International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

SCIENTIFIC VALIDATION OF PURIFICATION OF KADUKKAI (TERMINALIA CHEBULA) – A SIDDHA DRUG

Natarajan*[1], Sathiyarajeswaran[1], Kannan[1]

[1]Siddha Central Research Institute, (Central Council for Research in Siddha, Dept. of AYUSH) Arumbakkam, Chennai, Tamilnadu – 600106.

ABSTRACT

This article provides an overview of the purification methods of Terminalia chebula(TC) as mentioned in Siddha medical practice(2), a traditional medical system in India. According to this practice the purification of the herb is to remove the seed from the ripen fruit before processing into medicine. Changes in the chemical profile of Terminalia chebula before (with seed - TCS) and after purification (without seed - TCWS) is achieved through physiochemical, phytochemical and HPTLC analysis. The results revealed TCWS has 60% water soluble extract and 40% alcohol soluble extract to that of 25% and 15% respectively in TCS. The total ash content was very minimal in TCWS. There is increase in the concentration of tannins, anthroquinones, alkaloids, saponins and decrease in the protein content in TCWS. No change in the concentration of total phenols, flavones, glycosides, reducing sugar and quinines. The HPTLC analysis shows nine peaks for TCWS whereas seven for TCS. This study reassures that Terminalia chebula is pharmaceutically potent only after removal of seeds which substantiates intuition of Siddhars scientifically.

Correspondence Author

DR. S. NATARAJAN

1/3, Srinivasa Mudali Street, F 2, Sri Murugan Flats Perambur, Chenna, Tamilnadu – 600011

Email: drnatarajan78@hotmail.com

Keywords:- *Terminaliachebula, Kadukkai, purification, Siddha medicine*

INTRODUCTION

Herbal drugs account a major stock holder in healthcare of developing countries[1]. Ancient Siddha Literature defines the benefits of medicinal plants and their potential therapeutic applications[2]. The usages of herbs as home remedies are increased worldwide nowadays and represent substantial proportions in pharmaceutical industry and global drug market. Traditional medicines are still being used as primary health care in remote areas where modern medicines not reached.

Siddha medicine quotes that Terminalia chebula cares the patients as if a mother cares a child[3&4]. Terminalia chebula is used in fevers, cough, asthma, urinary diseases, piles, worms, rheumatism and scorpions ting. It is highly useful in chronic diarrhea, dysentery, flatulence, vomiting, hiccup, colic and enlarged spleen and liver[5].

Terminalia chebula(T. chebula) has been reported to exhibit a variety of biologicalactivities including anticancer[6], antidiabetic[7], antimutagenic[8], antibacterial[8], antifungal[10] and antiviralactivities[11] Purification is the first step in any standard operating procedures in preparing any medicine in Siddha.Purification of Terminalia chebula is to remove the seed and use fruit rind only.

MATERIAL AND METHODS

Collection of plant material and authentication:

The medicinal dried ripe fruit of Terminalia chebula was purchased from local crude drug market from Chennai. It was identified and authenticated by Department of Pharmacognosy, Siddha Central Research Institute, Chennai.

Drying and size reduction of plant:

Two samples were taken, one sample was powdered with seed(TCS) and the other without seed(TCWS), sieved(80 mesh) and stored in air tight container at 25^{O} C. Macroscopic characters were studied as per standards. Powder analyzed as per standard procedure. A Small quantity of powdered material was washed with water to remove sugar and cleared by heating gently with standard choral hydrate solution, cooled and mounted in glycerin for microscopic observation. Phloroglucinol stain and dilute iodine solution were used to observe lignified stone cell and starch grains respectively.

Determination of physicochemical parameters[12]:

The dried plant material was subjected for determination of physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, moisture content, foreign organic matter, crude fibre, alcohol soluble extractive and water soluble extractivewere calculated as per India Pharmacopoeia

Determination of Foreign Matter

Weigh a sample of plant material, taking the quantity indicated above unless other-wise specified in the test procedures for the plant material concerned. Spread it in a thin layer and sort the foreign matter into groups either by visual inspection, using a magnifying lens (6x or 10x), or with the help of a suitable sieve, according to the requirements for the specific plant material. Sift the remainder of the sample through a No. 250 sieve; dust is regarded as mineral admixture. Weigh the portions of this sorted foreign matter to within 0.05g. Calculate the content of each group in grams per 100g of air-dried sample.

Total ash

Place about 2-4g of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600°C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh. If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate R. Dry on a water-bath, then on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

Acid-insoluble ash

To the crucible containing the total ash, add 25 ml of hydrochloric acid (~70g/l) TS, cover with a watch-glass and boil gently for 5 minutes. Rinse the watch-glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ashless filter-paper and wash with hot water until the filtrate is neutral. Transfer the filter-paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of acid-insoluble ash in mg per g of air-dried material.

