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EXTRACTION AND COMPARATIVE STUDY ON PHYSICO-CHEMICAL, PHYTOCHEMICAL ANALYSIS OF FRUITS OF *TERMINALIA CHEBULA* AND RHIZOMES OF *CURCUMA LONGA*

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Abstract

India being a rich and varied collection of medicinal plants since the vedic age. Chemistry of natural product is an ancient science. Secondary products present in the plants are responsible for beneficial medicinal effects of plant materials. *Terminalia chebula* Retz (Combretaceae), commonly known as haritaki and black myrobalan, is an important plant used in indigenous systems of medicine as remedy for fever, cough, diarrhoea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections. *Curcuma longa*Linn (Zingiberaceae) is well-known and valued medicinal plant. It has a long history of traditional uses ranging from folk medicine to several culinary preparations. The aim of the present study was to evaluate physicochemical, qualitative phytochemical analysis of fruits of *Terminalia chebula* andrhizomes of *Curcuma longa* collected from Bhopal region of Madhya Pradesh. The present study provides evidence that successive solvent extracts of *Terminalia chebula* and *Curcuma longa* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases. The physicochemical evaluations carried out in terms of loss on drying, ash value, extractive values and acid insoluble ash value etc. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, carbohydrates, glycosides, tannin and phenolic compounds. These studies provided information for standardization and correct identification of these two plants material. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

***Keywords*:** *Terminalia chebula, Curcuma longa,* Physicochemical, Qualitative phytochemical analysis

Introduction

Plants and animals contain organic substances and could be obtained in both primary and secondary metabolic process and they also provide a source of medicine since the ancient times. The plant kingdom has proved to be the most useful in the treatment of many diseases and they provide an important source of all the pharmaceuticals in the world. Few of the bioactive constituents of these plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facets of life have served a valuable starting material for drug development (Ajayi, *et al.,* 2011). *Terminalia chebula* has been extensively used in ayurveda, unani and homoeopathic system. The Sanskrit name for *Terminalia chebula* is Haritaki which means yellowish dye (harita) that contains the god Siva (Hari, i.e. the Himalayas) and it is known to cure (harayet) all the diseases (Das, 1991). In Sanskrit Haritaki is also known as 'Abhaya' which refers to the 'fearlessness', as it provides in the face of the disease. In Indian mythology, this plant has been known to be originated from the drops of ambrosia (Amrita) which fell on the earth when Indra was drinking it (Srikanthmurthy, 2000). *Terminalia chebula* (Combretaceae) is medium to large-sized tree distributed throughout tropical and subtropical Asia, including China and Tibet. This tree is wild in the forests of Northern India, Uttar Pradesh, Bengal, Southern Maharashtra, Tamil Nadu and Karnataka. The fruit is used medicinally (Varrier, 1996). It is considered to be a rasayana (with literal meaning: Path (ayana) of the Juice (rasa), or Elixir vitae) for Vata, balances tridoshas (loosely translated to three energetic forces in the body), enhances digestion (dipanapachana), sharpens the senses (medhyam), displays alterative (medicinal substance that acts gradually to nourish and improve the system), astringent, expectorant, anti-inflammatory, anodyne, cardiotonic, laxative, antiseptic and antiemetic properties (Jagtap & Karkera, 1999; Ahmad *et al*., 1998 and Sato *et al.,* 1997). Seven different types of fruits are recognized (i.e. vijaya, rohini, putana, amrita, abhaya, jivanti and chetaki), based on the region of harvestation, as well as its colour and shape.*Curcuma longa* L (Turmeric, Zingiberaceae) includes more than 80 species of rhizomatous perennial herbs and has widespread existence in the tropics of Asia, Africa, and Australia (Purseglove *et al.,* 1981). It is a perennial herbaceous plant, which reaches a stature of up to 1 m. There are highly branched, yellow-to-orange, cylindrical, aromatic rhizomes. *Curcuma longa* commonly known as turmeric (Haldi) is a well-known plant which is used as a drug in Ayurvedic and Unani system of medicine (Maiti *et al.,* 2007). Others various common name includes Curcuma India (port.), geelwortel (Dutch), kurkum (Arab), Manjano (East Africa), manjal (Tamil), kunyit (Indonesia), temukunyit (Malaysian), and iyu-chin (Chin.). The most important chemical components of turmeric are a group of compounds called curcuminoids, which include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin (Priyadarsini, 2007; Rana *et al.,* 2016 and Vavilova, 1990). India is the largest producer, consumer, and exporter of turmeric in the world and contains highest diversity (40 species) of *C. longa*. The World Health Organization has suggested the use of turmeric as a spice (Afaq *et al.,* 2002). Comprehensively, Curcuma is attainment importance as a growing source of new drug (s) to fight a variety of ailments as the species contain molecules validated with anti-fungal properties (Ammon & Wahl, 1991), anti-inflammatory, hepatoprotective, antitumor, antiviral (Polasa

et al., 1991) and anticancer activities. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Materials and Methods

