic acid and other flavonoids were confirmed by qualitative tests followed by TLC. All the data of three extracts were compared with standard data, the ethanolic fruit extract was more same that the all two extracts.

Keywords: *Terminalia Bellerica,* Ash Values, Extractive Values, Gallic Acid

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INTRODUCTION

Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used popular folks medicine. It has been shown that in-vitro screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigation [1].

The research in area of use of herbal drugs and drugs from natural origin has provided a platform for lowering the symptoms of disease and some unwanted side effects of modern therapeutics. *Triphala* is regarded as an important rasayana in ayurvedic medicine. *Triphala* powder consists of three plant fruits (*Terminalia chebula, Terminalia bellerica and Phyllanthus embelica* ratio for *Triphala is* 1:1:1) [2]. *Terminalia bellerica* Roxb. (Combretaceae) is one of the ingredients of ayurvedic purgative medicament of '*Triphala*' available in the Indian market for the treatment of dyspepsia, diarrhea, and dysentery, inflammation of the small intestine biliousness, flatulence, liver disease and leprosy [3]. *Terminalia bellerica* has been found to be antidiabetic by various investigators [4, 5], also, it's have been found to promote healing of infected full thickness dermal wound [6]. The fruit is also reported to have antioxidant [7], anti-diarrheal [8], purgative [9] and hypotensive [10].

Chemically, the presence of β-sitosterol, gallic acid, ellagic acid, galactose, ethyl gallate, chebulagic acid, mannitol, glucose, galactose, fructose and rhamnose in the fruit of *Terminalia bellerica* have also been reported [11]. Active principle such as gallic acid (3, 4, 5-trihydroxybenzoic acid) has also been identified. It shows marked bile stimulating activity and has strong antioxidant properties [12]. The present

study has been undertaken the 3- extracts *viz*. chloroform, ethanol, methanol of plant fruit evaluated its pharmacognostic parameters by comparison of standard data.

MATERIALS AND METHODS

Collection and Authentication of *Terminalia bellerica*

The fruits of Terminalia bellerica were purchased from local market of Mathura. The plants were authenticated by the Dr. Sunita Garg (Emeritus Scientist Raw Materials Herbarium &Museum), NISCAIR, New Delhi. Voucher specimen No. NISCAIR/RHMD/Consult/2018/3138-87, Dated 16/01/2018 was preserved for further references. The fruits washed 2-3 times from distilled water then dried in shade and grinded into fine powder, stored in closed container separately with proper labeling for further use.

Microscopic characters

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 powder

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.

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

Acid-Insoluble Ash: Boil the total ash was obtained for 5 mins with 25 ml of dilute hydrochloric acid, collect the insoluble matter in a gooch crucible, it insoluble matter was wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

Water soluble ash: The ash obtained as described in the determination of total ash was boiled for 5 min. with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited at a temperature not exceeding 450 ^{0}C. The procedure was repeated until a constant weight was observed. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug.

Extractive values of powder

Alcohol-soluble extractive: 5 gm of accurately weighed powdered drug was taken in a stopper conical flask and add 100 ml of 90% alcohol, and shake constantly for 6 hr 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:

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

Water Soluble extractive: 5 gm of accurately weighed powdered drug was taken in a stopper conical flask and add 100 ml of chloroform water, and shake constantly for 6 hr 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:

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

Foreign matter

Weighed accurately 250 g of the crude sample was spread out in a thin layer. The sample was inspected with the unaided eye or with the use of a magnifying lens (10 X) and the foreign matter was separated manually as completely as possible and weighed. The percentage of organic matter was weighed and determined with reference to the weight of the drug taken.

Loss on drying
About 2-5g of the prepared air dried material was accurately weighed in a previously dried and tarred flat weighing bottle. The sample was distributed evenly and was placed in the drying chamber (Oven). Drying was carried out by heating to 100-150 ^{0}C, the bottle was removed from the oven and the bottle was closed promptly and allowed to cool to room temperature and then weighed. The experiment was repeated until two consecutive weighing did not differ by more than 5 mg, unless otherwise stated in the test procedure. The loss in weight on drying was then calculated [13].