Water-soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter-paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash.

Calculate the content of water-soluble ash in mg per g of air-dried material.

Preliminary phytochemical analysis[13]:

Phytoconstituents(triterpenoids, steroids, alkaloids, sugar, tannins, glycosides and flavonoids, etc) were detected.

Test for Phenol

Substance in water is added with 5 % alcoholic ferric chloride. Dark blue or greencolour shows presence of phenol.

Test for Tannin

Substance is shaken with water and added with lead acetate solution. White precipitate shows the presence of tannin.

Test for Flavonoids (Shinoda test)

Substance is dissolved in alcohol, added with magnesium bits and concentrated hydrochloric acid. On heating over a water bath, the appearance of majentacolour shows the presence of flavonoids.

Triterpenoids(Noller's Test)

To few mg of extract, addtin and thionyl chloride and heat in water bath. Purple colour indicates the presence of tritepenoids.

Test for Proteins (Biuret test)

To the sample solution in a test tube, add sodium hydroxide solution and then add a few drops of very dilute (1 %) copper II sulphate solution and mix gently. Appearance of purple colour indicates the presence of protein.

Test for Glycosides

Substance is treated with anthrone and concentrated sulphuric acid. On heating over a water bath, the appearance of green colour shows the presence of glycoside.

Test for Reducing sugar (Fehling's Test)

To the sample solution, Fehling's reagent is added. The appearance of brick red precipitate or colouration indicates the presence of reducing sugar.

Test for Anthraquinones

Few milligram of crude powder is shaken with 10 ml of benzene and filtered. To this filtrate, 0.5 ml of 10 % ammonia solution is added and the mixture is shaken well and the presence of the violet colour in the layer phase indicates the presence of the anthraquinone.

Test for Quinones

To few mg of extract, add few drops of concentrated sulphuric acid. Appearance of red colour shows the presence of quinone.

Test for Alkaloids (Mayer's Test)

To few milligrams of solvent free extract add few ml of HCl and filtered. To this, add2 drops of Mayer's Reagent. White creamy precipitate indicates the presence of alkaloids.

Test for Saponins

To fewmg of extract distilled water is added and shaken well. The formation of foam indicates thepresence of saponin.

Quantitative determination of the phytoconstituents

Saponins determination

The method employed was that of Obadoni and Ochuko (2001).The samples were ground and 20 g of each were put into a conicalflask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at55°C. The mixture was filtered and the residue re-extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added andshaken vigorously. The aqueous layer was recovered while the Omale et al. 89 ether layer was discarded. The purification process was repeated.60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. Theremaining solution was heated on a water bath. After evaporation the

samples were dried in the oven to a constant weight; thesaponins content were calculated as percentage.

Alkaloid determination

The determination of alkaloid was as described by Harborne (1973). A portion (5 g) of the sample was weighed into a 250 mlbeaker and 200 ml of 10% acetic acid in ethanol was added,covered and allowed to stand for 2 h. This was filtered and theextract was concentrated on a water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was addeddrop-wise to the extract and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. Theresidue is the alkaloid, which was dried and weighed.

Tannin determination

Tannin determination was done by Van – burden and Robinson (1981) method. A portion (500 mg) of the sample was weighed intoa 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Flavonoid determination

This was done following the method of Boham and Kocipai – Abyazan (1994). A portion (10 g) of the plant sample was extractedrepeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmanfilterpaper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath andweighed to a constant weight.

HPTLC finger printing:

For HPTLC study, sample (1g) was boiled in 2 N hydrochloric acid (5ml) for 30 min, cooled, filtered and extracted with diethyl ether(10ml, three times). Diethyl ether extract was concentrated and dried. Known quantity of extract was dissolved in methanol and used as test fg`solution for HPTLC.

Calculated quantities of methanolic extracts were applied on HPTLC (Pre coated silica gel G 60 F254 Merck) aluminum plate (20cm x 10 cm) by using CAMAG Linomat applicator IV. Plate was eluted to a distance of 8.5 cm at room temperature(25^{o}C) in a solvent system[Toulene: ethyl acetate: GAA: formic acid (20:45:20:5)] in previously saturated twin through chamber (CAMAG). Dried plate was scanned initially at 254nm for quantitative analysis and then at 366nm

The objective of comparing the changes in the chemical profile of Terminalia chebula before and after purification is achieved through conducting the following physio chemical analysis, phyto chemical analysis, HPTLC tests.