Plant material

The fruits of *Terminalia chebula and* rhizomes of *Curcuma longa* were collected from Bhopal and authenticated by botanist from the Department of Botany, Safia Science College Bhopal. Plant authentication number: *Terminalia chebula* (198/Saif/Sci./Clg/bpl) and *Curcuma longa* (101/Saif/Sci./Clg/bpl).

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction

Collected plant material washed under running tap water and kept in shade for drying. Dried plant materials were then powdered using blender and further observed for colour, odour and texture then placed in packed labeled air tight container for further use. Plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of fruits of *Terminalia chebula* and rhizomes of *Curcuma longa* were placed in thimble of soxhlet apparatus. Soxhlation was performed at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethyl acetate and methanol solvent. For each solvent, soxhlation was continued till no visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporated using rotary vacuum evaporator (Buchi type) at 40°C. Dried extract was weighed and percentage yield for each extract was determined.

Physicochemical Parameters

The various physicochemical parameters that were determined as per The Unani Pharmacopeia of India.

Determination of loss on drying

Two grams of crude powder was taken in an evaporating dish and then dried in an oven at 105°C till constant weight was obtained. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially.

Total ash

3 g of powdered drug was accurately weighed and taken in a tarred silica crucible which was previously ignited and weighed. The powdered drug was spread as a fine even layer on the bottom of the crucible. The crucible was incinerated gradually by increasing the temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The ash was weighed and the total ash content was calculated with reference to the air dried drug.

Acid insoluble ash

The ash obtained as described in total ash was boiled with 25ml of dilute hydrochloric acid for 5 minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. This insoluble ash was transferred into a silica crucible and it was ignited, cooled and weighed. The process was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the quantity of air dried crude drug.

Water soluble ash

Total ash obtained was boiled for 5 minutes with 25ml of water. The insoluble matter was collected in ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible and was ignited, cooled and weighed. The process was repeated to get constant weight. The weight of insoluble matter was subtracted from the weight of the total ash. The difference of the weight was considered as the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Solvent ether soluble extractive values

Accurately weighed 5gm of powdered air dried leaves was taken with 100 ml of solvent ether in a stopper flask and kept for 24 hours. The flask was shaken frequently (Maceration) then the solvent ether extract was filtered rapidly through filter paper to prevent excessive loss of solvent ether. 25 ml of solvent ether extract was evaporated to dryness on a water bath and complete the drying in an oven at 100^0C.Then the residue was cooled weighed and kept in desiccators. Then the percentage w/w of solvent ether soluble extractive with reference to the air-dried drug was calculated.

Alcohol soluble extractive values

Accurately weighed 5 gm of powdered air dried leaves was taken with 100ml of alcohol (90 % v/v) in a stopper flask and kept for 24 hours. The flask was shaken frequently (Maceration).Then the alcohol extract was filtered rapidly through filter paper to prevent excessive loss of alcohol. 25ml of alcoholic extract was evaporated to dryness on a water bath and complete the drying in an oven 100^0C. Then the residue was cooled, weighed and kept in desiccators. Then the percentage w/w of alcohol soluble extractive with reference to the air-dried drug was calculated.

Water soluble extractive values

Accurately weighed 5gm of powdered air-dried leaves was taken with 100 ml of water in a stopper flask and kept for 24 hours. The flask was shaken frequently (Maceration).Then the aqueous extract was filtered rapidly through filter paper. 25 ml of aqueous extract was evaporated to dryness on a water bath and complete the drying in an oven at 100^oC. Then the residue was cooled, weighed and kept in desiccators. Then percentage w/w of soluble extractive with reference to the air dried drug was calculated.

pH Value at 10% and 1% dilution

pH of 10% Solution. An accurately weighed 10 gm of drug was dissolved in accurately measured 100 ml of water and filtered and the pH of filtrate was checked with a standardized glass electrode.

pH of 1% Solution. An accurately weighed 1 gm of drug was dissolved in accurately measured 100 ml of water and filtered and the pH of filtrate was checked with a standardized glass electrode.