Successive solvent extraction
Extract about 5 g of the air dried powered plant material successively with the following solvent in a soxhlet assembly (Chloroform → Ethanol → Methanol).

Each time before extracting with the next solvent, dried the powdered material in air Oven below 50^{0}C. Finally, macerated the marc with chloroform water for 24 hours to obtain the aqueous extract. Concentrate the each extract by distilling off the solvent and then evaporated to the dryness on the water bath. The extract obtained with each solvent and calculated its percentage in terms of the air dried weight of the plant material [14].

Chemical test [15]
Test for alkaloids
Small portion of solvent free all the extract were stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents, such as Mayer's reagent, and Hager's reagent.

Mayer's reagent: Few drops of Mayer's reagent were added to the methanol, ethanol and chloroform extracts, a cream color precipitate was observed.

Hager's reagent: Few drops of Hager's reagent were added to the methanol, ethanol and chloroform extracts, a yellow precipitate was observed.

Test for amino acids
Millon's test: Few drops of Millon's reagent were added to the methanol, ethanol and chloroform extracts, a white precipitate was observed.

Test for flavonoids
Shinoda test: 5 ml of ethanol and few drops of concentrated hydrochloric acid were added to the methanol, ethanol and chloroform extracts, brown colour appeared.

Test for steroids and triterpenoids
Small portion of (2 mg) of extract was hydrolyzed with dilute hydrochloric acid for few hours in water bath and was subjected to Salkowaski test.

Salkowaski test
2 ml chloroform and 2 ml concentrated sulphuric acid was added to the 1 ml of extract, stirred, red colour was observed in chloroform layer and in acid layer greenish yellow fluorescence was observed.

Test for tannins
Ferric chloride test: Treat the extracts with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Test for proteins
Heat test- Heated the test solution in a boiling water bath, proteins get coagulated.

Detection of fixed oil and fats
Small quantities of petroleum ether and benzene extracts were pressed separately between two filter papers. Oil stains on the paper indicated the presence of fixed oil.

Detection of volatile oil
About 50 gm powdered material was taken in a volatile oil estimation apparatus and subjected to hydro distillation for the detection of volatile oil. The distillate is collected in the graduated tube of the assembly in which the aqueous portion is automatically separated from the volatile oil, if it is present in the drug, and returned back to the distillation flask.

Chromatographic Studies (T.L.C.) [16]
Thin layer chromatography was performed on silica gel G coated glass plates. The adsorbent silica gel G was coated to a thickness of about 0.3mm on previously cleaned TLC plates of 7× 3 cm using conventional spreader. The plates were placed in hot air oven at 105 ^{0}C for 30 min. for activation. The compounds were applied as a spot on the activated plate about 2 cm above from the

bottom. The spots were visualized by Spray the plate with 5% ferric chloride in methanol.

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 silica gel coated plate 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 105^0 C for 45 min in an electric oven.
Test Solution: Reflux the powdered drug (0.4 gm) with methanol (50 ml) for 30 min cool and filter. Evaporate the filtrate to dryness. Dissolve the residue in methanol (50 ml).

Standard solution: Dissolve gallic acid (2 mg) in methanol (5 ml).

Solvent system:
Ethyl acetate: Toluene: Methanol: Glacial acetic acid
7.5: 2.0: 0.5: 0.2

Visualization of spots: Spray the plate alcoholic 5% ferric chloride reagent.
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 which was carefully tited in order to moisten the filter paper and to equilibrate the chamber with solvent vapour. The closed chamber was allowed to saturate with solvent vapour. 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 RF value (relative to front).

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

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.

RESULTS AND 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 [17]. According to World Health Organization, 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.

Terminalia bellerica fruits are an important ayurvedic drug which has also been studied extensively by different investigators. *Terminalia bellerica* not only destroy pathogenic bacteria but it also used in wound healing, anti-inflammatory, analgesic, and antioxidant. Different parts of *Terminalia bellerica* such as fruits, leaves, and stem bark were used for the various pharmacological activities.

Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of fruits. The organoleptic or macroscopic studies yielded important characteristics, such as the fractured surfaces of dried fruits, typical tongue sensitizing tasteless and odourless of the fruits, which are useful diagnostic characters (Table 1).

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 as hand measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards (Table 2).

Table 1: Macroscopically study of fruit of *Terminalia bellerica*

S. No.	Parameter	Standard values of *Terminalia bellerica*	Test values of *Terminalia bellerica*
1.	Odour	Odourless	Odourless
2.	Colour	Yellow	Yellow
3.	Shape	Ovoid	Ovoid
4.	Taste	Astringent	Astringent
5.	Surface characteristics	Velvet, Irregular Wrinkled, Five Longitudinal Ridges.	Velvet, Irregular Wrinkled, Five Longitudinal Ridges.
6.	Size	1.3-2.5 Cm Diameter	2-3 Cm

Table 2: Physiochemical Standardization of drug powder

S. No.	Parameters	Standard values of *Terminalia bellerica*	Test values of *Terminalia bellerica*
1.	Total Ash value Water soluble Acid insoluble	#NMT 7% #NMT 2% #NMT 2%	4.66% 1.1% 0.77%
2.	Alcohol soluble extractive value	## NLT 25%	31.2%
3.	Water soluble extractive value	##NLT 35%	37.2%
4.	Loss in weight on drying at 105^{0}C	#NMT 12%	11.3%
5.	Foreign organic matter	#NMT 2%	0.98%

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

The plant material was subjected to preliminary phytochemical screening involving successive solvent extraction by different solvents in order of increasing polarity to obtain diverse polar and non-polarphyto constituents possessing different solubility pattern, followed by various chemical tests for qualitative detection of various chemical constituents. As per photochemical screening, the fruits of *Terminalia bellerica* contain mainly gallic acid, alkaloid and triterpenoids. Polyphenols and flavonoids were found to be appreciable as compared with other 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 (Table 3).

Table 3: Results of phytochemical screenings of successive extracts of fruits of *Terminalia bellerica*

Chemical test	Methanol extract	Ethanol extract	Chloroform extract
Test for alkaloids			
Hager's Test	-Ve	+ ve	-Ve
Mayer's Test	-Ve	+ ve	-Ve
Test for amino acid			
Millon's Test	-Ve	+ ve	—Ve
Test for flavonoids			
Shinoda Test	-Ve	+ ve	-Ve
Alkaline Reagent	-Ve	+ ve	+ ve
Zinc Chloride	-Ve	-Ve	-Ve
Test for steroids and triterpenoids			
Salkowaski Test	-Ve	+ ve	-Ve
Test for tannins			
Ferric chloride Test	-Ve	+ ve	-Ve
Catechine Test	-Ve	+ ve	-Ve
Test for Protein			
Heat Test	-Ve	+ ve	-Ve
Test for Carbohydrate			
Fehling Test	+ ve	+ ve	+ ve
Test for Lignins	-Ve	-Ve	+ ve
Test for Glycoside			
Legal Test	-Ve	-Ve	-Ve
Test for Resin			
Turbidity Test	-Ve	-Ve	+ ve
Test for Napthaloquinone			
Dam-Karrer Test	-Ve	-Ve	-Ve

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. As per photochemical screening the fruit of *Terminalia bellerica* contains mainly gallic acid, ellagic acid and other polyphenols. 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 (Table 4).

Table 4: T.L.C. Identification of *Terminalia bellerica* fruit extracts

S. No.	*Terminalia bellerica* extracts	R_f Values of spot of standard	R_f Values of spot of test
1.	Ethanol extract	0.70	0.66
2.	Methanol extract	0.86	0.65
3.	Chloroform extract	0.78	0.17

After present investigation it can be concluded that the pharmacognostical study of fruit of *Terminalia bellerica* 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, *Terminalia bellerica* being morphologically variable species, these information will also be helpful to differentiate *Terminalia bellerica* from the closely related other species and varieties of *Terminalia bellerica* fruit. The all three extracts were compared with standard data; the ethanolic extract was more same pharmacognostical parameters as standard data.

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