RESULTS

The results of physicochemical analysis are given in the Table-1. The total ash value, acid insoluble ash value and water soluble ash value of TCS were found to be 4.56, 2.75 and 2.07 % respectively. Alcohol soluble and water soluble extractive values were found to be 14.23 and 25.41% respectively. The percent yields of differentextracts are given in Table-1. The total ash value, acid insoluble ash value and water soluble ash value of TCWS were found to be 5.00, 0.50 and 2.07 % respectively. Alcohol soluble and water soluble extractive values were found to be 42 and 65 % respectively. The percent yields of different extracts are given in Table-1.The results of preliminary phyto- chemical analysis of different extracts are givenin Table-2. Secondary metabolites were found in goodproportion inTCWS whencompared with TCS.These secondary metabolites may be responsible forvarious pharmacological effects In the HPTLC Nine active peaks are identified in the TCWS whereas there are seven peaks in the TCS.

DISCUSSION

Kadukkai is one of the important drug in Siddha Medicine. There are number of formulations like Chooranam (Powder), Ennai(Medicated Oil), Ilagam(Confectionary) Mathirai(Pills) in which kadukkai is a main ingredient. Kadukkai is also a rejuvenator drug. In

spite of variety of usage of this drug, the purification of the drug stands vital.

The comparative study of TCWS and TCS reveals that the secondary metabolites like phenols, tannins, alkaloids, anthroquinones, glycosides, flavanoids etc. are higher in TCWS than the TCS. This support the claim of TCWS is pharmacogically potent than TCS. Moreover there is absence of alkaloid in the TCS evinces the degradation of phytochemicals. In the HPTLC Nine active peaks are identified in the TCWS whereas there are seven peaks in the TCS. This also confirms the TCWS is more potent than TCS. Futher more studies are requested to understand the rationale of traditional purification methods in Siddha Medicine.

CONCLUSION

The Wisdom of Siddhars are substantiated scientifically with all evidence bases which make the system alive and wide spreading. The expertize of Siddhars in selection, purification and Standard Operating Procedures are thought provoking which is once again proven in this comparative study. The traditional standard even though arise out of intuition have a scientific evidence base which has been proved. The study reveals that Terminalia chebula is useful only after removal of seeds to be pharmaceutically potent.

Table1: Physico-chemical parameters of Terminalia chebula with seed(TCS) and Terminalia chebula without seed(TCWS)

S.No	Parameters	TCS (**Mean % w/w)**	TCWS (**Mean % w/w)**
1	Crude fiber content	13.45	
2	Total ash	4.56	5.00
3	Acid insoluble ash	2.75	0.5
4	Alcohol soluble extractive	14.23	40
5	Water soluble extractive	25.41	60
3	Acid Soluble Ash	1.95	
5	Water Soluble Ash	2.07	
6	Water Insoluble Ash	2.87	

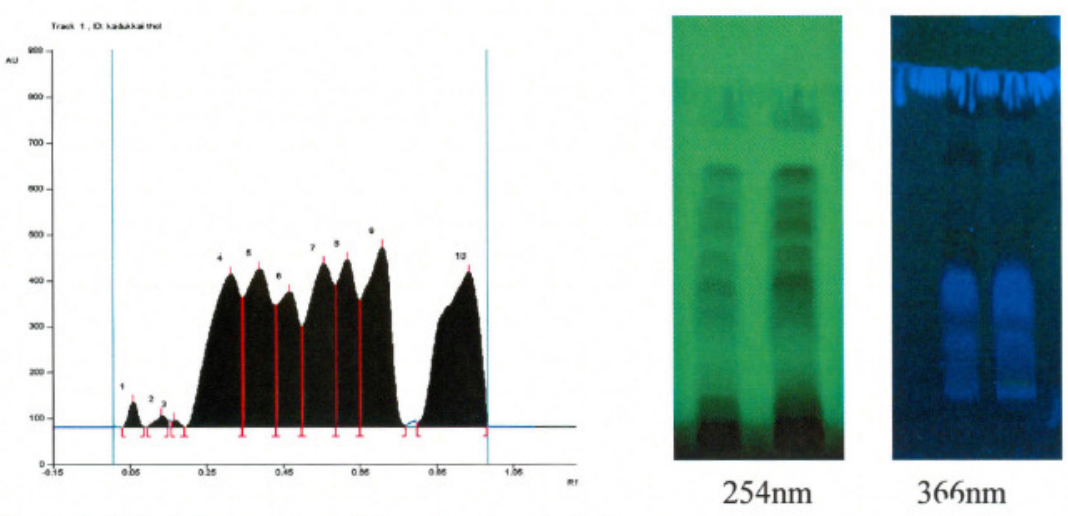
Table 2: Preliminary phytochemical test of Terminalia chebula with seed(TCS) and Terminalia chebula without seed(TCWS)