Moisture content

About 10 gm of drug will be taken in a evaporating dish and dried in the hot air oven at 105^0C for 5 hour and weighed, continue the drying and weighed after 1 hour interval until difference between two successive weighing correspond to not more than 0.25% constant weight. Constant weight is reached when two consecutive weightings after drying for 30 min, in a desiccator, show not more than 0.01 gm difference. Then the percentage of loss on drying will be calculated with reference to the air dried drug.

Qualitative phytochemical analysis of plant extract

The *Terminalia chebula* and *Curcuma longa* extracts obtained was subjected to the preliminary phytochemical analysis (Jain *et al*., 2012 & Jain *et al*., 2019). The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Test for carbohydrates

Molisch's test: In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α-napthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.

Benedict's test: In a test tube containing extract of drug add benedict's solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.

Barfoed's test: The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. Formation of red precipitate of copper oxide was indicated the presence of carbohydrates.

Anthrone test: To the two ml of anthrone test solution, add the extract of drug. A green or blue colour indicated the presence of carbohydrate.

Test for alkaloids

Dragendorff's Test: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.

Wagner's test: Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner's reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.

Mayer's Test: Two ml of extract solution was treated with 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.

Hager's Test: Extract of the drug solution was treated with 3 ml of Hager's reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

Test for glycosides

Legal's test: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.

Baljet's test: To the drug extract, sodium picrate solution was added, yellow to orange colour was indicated the presence of glycosides.

Borntrager's test: Few ml of dilute sulphuric acid solution, the test solution of extract was added. It was filtered and the filtrate was boiled with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet colour was produced in orange layer confirmed the presence of glycosides.

Keller Kiliani test: Methanolic extract was dissolved in glacial acetic acid containing trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquid indicated the presence of glycosides.

Test of saponins

1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

Test for flavonoids

Shinoda test: In the test tube containing alcoholic extract of the drug added 5-10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

Test for tannins

To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

To the sample of extract, potassium cyanide was added, deep red colour was confirmed the presence of tannins.

To the sample of extract, potassium dichromate solution was added, yellow precipitate was produced.

Test for protein and amino acid

Biuret's test: To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.

Ninhydrin's test: Two drops of freshly prepared 0.2 % ninhydrin reagent was added to the extract and heated to boiling for 1 - 2 min. and allow cooling. A blue colour developed that indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract in a test tube, add conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. Added 20 % of sodium hydroxide solution in excess orange colour indicated presence of aromatic amino acid.

Millon's test: The small quantity of extract of the drug dissolved in distilled water added 5 - 6 drop of millon's

reagent. A white precipitate was formed which turned red on heating, indicated the presence of proteins.

Lead acetate test: The extract was taken and two ml of 40 % sodium hydroxide solution was added and boiled, glacial acetic acid was added and cooled than added 1 ml of lead acetate solution, gray black precipitate was formed which indicated presence of sulphur containing amino acid.

Test of fats or fixed oils

Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then added 10 % sodium hydroxide solution a clear blue solution was obtain which showed glycerin present in sample.

Using sodium hydrogen sulphate: The extract was taken in test tube added a pinch of sodium hydrogen sulphate pungent odour was formed which showed glycerin present in sample.

Saponification: Four ml of 2 % sodium carbonate solution was taken and the extract was added. Shaked vigorously and boiled. A clean soapy solution was formed cooled and added few drops of conc. HCl and observed that fatty separate out and float up.

Results and Discussions

The crude extracts so obtained after each of the successive soxhlet extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the fruits of *Terminalia chebula* and rhizomes of *Curcuma longa*of the plants using petroleum ether, ethyl acetate and methanol as solvents are depicted in the Table 1.

The physical constituent's estimation of the drugs is an essential parameter to determine adulteration or inappropriate handling of drugs. The physicochemical characters of powder drug of fruits of *Terminalia chebula* and rhizomes of *Curcuma longa* such as total alcohol soluble extractive, water soluble extractive, ash value, acid insoluble ash, and water soluble ash, loss after drying and foreign substances are given in Table2. The *Curcuma longa* rhizomes showed less moisture content; it was 5.23% than fruits of *Terminalia chebula*. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. These can serve as a valuable basis of information and provide suitable standards to establish the quality of this plant material as future prospects. An ash values are used to decide quality and purity of crude drug, it indicates presence of various impurities like, silicate, oxalate and carbonate. The water soluble ash is used to determine the quantity of inorganic compounds present in drugs. The acid insoluble ash helps to estimate the amount of silica present in the material. The total water soluble portion of the ash is considered as water soluble ash. Less amount of these three parameters indicate that the inorganic matter and silica were less in fruit of *Terminalia chebula* and rhizomes of *Curcuma longa*.