S.No	Natural products	TCS	TCWS
1	Total phenols	+++	+++
2	Tannins	++	+++
3	Flavones	++	++
4	Proteins	++	+
5	Glycosides	-	-
6	Reducing sugars	+++	+++
7	Anthraquinones	+	+++
8	Quinones	+	+
9	Alkaloids	-	++
10	Saponins	+	+++

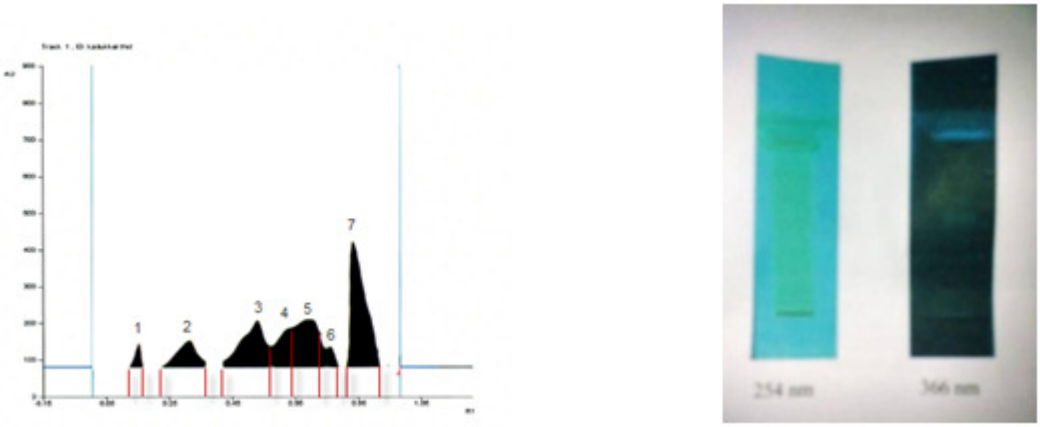
+ - present ++ - Moderately present +++ - Abundant

HPTLC:

Terminalia chebula without seed(TCWS)



Terminalia chebula with seed(TCS)



REFERENCES

1. World Health Organization Geneva, Quality control methods for medicinal plant materials, Type set in Hong kong, Printed in England, ISBN 92 415 45100 (NLM classification QV766)].[1]
2. Subbarayappa BV. Siddha medicine : an overview. Lancet. 1997.20-27: 350(9094):1841-4.
3. Hakkim. Mohammed Abdullah., 1992. KadukkaiVallaraiThaniManbu, third ed. Directorate of Indian Medicine and Homeopathy, Chennai.
4. C.KannusamyPillai., 1974. ChikitcharathnaDeepam, third ed. Rathinanaicker Sons, Chennai.
5. Dr. K.M. Nadkarni., 1976. Indian MateriaMedica, Revised reprint of third ed, Popular PrakashanPvt Ltd, New Delhi.
6. Saleem A, Husheem M, Härkönen P, Pihlaja K (2002) Inhibitionof cancer cell growth by crude extract and the phenolicsofTerminaliachebula Retz. fruit. J Ethnopharmacol 81:327–336
7. Sabu MC, Kuttan R (2002) Anti-diabetic activity of medicinalplants and its relationship with their antioxidant property.JEthnopharmacol 81:155–160
8. Kaur S, Grover IS, Singh M (1998) Antimutagenicity of hydrolysable tannins from Terminalia chebula in Salmonella typhimurium.Mutat Res 419:169–179

9. Aqil F, Khan MS, Owais M, Ahmad I (2005) EVect of certain bioactiveplant extracts on clinical isolates of beta-lactamaseproducing methicillin resistant Staphylococcus aureus. J BasicMicrobiol 45:106–114
10. Vonshak A, Barazani O, Sathiyamoorthy P, Shalev R, Vardy D,Golan-Goldhirsh A (2003) Screening South Indian medicinalplants for antifungal activity against cutaneous pathogens.Phytother Res 17:1123–1125
11. Badmaev V, Nowakowski M (2000) Protection of epithelial cellsagainstinXuenza a virus by a plant derived biological responsemodiWer Ledretan-96. Phytother Res 14:245–249
12. Trease, G.E. and W.C. Evans, 1989. Pharmacognosy.11th Edn. Brailliar Tiridel and Macmillian Publishers, London.
13. Harbone JB. Phytochemical Methods. London: Chapman and Hill; 1973.

*****