The results of qualitative phytochemical analysis of the crude powder fruit of *Terminalia chebula* and rhizomes of *Curcuma longa* are shown in Table 3 & 4. Petroleum ether and methanolic extract of fruit of *Terminalia chebula* sample showed the presence of carbohydrates, glycosides, alkaloids, flavonoids, triterpenoids, steroids, tannin and phenolic compounds. Petroleum ether and methanolic extract of rhizomes of *Curcuma longa* sample showed the presence of glycosides, alkaloids, flavonoids, saponins, triterpenoids, steroids, tannin and phenolic compounds.

Conclusion

Suitable parameters for the phytochemical screening and physicochemical characterization have been for fruit of *Terminalia chebula* and rhizomes of *Curcuma longa*. These parameters can serve as quality characters and criteria for the evaluation of the identity and authenticity of the plant. Further studies are recommended to isolate and characterize the chemical constituents which may be responsible for the pharmacological activities of the plant.

Table 1 : Results of percentage yield of *Terminalia chebula, Curcuma longa*

Plant Name	Percentage yield (%)		
	Pet. ether	ethyl acetate	Methanol
Terminalia chebula	3.5	4.3	6.3
Curcuma longa	3.8	4.9	7.6

Table 2 : Physico-chemical parameters of fruit of *Terminalia chebula* and rhizomes of *Curcuma longa*

S. No.	Parameters	Results	
		Terminalia chebula	***Curcuma longa***
1	Description	Brownish	Yellowish
2	Loss on drying at 105^0C	1.41% w/w	2.21% w/w
3	Total Ash	3.48 % w/w	4.21 % w/w
4	Acid-insoluble ash	0.43 % w/w	0.23 % w/w
5	Water-soluble extractive	44.11 % w/w	33.12 % w/w
6	Alcohol-soluble extractive	31.32 % w/w	27.32 % w/w
7	pH of 1.00% w/v soln	3.20	2.10
8	pH of 10.00% w/v soln	3.23	2.21
9	Moisture content	6.31%	5.23%

Table 3 : Phytochemical evaluations of different extracts of *Terminalia chebula*

S. No.	Experiment	Results	
		Petroleum etherextract	**Methanolic extract**
	Test for Carbohydrates		
1	Molisch's Test	-ve	+ve
2	Fehling's Test	-ve	+ve
3	Benedict's Test	-ve	+ve
	Test for Protein & Amino acids		
4	Biuret's Test	-ve	-ve
5	Ninhydrin Test	-ve	-ve
	Test for Glycosides		
6	BorntragerTest	+ve	+ve
7	Killer killaniTest	+ve	+ve
	Test for Alkaloids		
8	Mayer's Test	-ve	+ve
9	Hager's Test	-ve	+ve
10	Wagner's Test	-ve	+ve
	Test for Saponins		
11	Froth Test	-ve	-ve
	Test for Flavonoids		
12	Lead acetate	-ve	+ve
13	Alkaline reagent test	-ve	+ve
	Test for Triterpenoids and Steroids		
14	Libermann-Burchard Test	-ve	+ve
15	Salkowski Test	-ve	+ve
	Test for Tannin and Phenolic compounds		
16	Ferric Chloride Test	-ve	+ve
17	Gelatin Test	-ve	+ve
18	Lead Acetate Test	-ve	+ve

Table 4 : Phytochemical evaluations of different extracts of *Curcuma longa*

S. No.	Experiment	Results	
		Petroleum ether extract	**Methanolic extract**
	Test for Carbohydrates		
1	Molisch's Test	-ve	-ve
2	Fehling's Test	-ve	-ve
3	Benedict's Test	-ve	-ve
	Test for Protein & Amino acids		
4	Biuret's Test	-ve	-ve
5	Ninhydrin Test	-ve	-ve
	Test for Glycosides		
6	BorntragerTest	-ve	+ve
7	Killer killaniTest	-ve	+ve
	Test for Alkaloids		
8	Mayer's Test	-ve	+ve
9	Hager's Test	-ve	+ve
10	Wagner's Test	-ve	+ve
	Test for Saponins		
11	Froth Test	+ve	+ve
	Test for Flavonoids		
12	Lead acetate	-ve	+ve
13	Alkaline reagent test	-ve	+ve
	Test for Triterpenoids and Steroids		
14	Libermann-Burchard Test	-ve	+ve
15	Salkowski Test	-ve	+ve
	Test for Tannin and Phenolic compounds		
16	Ferric Chloride Test	-ve	+ve
17	Gelatin Test	+ve	+ve
18	Lead Acetate Test	+ve	+ve

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